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TITLE: Services to develop, standardize, and validate polymerase chain reaction (PCR) protocols for the detection of leishmaniasis in clinical samples

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## 1. Objectives

As set forth in the Statement of Work for contract DAMD17-92-C-2097 entitled "Services to develop, standardize, and validate polymerase chain reaction (PCR) protocols for the detection of Leishmaniasis in clinical samples", the objective of this study is comprised of two phases. In the first phase, the contractor shall "Develop and standardize the technology known as the polymerase chain reaction (PCR) for the detection of leishmaniasis in clinical samples". Phase II defines the task of the contractor to "Validate PCR testing protocols developed as a result of the first objective in a large select military population of approximately 2,500 to 3,000 individuals". The mid term report (October 20, 1993) described the development of a polymerase chain reaction (PCR) assay for the detection of leishmaniasis and testing of a limited number of clinical samples. This final report includes the summary of the previous report and the additional work carried out to optimize the PCR methods for Old World and New World species of *Leishmania* and testing of additional clinical samples from different geographic areas.

## 2. Introduction

Leishmaniasis, a very important zoonotic disease of humans in the developing countries, became a problem of considerable concern in the United States, following Operation Desert Storm (ODS) (1). The etiologic agent of leishmaniasis is a protozoan of the genus *Leishmania*. Members of the genus *Leishmania* are grouped broadly into the New World (*L. mexicana* and *L. braziliensis* complexes) and Old World (*L. donovani* and *L. tropica*) species. Four species and at least 15 subspecies are generally recognized. All are similar in morphology and life history. The parasites which infect humans are transmitted by the Old and New World sandflies, *Phlebotomus* and *Lutzomyia*, respectively. In the insect vector, the protozoan is found as the flagellated or promastigote form. Upon introduction into the human host, and if phagocytized by a macrophage, the parasite transforms into the non-flagellated, ovoid form, the amastigote.

Three major types of pathologies result from active infection with *Leishmania*. Cutaneous leishmaniasis is characterized by fairly localized cutaneous lesions. If the organism spreads from the site of infection to the nasal cavity and naso-pharyngeal region, the severely disfiguring mucocutaneous leishmaniasis can result. The most severe form of the disease occurs if the parasite metastasizes through the reticuloendothelial system, to infect the spleen, bone marrow, and other tissues. Visceral leishmaniasis, or Kala-azar, is frequently fatal, if untreated.

Increased travel between the industrialized countries of the West and the developing nations, by businessmen, military personnel and tourists, mandates the development of more appropriate tools for laboratory diagnosis of this potentially very serious disease. Effective drug treatments to control the infection are available, but a proper diagnosis is required before starting treatment. To apply laboratory diagnostic methods in developing nations,

simplified assay formats are desirable. A further reason to pursue development of nucleic acid based tests which are simplified and amenable to large scale screening is their potential for preventing the dissemination of these and similar parasitic infections through the blood donor pool.

For the purposes of this study, the primary interest is in Old World species, as these are the organisms native to the geographic region where ODS took place. The strains *L. tropica*, *L. major*, and *L. donovani* predominate in south west Asia, and are the 3 species against which most of the PCR primer sets described in this report were tested. When patient samples were used as positive controls, the majority of these were from geographic regions where *L. donovani* predominates. During the course of this contract, however, several New World and Old World species have also been examined, as well as patient samples from South and Central America, Africa and India.

## **2.1 Polymerase Chain Reaction (PCR) Assay**

The polymerase chain reaction (PCR) is *in vitro* DNA replication. Rather than the DNA double helix being unwound by replication-associated enzyme complexes, DNA is heat-denatured in the presence of a thermostable DNA dependent DNA polymerase, oligonucleotide primers complementary to target sequences to be amplified, and deoxynucleoside building blocks for DNA synthesis. Alternating cycles of denaturation, primer annealing (hybridization), and extension, result in the accumulation of double-stranded DNA fragments of discrete length, termed amplicons. This process operates under defined conditions and for a limited number of cycles as a quantitative exponential amplification of the target sequences. PCR thus results in a vast increase in copies of the target sequence and probably constitutes the most sensitive analytical technique currently available for molecular diagnostics,

being capable of detection of a single copy of nucleic acid per reaction.

### 2.1.1 Quality Control For PCR

SRA has more than 6 years experience using PCR in both a diagnostic and a developmental testing atmosphere, and is well versed in techniques required to minimize or eliminate the chances of contamination from different sources (2,3). Both physical and biochemical methods of avoiding cross contamination that SRA has followed in testing *Leishmania* specimens have been described in the mid-term report (October 20, 1993). In brief, these measures include, physical separation of pre- and post- amplification laboratories equipped with dedicated equipments, including certified Biosafety cabinets equipped with HEPA filters for all steps involving potentially infectious materials; strict QC of all reagents; single-use frozen aliquots of critical reagents; use of either positive displacement pipettors with single-use tips and pistons or special commercially-available pipet tips with an aerosol-barrier; proper use of positive and negative controls, hot start method for PCR and use of the enzyme uracil-N-glycosylase (uracil DNA glycosylase, UNG), in a method analogous to the excision repair system of living cells (4).

In this method, dUTP substitutes for dTTP in all PCR reactions, and UNG is included in all PCR reactions. Prior to temperature cycling, this moderately heat stable enzyme selectively excises uracil residues which have been incorporated into DNA during previous amplifications. During the initial heating step in PCR, the DNA backbone of any contaminating previously amplified material is broken at these apyrimidinic sites, thus preventing the U-containing DNA from serving as a template for polymerization. Since native DNA templates do not contain U residues and since *Taq* DNA polymerase efficiently incorporates dUTP as well as dTTP during PCR, this technique can be made to operate without decreasing sensitivity or specificity of PCR reactions. Pretreatment of all

PCR reactions eliminates the most common cause of a false positive result - carryover of amplified DNA from a previous amplification. SRA has incorporated this technique into PCR protocols for the amplification of HIV-1 and HTLV-I/II, as well as all PCR methods which have been used by us to amplify *Leishmania* sequences.

### **2.1.2 Design of an Appropriate Testing Algorithm**

In the course of conducting PCR testing prior to the advent of UNG-mediated carryover prevention, we found it necessary to design PCR testing algorithms to minimize the chance of false positive test results arising from PCR product contamination (5). Specifically, we have tested (and continue to test) all specimens as duplicate reactions plus a negative control spatially unique to the duplicate reaction set, with a primary primer set. Reaction products are subjected to hybridization analysis using an oligonucleotide probe to sequences bracketed by but not overlapping the primers. A result of "reactive" is then defined as the detection of specific hybridization signal in both duplicates, with no specific signal in the corresponding negative control reaction. The detection of specific hybrids in only one duplicate is defined as a result of "non-diagnostic," necessitating repeat testing. "Non-reactive" refers to the absence of specific signal in both duplicates, with low copy number positive controls being detected. In spite of the introduction of sophisticated biochemical methods for detection of product cross-contamination, the ever-present possibilities for operator error support the continued use of carefully-designed PCR testing algorithms.

### **2.1.3 Optimization of Reaction Parameters**

In addition to the techniques designed to eliminate contamination and resulting false positives, all PCR reactions are optimized for both specificity and product yield. These procedures include empirical determination of optimal oligonucleotide ratios and

concentrations, magnesium ion concentration, Taq polymerase concentration, and annealing temperature. Despite these precautions, some non-specific annealing of PCR primers does occur, even with single copy gene detection, and more so with the detection of retrovirus or parasite DNA in the presence of a high background of human genomic DNA. Since annealing of primers to template is not 100% specific under all conditions encountered during the course of a PCR reaction, it is necessary to adjust reaction conditions to maximize synthesis of specific product.

While post-PCR hybridization detection ensures that non-specific products will not be detected, the synthesis of these spurious amplicons affects the amplification process. Non-specific reaction products do incorporate PCR primers such that subsequent amplification cycles result in their specific amplification as "quasi-specific" templates. This detracts from the overall efficiency of the reaction, as both non-specific and specific products compete for primer and Taq binding. Careful adjustment of reactant concentrations to strike a balance between maximization of primer hybridization and minimization of non-specific annealing can significantly increase PCR product yield and also extend sensitivity into the < 10 copy range. In our experience, with some primer sets, rigorous optimization can extend the detection limit 2 to 3 orders of magnitude. SRA has pioneered the development of HPLC protocols for quantitation of PCR products (6). The analytical precision of HPLC analysis allows more precise determination of PCR product yield, with very fast turnaround, often allowing complete optimization of reaction conditions for a new primer set within two days.

At the start of this contract, HPLC was used to evaluate PCR reaction products. While it has the aforementioned advantages of precise quantitation, it does not have the sensitivity of our current microplate based capture assays. A basic protocol for

using the HPLC for PCR product detection is provided, in the Methods and Results section.

#### 2.1.4 Design of PCR Primers and Probes

Design of synthetic oligonucleotide primers and probes is facilitated by the use of computer software dedicated to that purpose (e.g., Oligo, National Biosciences; Primer Detective, Clontech). After candidate sequences are designed, these sequences are compared to DNA sequences of both related and unrelated organisms by computer homology searches from the Genbank database using the Lasergene DNASTar program running on a Macintosh IIci. These preliminary steps reduce the chance of PCR artifacts (primer-dimers) due to primers that share significant sequence homology, or secondary structure that would reduce the overall efficiency of the PCR amplification. In addition, with highly variable sequences or sequences which are only partially known (e.g., *Leishmania* minicircle kDNA), it has been suggested that PCR primers preferentially end in 3'-T, to minimize the effects of possible 3'-mismatch (7). Other strategies to lessen the effect of random non-homologies with the target sequence include the synthesis of primers with degenerate positions and/or inosine substitutions (8). However, excessive degeneracy should be avoided, in order to maintain specificity. We have exploited this latter technique in the design of some "second generation" primers that show improved detection of New World *Leishmania* strains in our testing.

In the case of *Leishmania*, almost 20 different PCR primer combinations have been evaluated to date. These include multiple sets that amplify sequences found in the kinetoplast (kDNA) minicircles, one set directed against sequences found in the kDNA maxicircles (equivalent to mitochondrial DNA), one set directed against ribosomal RNA sequences (rRNA), and one set specific for conserved sequences from one nuclear gene (DHFR). Finally, it should be noted that, even though primer and probe sequences have

been carefully chosen based on predicted homology to the desired sequences and the lack of homology to other (especially human) sequences in GenBank, it is still necessary to test these primers sets against actual specimens of related and unrelated organisms. This has been done for all primer sets that show acceptable sensitivity against the *Leishmania* strains of interest.

### **3. Methods and Results**

While there has been significant improvement in the sensitivity of the PCR detection protocol over the two year course of this contract, the basic elements of the procedure employed are common to many of the PCR protocols already in use by SRA. Sample preparation steps and the basic PCR protocols remain essentially unchanged from that described at the start of the contract, since they were validated previously. Similarly, the capture plate procedure for PCR product detection was developed for other applications and its adaptation to this use was governed solely by the design and implementation of *Leishmania*-specific probes. The PCR reaction and detection protocols are given in the following sections, along with a detailed discussion of results obtained with the various PCR primer sets tested to date.

#### **3.1 Sample Preparation**

The sensitivity of PCR permits the detection of the low level of parasites in the peripheral blood, at least during active infection. In many cases, however, peripheral blood samples from a given individual were negative, while splenic or bone marrow aspirates were positive by PCR. Although the major specimen type is whole blood, the protocol given below works equally well for bone marrow and splenic aspirates, as well as cutaneous lesion lavage specimens, thus simplifying the overall test.

We have successfully employed differential lysis for the selective removal of RBC's from blood and bone marrow samples prior to DNA extraction for PCR. This method is based on the specific RBC-lytic activity of saponin, and is quite simple, requiring only the use of a tabletop centrifuge. The blood is gently mixed with 0.3% saponin (Mallinckrodt) in slightly hypotonic saline and allowed to remain at ambient temperature for 5 minutes, during which time RBC's are lysed. Centrifugation recovers leukocytes, which are again washed with saponin to remove residual RBC's. Some specimens need to be washed more than 1-2 times depending upon the amount of contaminated RBC's lysed with saponin. The final cell pellet contained total leukocytes, and appeared to be free of inhibition to PCR, by either heme or the saponin itself. The cell pellet was then lysed by the addition of proteinase K, and, following heat inactivation of the proteinase K, the crude lysate was either used directly in diagnostic PCR reactions or stored frozen until the PCR reaction was set up.

### **3.1.1 Sample Preparation by Total Leukocyte Separation**

1. For specimens received in LeukoPREP tubes
  - a. Centrifuge at 3000 rpm for 20 min. Pipet off the supernatant into a 50 mL polypropylene centrifuge tube
  - b. Count cells using ZAP-OGLOBIN. (40  $\mu$ L specimen + 20 mL Isoton II + 5-6 drops ZAP-OGLOBIN)
  - c. Add 20 mL of 0.1% saponin in 0.6% NaCl. Mix well by inversion. Maintain at room temperature 5 min.
  - d. Centrifuge at 1500 rpm for 15 min. Decant the supernatant.
2. Whole Blood specimens
  - a. Count cells using ZAP-OGLOBIN. (40  $\mu$ L specimen + 20 mL Isoton II + 5-6 drops ZAP-OGLOBIN)
  - b. Add 10 volumes of 0.1% saponin in 0.6% NaCl. Mix well by inversion. Maintain at room temperature 5 min.

- c. Centrifuge 1500 rpm for 15 minutes. Decant the supernatant.
- d. Resuspend the pellet with 15 mL 0.1% saponin in 0.6% NaCl
- e. Centrifuge 1500 rpm for 15 minutes. Decant the supernatant.

### 3.1.2 Cell Lysis

1. Use the cell count taken at the beginning of this procedure and determine lysis buffer volume for  $30 \times 10^6$  cells/ mL. Add the determined volume of lysis buffer containing 2X proteinase K. Vortex briefly.
3. Incubate in a water bath at 55° C - 60° C. for 1 h. Vortex briefly. If a large number of cells are being lysed, it may be necessary to vortex several times during this hour or extend the incubation time.
4. Transfer lysate to 1.5 mL screw-cap microcentrifuge tube. Label tube with specimen number, date lysed, tech initials.

NOTE: Lysates prepared by this protocol should be labelled with an "S."

5. Heat-inactivate the proteinase K by keeping the tubes at 95° C for 15 min. in dry-bath.
6. Quench on ice. Store at -20° C in freezer boxes in pre-PCR lab.

### 3.1.3 PCR Cycling Conditions

1. Prepare lower layer PCR mix as follows:

|                           |         |
|---------------------------|---------|
| H <sub>2</sub> O          | 10.9 µl |
| 10X buffer (Promega)      | 4.0 µl  |
| MgCl <sub>2</sub> (25 mM) | 4.0 µl  |
| dNTP (AUCG)               | 16.0 µl |
| UNG                       | 0.1 µl  |
| JW11/B-JW12 (10 µM each)  | 5.0 µl  |
| -----                     |         |
| TOTAL                     | 40.0 µl |

2. Prepare an upper layer PCR mix as follows:

|                      |              |
|----------------------|--------------|
| H <sub>2</sub> O     | 7.9 $\mu$ l  |
| 10X buffer (Promega) | 1.0 $\mu$ l  |
| UNG                  | 0.1 $\mu$ l  |
| Taq polymerase       | 1.0 $\mu$ l  |
| -----                |              |
| TOTAL                | 10.0 $\mu$ l |

3. Aliquot 40  $\mu$ L of the lower mix to each tube.
4. Add one bead of ampliwx. Number tubes. Place in the heating block at 65° C for up to 5 minutes to melt the wax. Allow to cool.
5. Pipet 10  $\mu$ L of the upper mix in the tube.
6. Add 50  $\mu$ L of the appropriate specimen lysate to each tube. Do not add positive control template in the pre-PCR lab.
7. In the positive control lab add 50  $\mu$ L *Leishmania* lysate of known copy numbers (1, 10 and 100) to the appropriate PCR tubes.
8. Immediately carry the reactions to the cycler. Proofread the program before starting. Fill out cycler log book.

#### CYCLER CONDITIONS

-----  
PRIMERS JW 11/B-12 or JW 11i/B-12i  
-----

Time Delay file

94° C 5' 0"  
-----

Step Cycles

97° C 0'15"

55° C 1' 0"

72° C 1' 0"

CYCLES 10  
-----

- Step Cycles

92° C 0'15"

55° C 1' 0"

72° C 1' 0"

CYCLES 30  
-----

SOAK 72° C

NOTE: products be frozen immediately upon removal from the cyclor, unless they can be assayed within 1 h due to the presence of un-denatured UNG and it's ability to degrade PCR products at room temperature and 4° C over time.

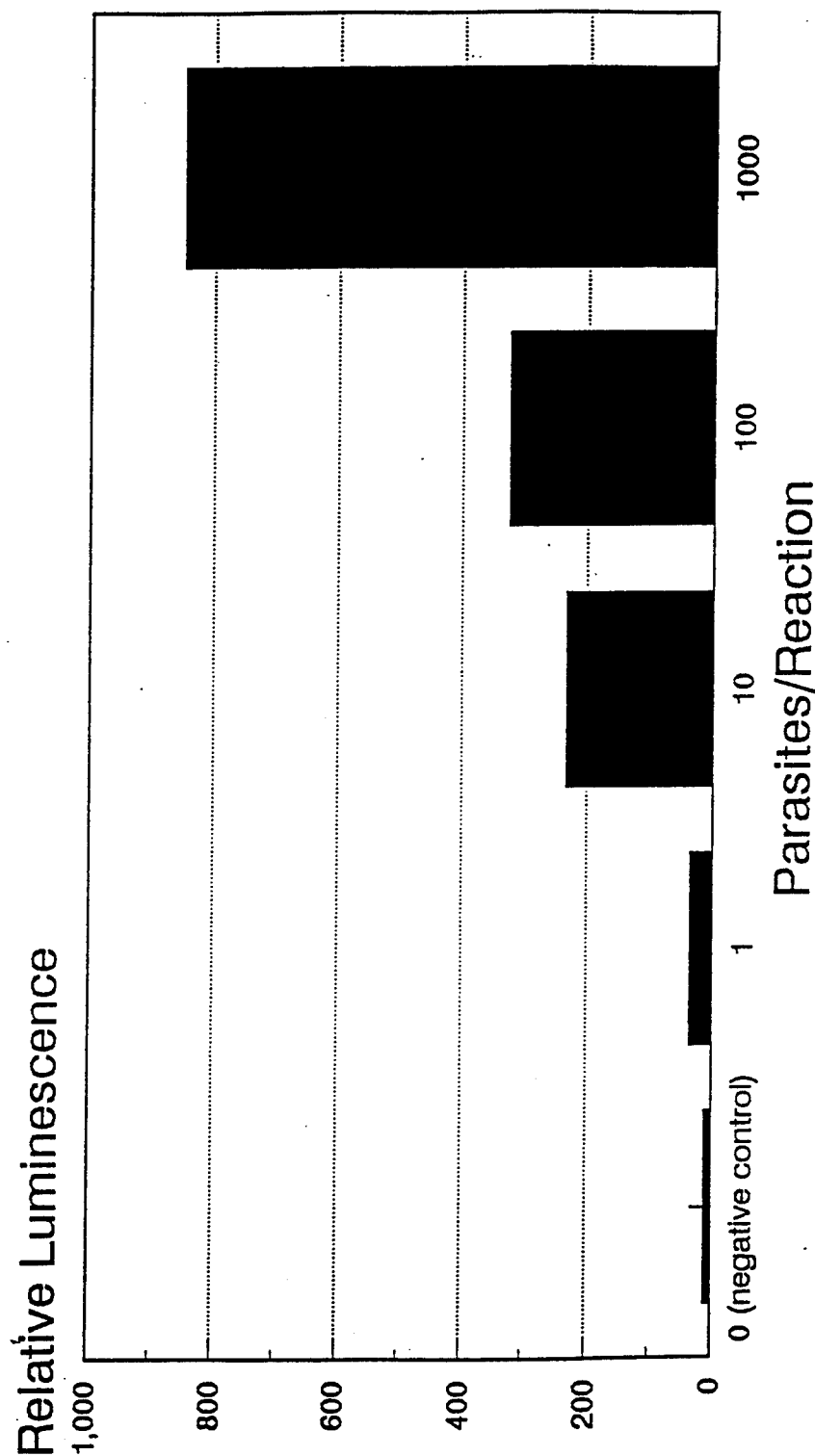
### **3.2 PCR Product Detection**

PCR products were analyzed using the affinity-based hybrid capture assay. We have used two slightly different detection systems with equivalent results. Earlier testings, utilized an Alkaline Phosphatase (AP) labeled specific oligonucleotide and a chemiluminescent substrate (Lumiphos) and later testings used the same oligo labeled with Horseradish Peroxidase (HRP) and a colorimetric substrate (OPD). The sensitivity obtained with either probe system is approximately equivalent. One distinct advantage with using and HRP probe is that the color produced from even 3 to 10 initial copies, after 40 cycles of amplification, is readily discernible by eye. Thus, it becomes possible to interpret results visually, by comparison with standards. Such an approach is acceptable for qualitative, though not quantitative, assays, and may be advantageous for application in developing nations. An example of the sensitivity of this system showing the detection of *L. tropica* by PCR using the AP-labeled probe and capture plate system is given in Figure 1.

#### **3.2.1 Capture Plate Procedure**

For simplicity, only the alkaline phosphatase (AP)-coupled protocol is described here. The primary differences include, obviously, use of a horseradish peroxidase (HRP) labeled oligonucleotide probe, OPD for colorimetric detection, and the use of clear rather than opaque plastic microwells in an ELISA-type plate reader rather than a luminometer. A diagram of the principles of operation of the capture plate is given in Figure 2.

Figure 1: Detection of *Leishmania tropica* minicircle DNA by PCR



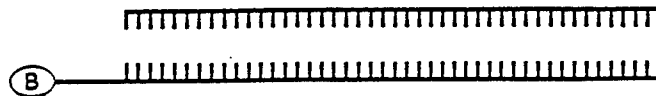
Dilutions of total DNA extracted from *L. tropica* promastigotes were prepared to correspond to the indicated numbers of parasites per reaction. Amplification was performed for 40 cycles using primers designed to amplify Old World *Leishmania*, and designated as sequences JW11 (5'-CCTATTTCACCAACCCMAGTTT, where M denotes a mixed base position: C,T) and JW12 (5'-CGGGTAGGGCGTTCTCGGAAANT, where M denotes A,T). Specific detection of the amplified sequences by alkaline phosphatase-labelled JW14 (5'-ATTGAACGGGGTTCTGTATGCATTTTCGAA) was performed in a 96 well plate, with chemiluminescent detection.

**Figure 2: Capture Plate PCR Product Analysis**

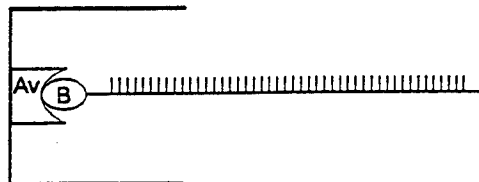
- ① PCR reaction is run using 1 biotinylated primer  
Specific product is produced



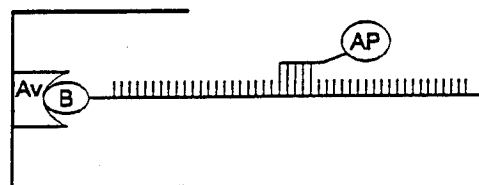
- ② PCR products are denatured by heating



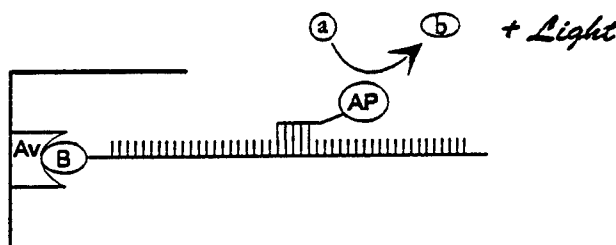
- ③ The biotinylated strand is captured in an avidin-coated microplate well



- ④ A specific synthetic oligonucleotide coupled to Alkaline Phosphatase (AP) is allowed to hybridize to the bound PCR product



- ⑤ A chemiluminescent substrate is added. The breakdown of this substrate produces light, which is detected by a microplate luminometer



1. Prepare an avidin-coated and blocked microwell plate according to the following procedure.
  - a. Pipet 120  $\mu$ l of 100 g/ml avidin D (Vector Labs) into each well of a high-binding plate (e.g., MaxiSorp, Nunc; Immulon 4, Dynatech). Incubate overnight at ambient temperature.
  - b. Remove the solution, and wash 4 times with Wash Buffer (1% Tween 20 in PBS).
  - c. Pipet 200  $\mu$ l of 1% casein (Hammarstein Grade, BDH) in PBS into each well. Incubate for 1 h to overnight at ambient temperature.
  - d. Remove the solution. Store the plate frozen, under which conditions it remains stable for at least several weeks.
2. Heat denature PCR products by incubation at 95° C for 5 min., followed by quick-cooling to approximately 4° C.
3. Pipet 90  $\mu$ l of Hybridization Buffer (1% casein in PBS) containing 1 pmol of AP- or HRP- conjugated probe JW-14 (Synthetic Genetics) into each well.
4. Pipet 10  $\mu$ l of PCR product into the appropriate well.
5. Incubate at 42° C for 20 min. to allow both hybridization and capture.
6. Remove the hybridization solution and discard. Wash the plate 4 times with Wash Buffer.
7. Pipet 100  $\mu$ l LumiPhos into each well. For HRP-labeled probes, 100  $\mu$ l of OPD is used as the substrate.
- 8
  - a. AP-labeled Probes: Incubate at 37° C for 30 min. Read immediately in the ML1000 microplate luminometer (Dynatech).
  - b. HRP-labeled Probes: Incubate at room temperature for 15 min and OD<sub>490</sub> is read in a Molecular Devices ELISA plate reader.

### 3.2.2 PCR Product Detection by HPLC

1. Inject 30  $\mu$ l of each PCR product onto a TSK-DEAE NPR column. For greater precision, an automatic sample injector should be used, such as the ISS-200 (Perkin Elmer).

2. The following gradient (requiring approximately 9 minutes per run) is used to separate specific and non-specific PCR products:
  - a. Equilibrate column 5 minutes at 46% A.
  - b. Ramp linearly to 54% A over 0.1 minute following injection.
  - c. Ramp linearly to 60% A over 3.9 minutes.
  - d. Ramp linearly to 75% A over 1 minute.
  - e. Return to 46% A over 0.1 minute.

Buffer A: 25 mM Tris-Cl, pH 9.0, 1.0 M NaCl, 1% acetonitrile  
Buffer B: 25 mM Tris-Cl, pH 9.0, 1% acetonitrile
3. Products are detected by UV absorbance monitoring at 260 nm.
4. Integration of chromatographic peaks is by an automatic integrator (Perkin Elmer Nelson Model 1020). Specific peaks are identified by characteristic retention times as compared with strong positives and molecular weight standards (2.5  $\mu$ g of 250  $\mu$ g/ml HaeIII digest of pBR322).
5. If quantitation is desired, data should be plotted as "peak area vs. log initial copy number." A linear plot should be obtained over the range of 30 to 30,000 initial DNA template copies. Linear regression permits the estimation of copy number in unknown samples.

### 3.3 PCR Primer Selection and Development

The principle area of development for this contract has been in the design and testing of various *Leishmania*-specific PCR primer and probe combinations. For all designs, the following rationale was used. Since the *Leishmania* parasites are suspected to be present in very low numbers in peripheral blood of infected individuals, it was deemed that maximal sensitivity was the key requirement for the assay.

Toward this end, it was reasoned that directing the PCR primers against a "pre-amplified" target was, if possible, the best way to increase signal strength, and hence assay sensitivity, going into

the PCR reactions themselves. Then the reaction conditions would be optimized as described previously in the Introduction to produce the maximum specific yield from each primer set. For these reasons, several PCR primer sets directed against *Leishmania* target sequences that exist in more than one copy per parasite were designed. These included sequences in the ribosomal RNA (rRNA) genes, present in 5-20 copies per organism, nuclear Dihydrofolate Reductase (DHFR) genes, that exist in 2-10 copies per parasite, certain maxicircle sequences, present in 10-100 copies per organism, and several different minicircle sequences.

The minicircle sequences offer the highest possible target number as they are present in 100-10000 copies per organism. One significant problem targeting minicircle sequences, however, is the extreme sequence heterogeneity and the observation that multiple distinct "families" of minicircles exist not only within a given parasite, but also between differing species of *Leishmania*, with the largest differences found between the New World and Old World strains. A diagram showing a comparison of the "conserved" sequence regions of a number of *Leishmania* strains is presented in Figure 3. A summary of the sequences tested is given in Table 1.

The sequences of the individual primers are given in the first section of the table, along with the target of amplification. In the JW11/12 primer set, the sequences indicated in parenthesis are mixed base positions in the synthetic oligonucleotides. The I in several other sequences indicate an inosine (I) base substitution at that position, that allows hybridization with any other base. An NW in the amplification target site indicates those sequences are specific for New World *Leishmania* strains. The JW and TW series of primers were designed in-house at SRA Technologies utilizing computer software for PCR primer design (Primer Detective, Oligo) and evaluated against potential cross-reactive sequences in Genbank using the Lasergene DNASTAR molecular biology software package running on a Mac IIci. Primer set DC 11/12, also

Alignment Workspace of Leish consensus II  
Tuesday, March 30, 1993 5:46 PM

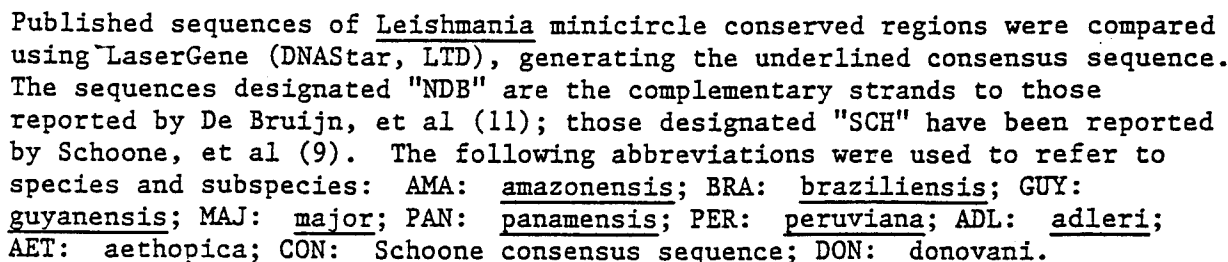


Table 1. Sequences of *Leishmania* Specific PCR Primers.

| Primer Name | Primer Sequences                    | Primer Location |
|-------------|-------------------------------------|-----------------|
| JW11        | CCTATTTTACACCAACCCC (C/T) AGTTT     | minicircle      |
| JW12        | CGGGTAGGGGCGTTCTGCGAAA (A/T) T      | minicircle      |
| TW01        | GCGTCTCCGACCCTCATCTTCAAGG           | DHFR (nuclear)  |
| TW02        | GACACCCTCCTCTCTATAACGGC             | DHFR (nuclear)  |
| TW03        | ATTGAAATAATAAAAGGTTGAGC             | maxicircle      |
| TW04        | AATTACAAATAATAGATCCTTGCG            | maxicircle      |
| JW16        | GAATTCGATTTTCGCAGAACGCCCT           | minicircle      |
| JW17        | GAATTCAAACTGGGGGTGGTGTGTAAAAT       | minicircle      |
| R222        | TATTGGAGATTATGGAGCTG                | rRNA gene       |
| R332        | GGCCGGTAAAGGCCGAATAG                | rRNA gene       |
| LK1S        | CCTATTTTACACCAACCCC                 | minicircle      |
| LK2R        | GGGTAGGGGCGTTCTGCGA                 | minicircle      |
| LS1         | GGGGTTGGTGTAAAATAG                  | minicircle      |
| LS2         | CCAGTTTCCCGCCCCG                    | minicircle      |
| B1          | GGGGTTGGTGTAAATATAGTGG              | minicircle NW   |
| B2          | CTAATTGTGCACGGGGAGG                 | minicircle NW   |
| B3          | CCCGACATGCCTCTGGGTAG                | minicircle NW   |
| PROBE P1    | CAGAAACCCCGTTCAAAAAT                | minicircle NW   |
| JW-11-i     | CCTATTTTACACCAACCCCIAGTTT           | minicircle      |
| JW-12-i     | CGGGTAGGGGCGTTCTGCGAAAIT            | minicircle      |
| C-JW11-1    | CCTATTTTACACCAACCCCIATTTI           | minicircle      |
| C-JW11-2    | CCTATTTTACACCAACCCCIATTT            | minicircle      |
| C-JW11-3    | CCTATTTTACACCAACCCCIAT              | minicircle      |
| C-JW12-1    | CGGGIAGGGGCGTTCTGCGAAAI             | minicircle      |
| C-JW12-2    | CGGGTAGGGGCGTTCTGCGAAAA             | minicircle      |
| C-JW14-1    | ATTGAACGGGTTTTCTGTATICTTTTTTCGAA    | minicircle      |
| C-JW14-2    | ATTIGAACGGGTTTTCTGIAIICIAATTTTTIGAA | minicircle      |
| C-JW14-3    | GAACGGGTTTTCTGIAIICIAATTTTCGTTTT    | minicircle      |
| JW-21       | TGAACGGGTTTTCTGIAIICATTT            | minicircle      |
| JW-22       | GGGGTTGGTGTAAAATAGGICIG             | minicircle      |
| JW-24       | CATTTTTTCIIITTTTCGCAGAACGCCCTACC    | minicircle      |
| DC-11       | CCCTATTTTACACCAACCCCCAGTTT          | minicircle      |
| DC-12       | CGGGTAGGGGCGTTCTGCGAAATT            | minicircle      |
| DC-LT-1     | ACCACCCGGCCCTATTTTA                 | minicircle      |
| DC-LT-2     | ATGTAGTAGCCCTCCGGGT                 | minicircle      |

designed in-house at SRA, is similar to JW 11/12 primer set except that the mixed base positions (bold face) were a perfect match for *L. tropica* kDNA sequence. The sources of the other primer sequences are as follows: R222/332 (ref. 9); LK1S/2R (Personal communication from G. van Eys to Maj. E. Nuzum) and LS1/LS2 (ref. 10).

### 3.3.1 PCR primer sets against Old World species of *Leishmania*

During Phase I of this project, we tested the sensitivities of JW 11/12 primer set against the strains of Old World *Leishmania*. Table 2 indicates the detection limits of primer set JW 11/12 when compared against control strains of *Leishmania* parasites.

**Table 2: Detection of various *Leishmania* species using the minicircle-specific PCR primers JW 11/12.**

| Species<br>of <i>Leishmania</i>  | # <i>Leishmania</i> Detected<br>with (JW 11/12) |     |    |   |
|----------------------------------|-------------------------------------------------|-----|----|---|
|                                  | 1000                                            | 100 | 10 | 1 |
| 746 ( <i>L. panamensis</i> )     | +                                               | +   |    |   |
| 842 ( <i>L. mexicana</i> )       | +                                               |     |    |   |
| 1031 ( <i>L. guyanensis</i> )    | +                                               | +   | +  | + |
| 1041 ( <i>L. major</i> )         | +                                               |     |    |   |
| 1063 ( <i>L. tropica</i> )       | +                                               | +   | +  | + |
| 1077 ( <i>L. major</i> )         | +                                               | +   | +  |   |
| 2053 ( <i>L. donovani</i> )      | +                                               | +   |    |   |
| 669 ( <i>L. amazonensis</i> )    | +                                               |     |    |   |
| 746 ( <i>L. panamensis</i> )     | +                                               | +   | +  |   |
| 1003 ( <i>L. braziliensis</i> )  | +                                               | +   |    |   |
| 2086a ( <i>L. braziliensis</i> ) | +                                               | +   | +  |   |
| 2086b ( <i>L. braziliensis</i> ) | +                                               | +   | +  |   |

Note: A plus (+) indicates that signal at least 5-fold above assay background was consistently obtained against that species, with lower numbers indicating greater sensitivity)

The sensitivity of various combinations of inosine-substituted primer sets against *L. tropica* and *L. donovani* is presented in Charts 1 and 2, respectively. The sensitivity of JW 11/12 and JW 11i/12i primer sets was retested during the Phase II of the project. Two separate sets of *L. tropica* cultures (stock 1063/5-24-94 and a stock of three cultures (6-3-94) grown at WRAIR were used for this testing. Chart 3 summarizes the results of this testing. The results indicate a sensitivity limit of 10 parasites for both primer sets against *L. tropica*. Table 3 summarizes the results of testing various combinations of primer sets against *L. tropica*.

Table 3: Sensitivity of various primer sets against *L. tropica*

| Primer Combinations | Detection Limits on <i>L. tropica</i> |
|---------------------|---------------------------------------|
| JW11/12             | Approximately 1-100 parasites         |
| TW03/04             | Approximately 100 parasites           |
| TW01/02             | Greater than 1000 parasites           |
| R222/332            | Greater than 1000 parasites           |
| JW16/17             | Greater than 1000 parasites           |
| LK1S/2R             | Greater than 1000 parasites           |
| LS1/2               | Greater than 1000 parasites           |
| B1/B2               | Greater than 1000 parasites           |
| JW11i/JW12i         | Approximately 1-100 parasites         |
| JW21/JW22           | Approximately 100 parasites           |
| DC11/12             | Approximately 100 parasites           |
| DC-LT-1/DC-LT-2     | Approximately 1 parasite              |

Similar testing was done with other Old World species of *Leishmania* during the course of this contract. The results are presented in Charts 4 and 5 for *L. donovani* and in Charts 6 and 7, for *L. chagasi*. These combinations were tested using our optimized PCR protocols and the HRP-coupled capture plate assay.

Chart 1. PCR Primers Tested Against *L. tropica*

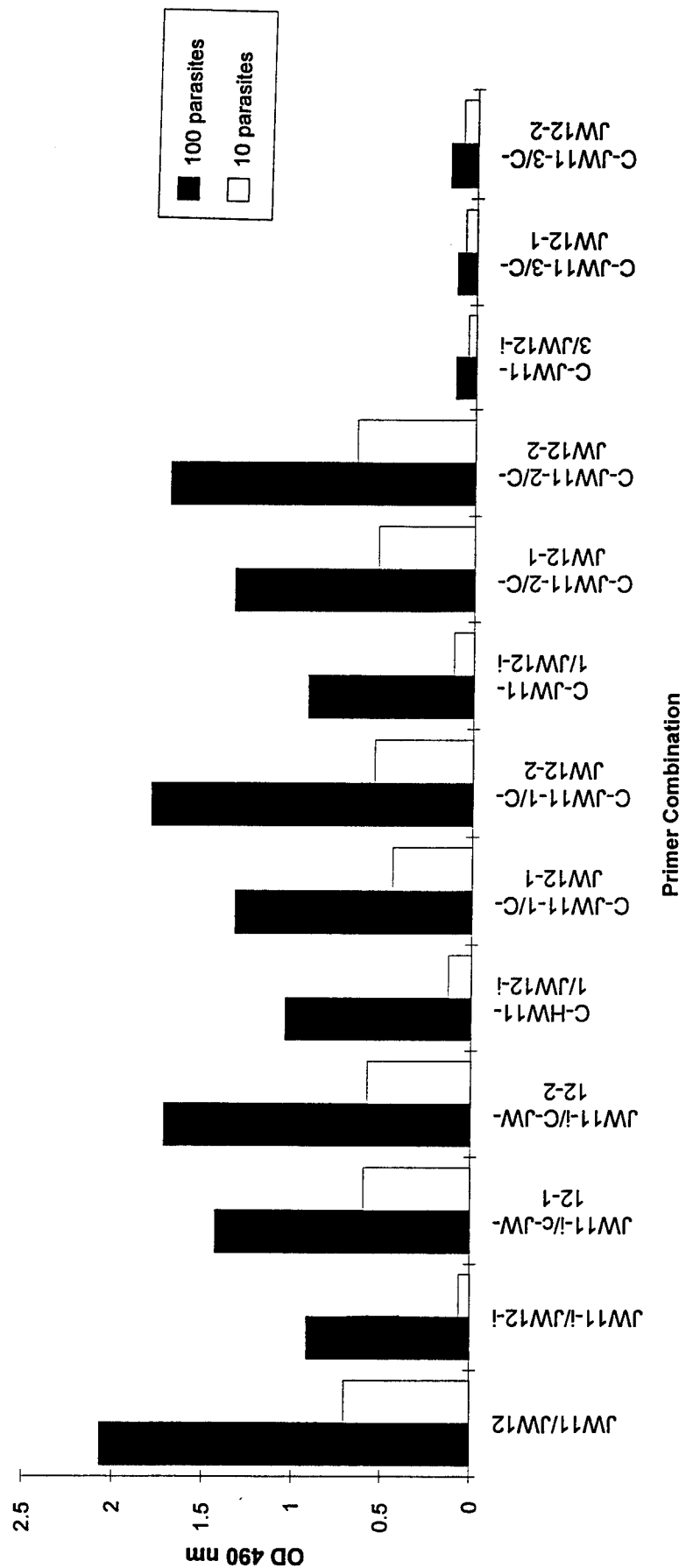


Chart 2. PCR Primers Against *L. donovani*

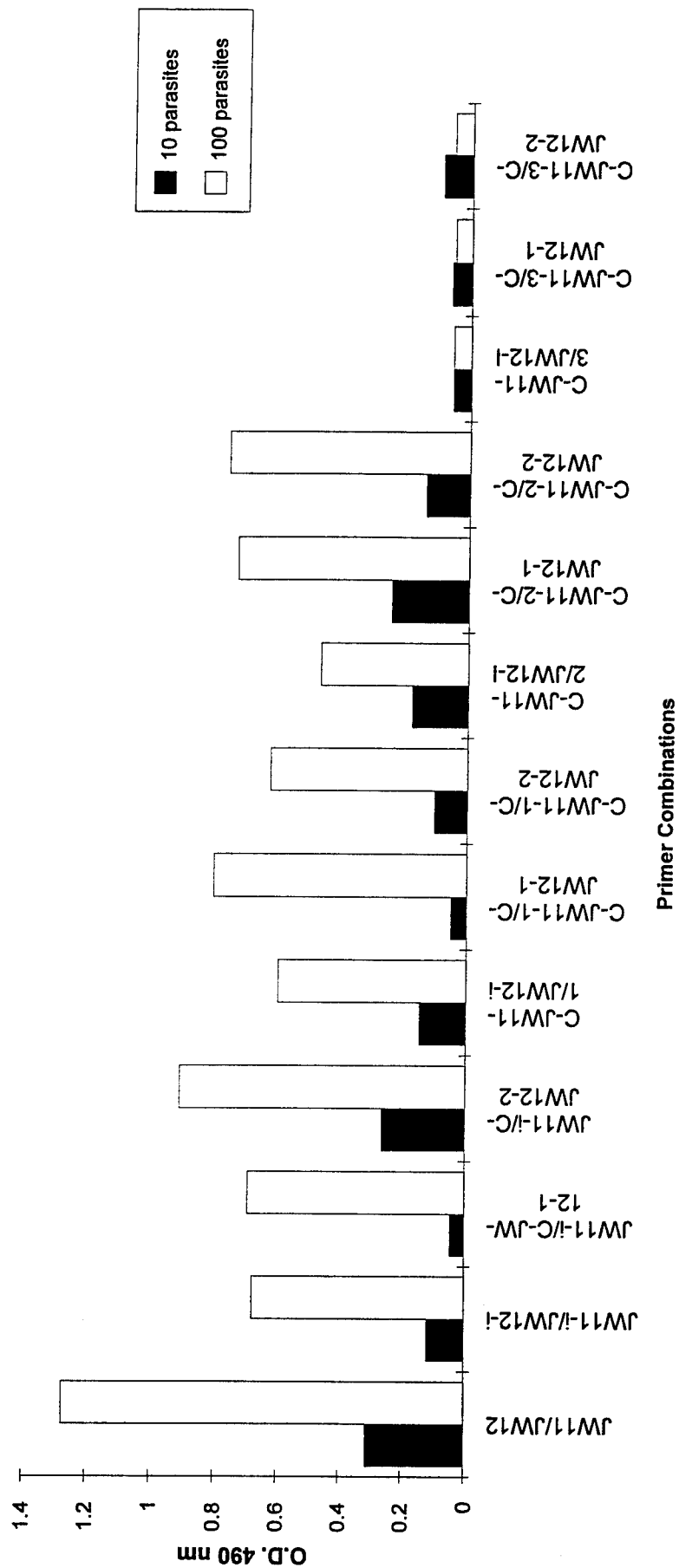


Chart 3. L. tropica (6/3/94) Testing of cultures with JW 11i/12i primer set

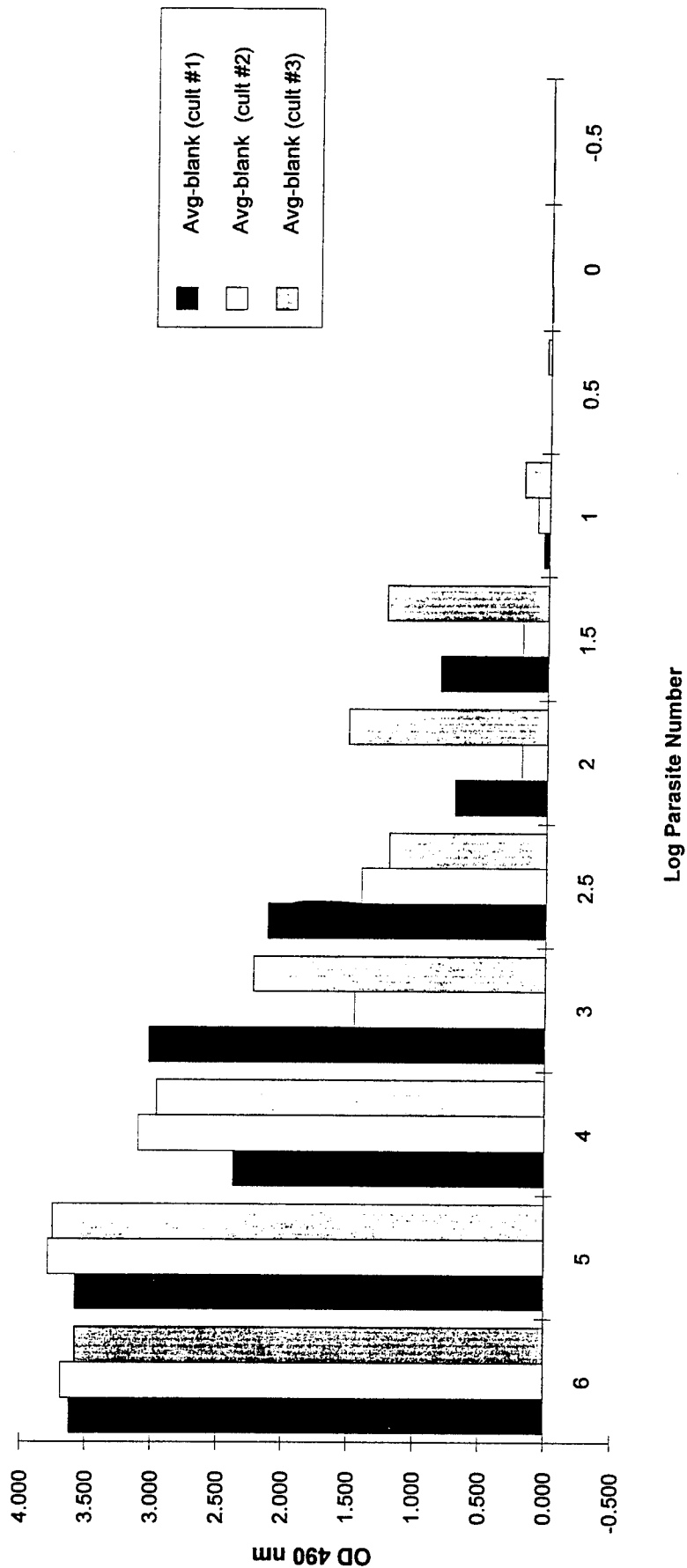


Chart 4. L. donovani (Stock 1073) Testing of JW 11/12 and JW 11i/12i primer sets

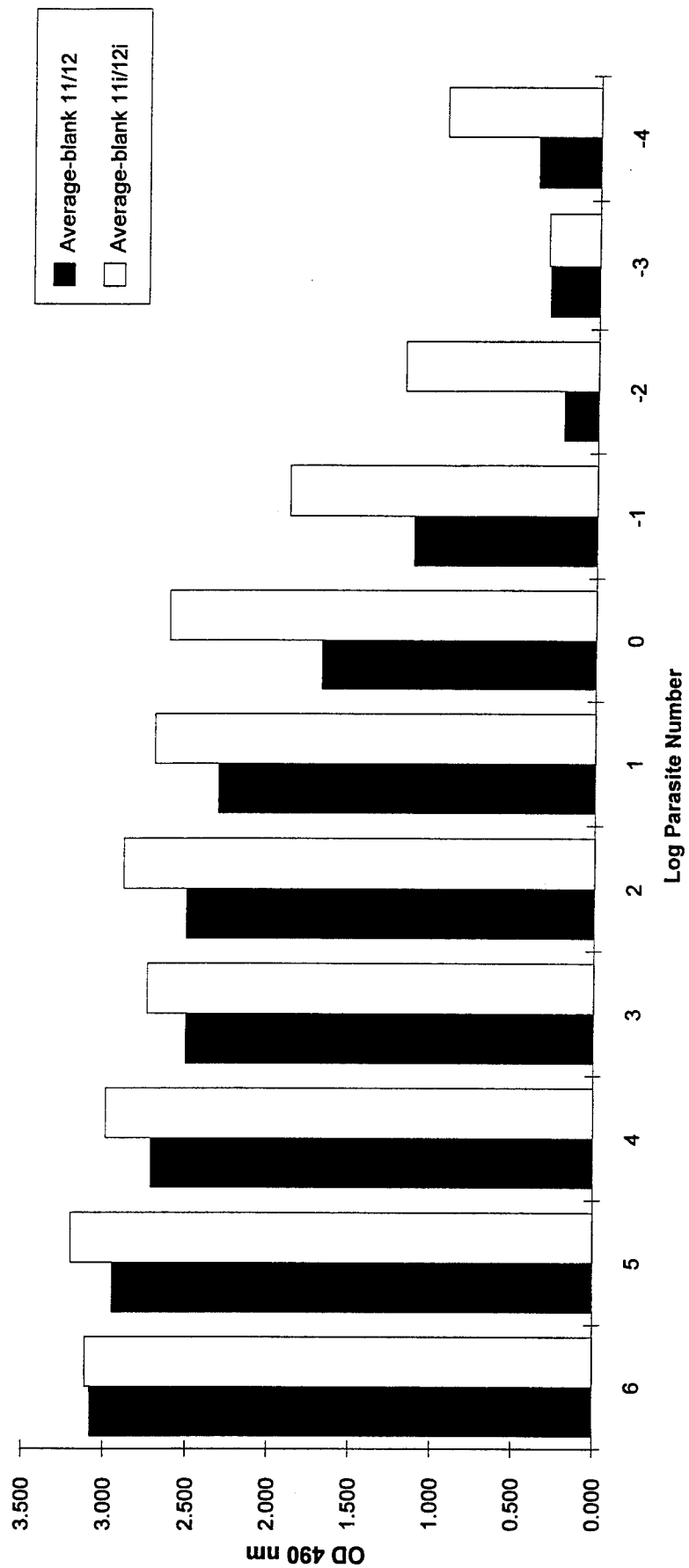


Chart 5. L. donovani (stock 7-13-94) Testing with primer sets JW 11/12 and JW 11i/12i

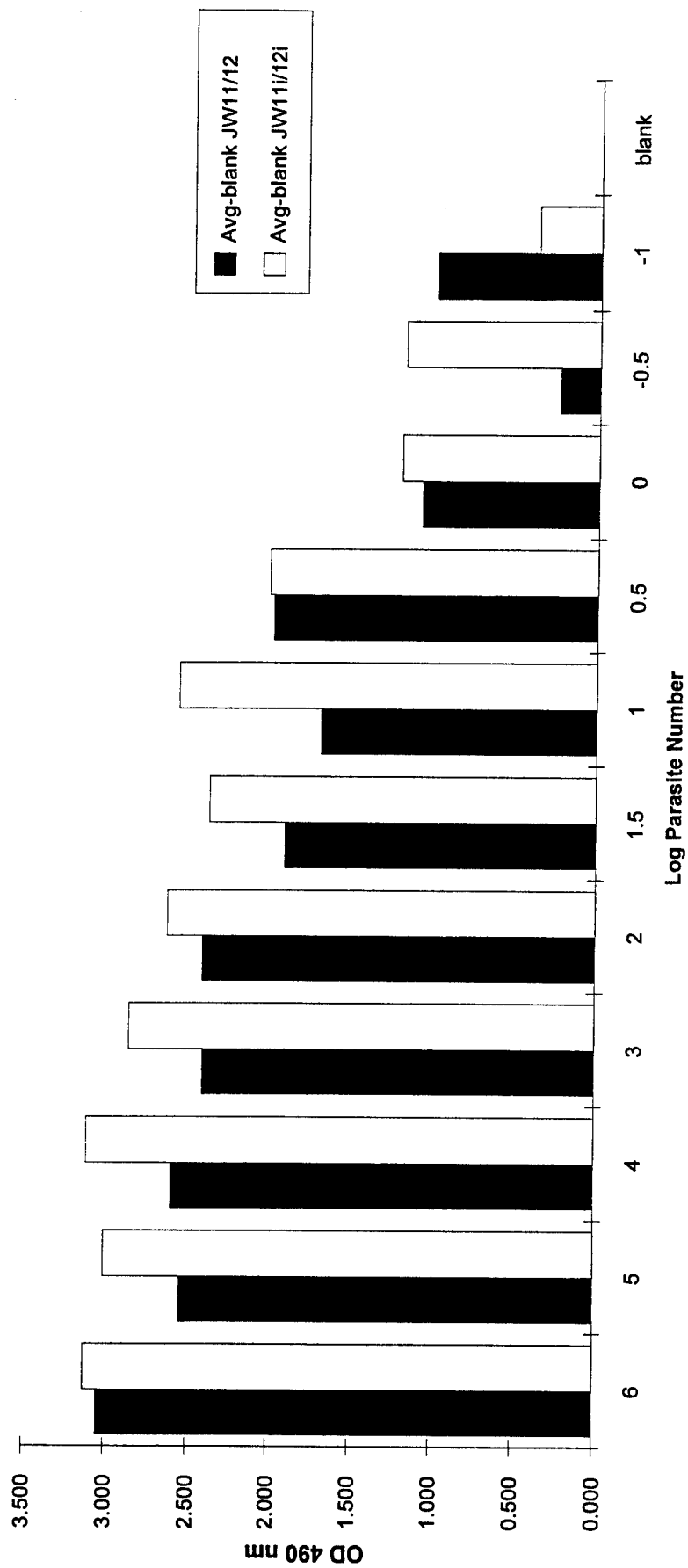


Chart 6. L. chagasi (testing with primer sets JW 11/12 and JW 11i/12i)

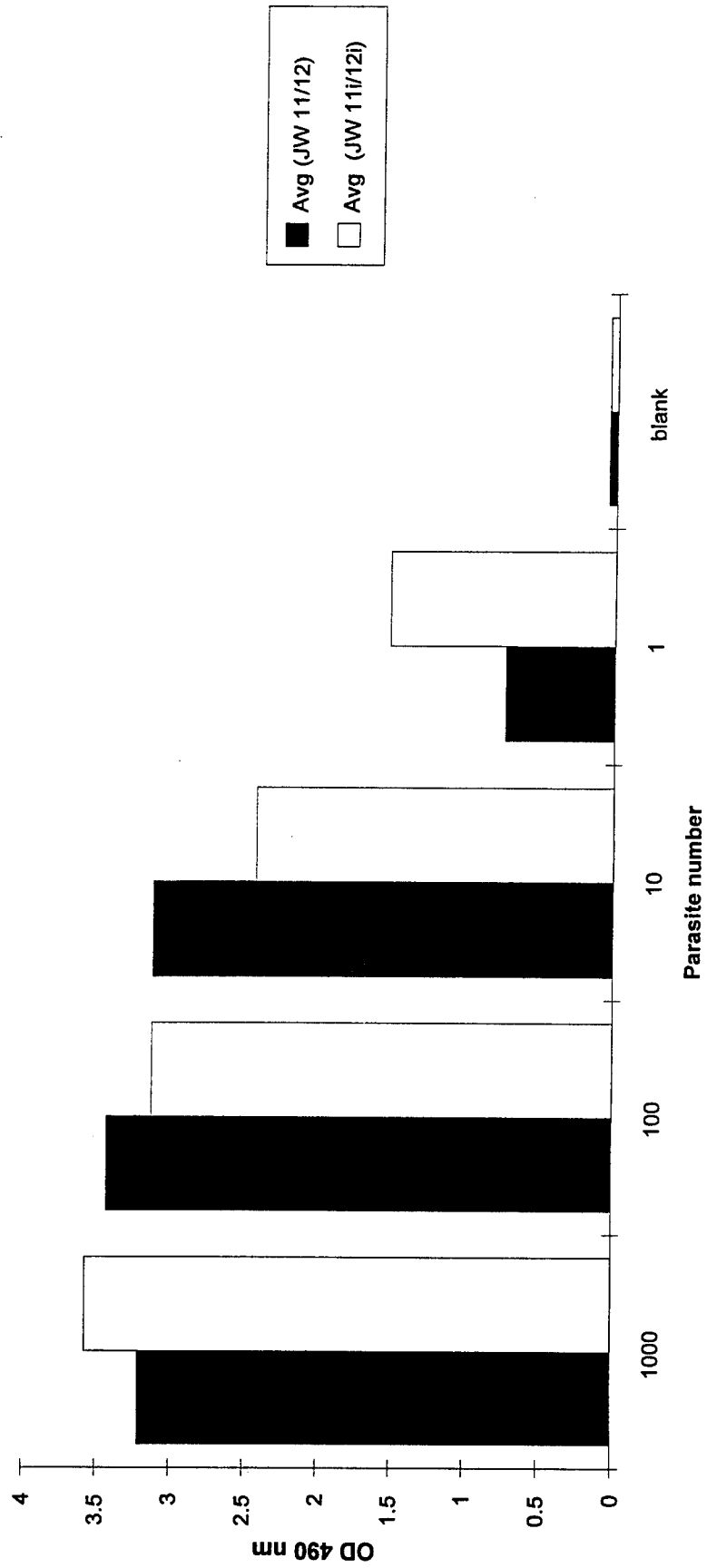
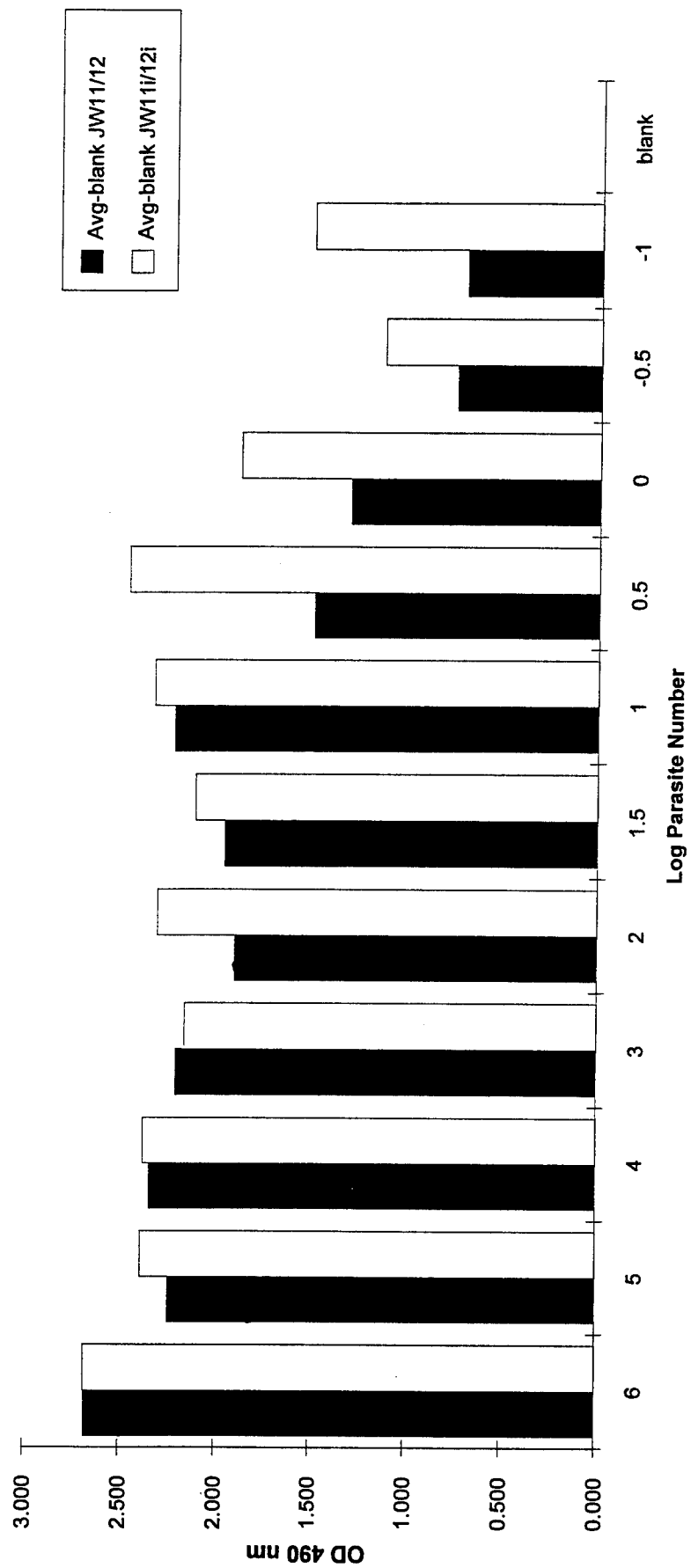


Chart 7. L. chagasi (stock 7-13-94) Testing with primer sets JW 11/12 and JW 11i/12i



To date, the most consistent and sensitive primer sets against the strains of old world *Leishmanias* examined are JW 11/12 and JW 11i/12i. The sensitivity limit is less than one parasite, for *L. donovani* and *L. chagasi*. However for *L. tropica*, the detection limit is one parasite with the newly tested primer set, DC-LT-1 and DC-LT-2; and it varies from 1-100 parasites with JW 11/12 and JW 11i/12i primer sets. As can be readily seen from the previous tables and charts, the inosine substituted primer set JW 11i/12i, seem to provide slightly higher sensitivity against control samples from *L. donovani* (presumably the causative agent of Kala-Azar) and *L. chagasi*. Their sensitivity, however remains the same, particularly against *L. tropica*. Other primer sets, except DC-LT-1/DC-LT-2 (a primer set designed in-house with sequences optimized to *L. tropica*), that have been tested to date are significantly less sensitive when tested against *L. tropica* parasites (Table 3).

### **3.3.2 PCR Primers with Multiple Inosine Substitutions for New World species of *Leishmania***

During the course of this study, a number of samples from South and Central America, both as controls and as patients with suspicious disease, were provided for testing. It became readily obvious when testing against control strains provided by MAJ M. Grogl that the sensitivity of the basic JW11/12 PCR primer set was much better against Old World than the New World strains. It was the opinion of the *Leishmania* Working Group that in addition to the original requirement for a PCR based detection test that was sensitive against the Old World strains prevalent in South West Asia, it would be useful to have a test usable against at least some of the New World strains of *Leishmania* for use with patient samples obtained from service personnel stationed in South and Central America. Toward these ends, it was decided to try designing PCR primers with multiple inosine substitutions at those DNA base positions that differ between characterized New and Old World *Leishmania* strains (see Figure 3).

A collection of inosine-substituted primers based on the most sensitive PCR primer set tested to date (JW11/12) was developed and tested in various pairwise combinations. The results of these tests for *L. braziliensis* comparing the pairwise combinations of these primers are given in Chart 8. These combinations were tested using our optimized PCR protocol and the HRP-coupled capture plate assay against 10 and 100 parasite equivalents of parasite DNA for *L. braziliensis*. Higher signal indicates greater PCR yield, and therefore, greater sensitivity of that particular PCR primer combination against the species of *Leishmania* in question. Based on the results obtained from this experiment, primer pair combinations were retested during Phase II of this contract, against two New World species of *Leishmania*, namely *L. braziliensis* and *L. panamensis*. All primer combinations efficiently detected one parasite under the conditions used for testing. Two primer sets, c-JW11-3/c-JW12-1 and c-JW11-3/c-JW12-2, showed a sensitivity, 2- to 5-fold higher than others (Charts 9 and 10). The sequences of the second primer set is compared below with the original JW 11/12 primer set.

JW-11       CCTATTTTACACCAACCCC (A/T) AGTTT

JW-12       CGGGTAGGGGCGTTCTGCGAAA (A/T) T

C-JW-11-3 CCTAT**TTT**ACACCAACCCC**IAI**

JW-12-2    CGGGTAGGGGCGTTCTGCGAAAA

The sequence differences are indicated in *bold italics*. The A/T indicates a mixed base composition at this position. Using this primer set, while not sufficiently different to be a truly independent second set, PCR conditions were reoptimized for *L. braziliensis*, as a representative of New World species of *Leishmania*. The reoptimized parameters included the testing of annealing temperatures of the primers and the concentrations of magnesium ions in the PCR mix. The results of three typical experiments are presented in Table 4. Additionally, a comparison of the standard PCR conditions and conditions optimized for *L. braziliensis* is presented

Chart 8. PCR primers against *L. braziliensis*

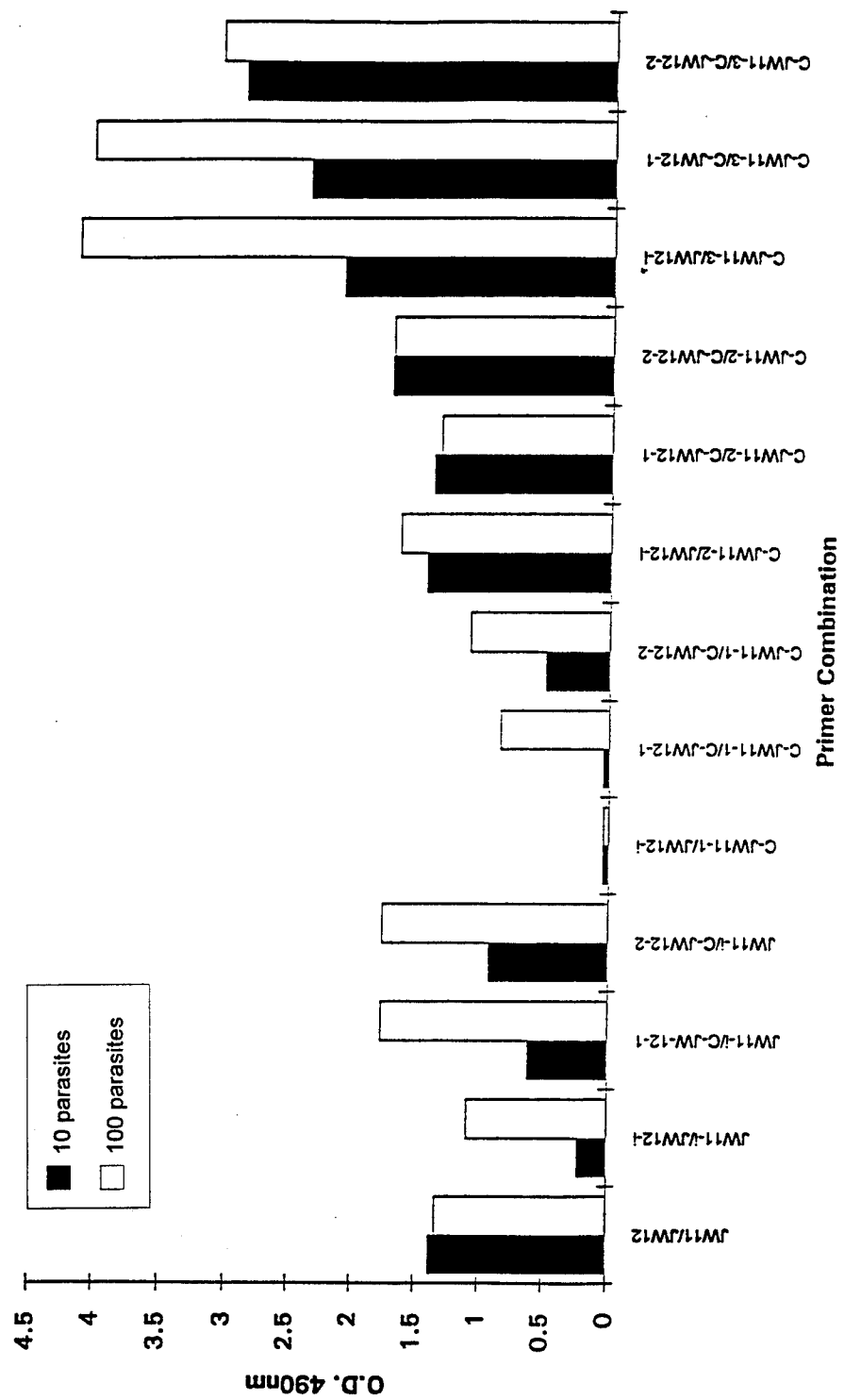


Chart 9. *L. braziliensis* (Testing of primer combinations)

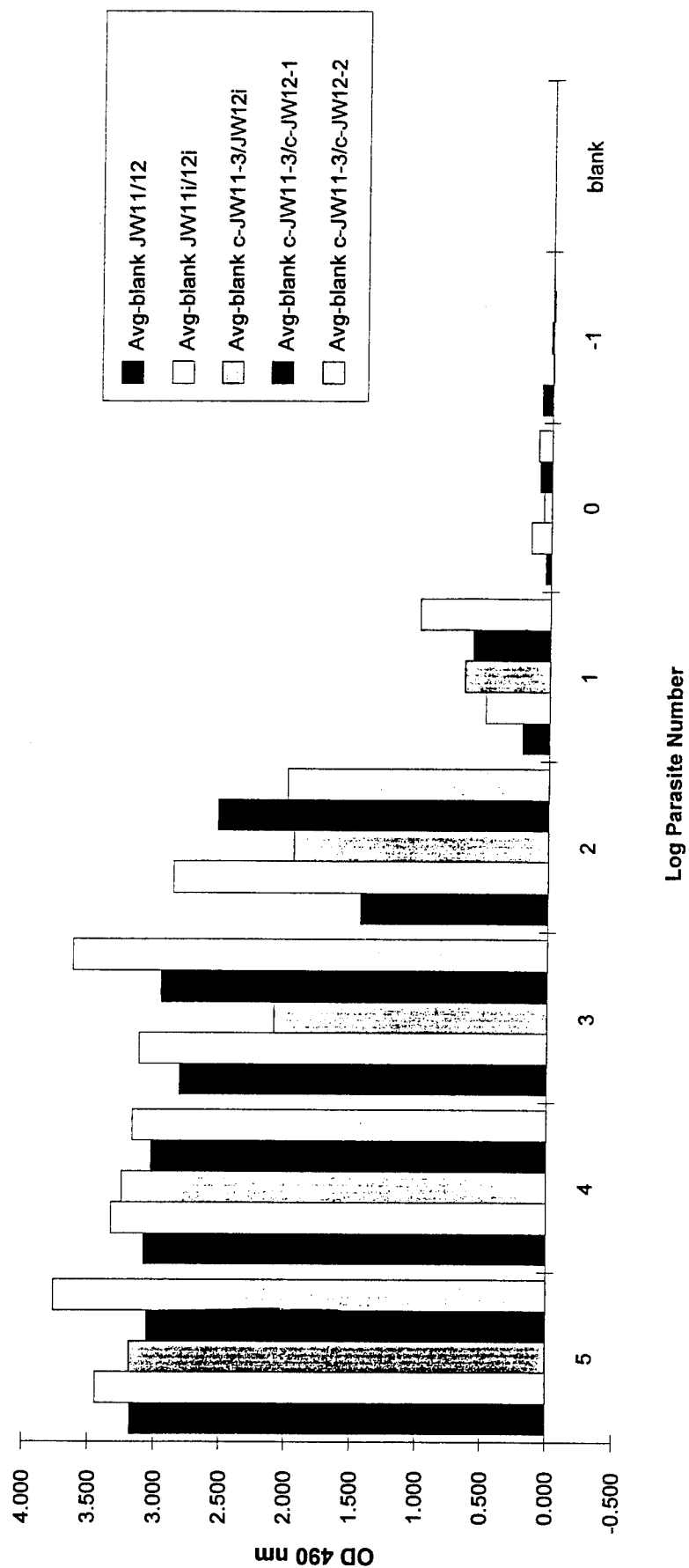
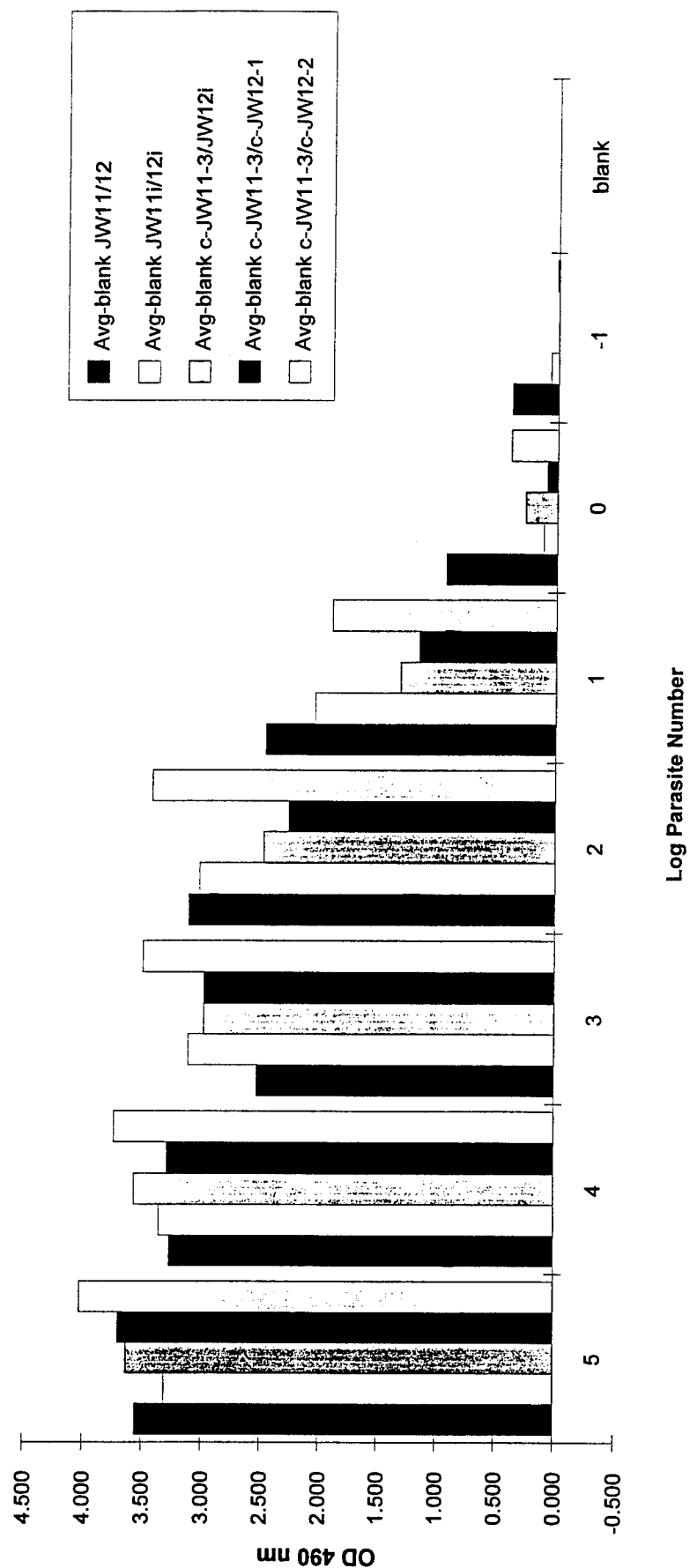


Chart 10. *L. panamensis* (Testing primer combinations)



in Chart 11. Further HPLC analysis of the PCR products of this optimization is needed to confirm the absence of any non-specific amplification products.

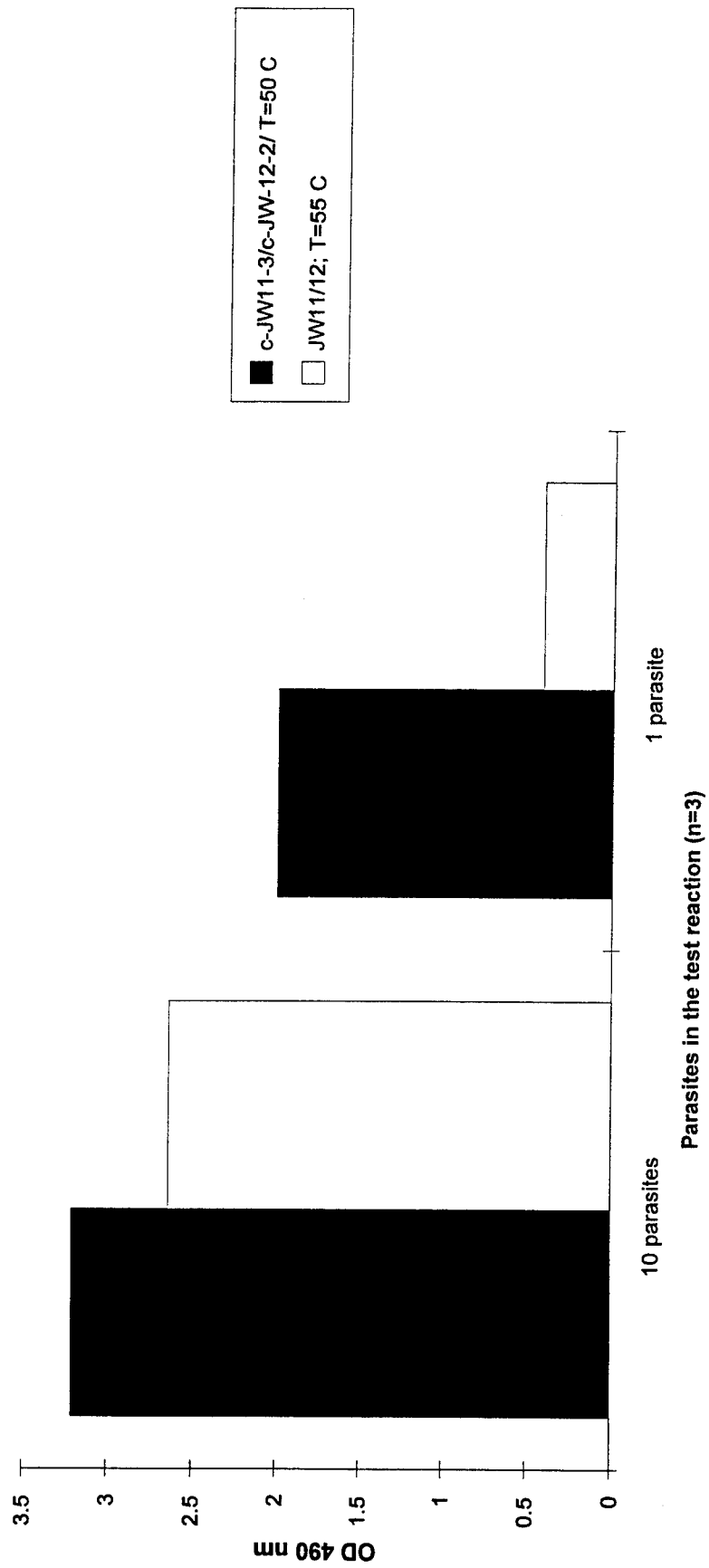
**Table 4. Results of PCR optimization for *L. braziliensis***  
(the values represent colorimetric readout ( $A_{490}$ )  
from HRP capture plate assay of PCR products)

| Annealing temperature           | 45° C                 | 50° C  | 55° C                                                 | 60° C  |
|---------------------------------|-----------------------|--------|-------------------------------------------------------|--------|
| 10 parasites (Test 1)           | 2.726                 | 3.046  | 2.679                                                 | 0.430  |
| Annealing temperature           | 45° C                 | 48° C  | 50° C                                                 | 53° C  |
| 10 parasites (Test 2)           | 2.423                 | 2.593  | 2.235                                                 | 1.079  |
| 1 parasite                      | 0.526                 | 0.940  | 0.999                                                 | 0.430  |
| MgCl <sub>2</sub> concentration | 1.5 mM                | 2.0 mM | 2.5 mM                                                | 3.0 mM |
| 10 parasites (Test 3)           | 2.768                 | 2.739  | 2.792                                                 | 2.698  |
| 1 parasite                      | 1.139                 | 1.143  | 0.703                                                 | 0.713  |
| New Parameters                  | Annealing Temp. 50° C |        | MgCl <sub>2</sub> conc. 2-2.25 mM<br>(same as before) |        |

Briefly, the more conservatively inosine-substituted primers give at least equivalent sensitivity to the JW 11/12 primer set for *L. tropica*, *L. chagasi* and *L. donovani*. Some of the more extensively inosine-substituted primers give increased sensitivity against the New World strain *L. braziliensis*, but show reduced signal when tested against the Old World strains of *L. tropica* and *L. donovani*. Based on these results, it was decided that since sensitivity against the Old World strains was of primary importance, the use of the more extensively inosine-substituted primers (eg. C-JW11-3/C-JW12-2) would be restricted to samples with origins in geographic regions where New World stains of *Leishmania* predominate.

Another idea for an additional primer set was explored, using JW-21/JW-22 primer set and JW-24 probe (modification of the conserved

Chart 11. L. braziliensis (comparison of standard vs optimized PCR conditions)



regions of of the original JW-11/12 primer set and JW-14 probe; Table 2). Unfortunately, the results of such testings were disappointing in terms of sensitivity when compared to the basic JW 11/12 primer set.

### **3.4 Decatenation of *Leishmania* kDNA minicircles**

Additional experiments were done to further increase the sensitivity of the PCR detection test. One set of experiments proposed in SRA's response to the contract proposal was the use of procedures to "decatenate" the *Leishmania* kinetoplast DNA, theoretically releasing most of the minicircle and maxicircle DNA fragments into solution. This could potentially increase the chance of detection of *Leishmania* parasites by making more target DNA templates accessible for PCR. After evaluating a number of potential treatments our results are reported below.

#### **3.4.1 Decatenation Protocol**

##### Protocol 1

Equal quantities of *Leishmania* parasite lysates were used for each treatment. Controls were lysates stored at -20° C. Treatments included;

1. Incubation at 37° C overnight.
2. Digestion with the restriction enzymes DraI, EcoRI, or BamHI at 37° C overnight using 20-50 units of enzyme. These enzymes have each been reported to introduce a single specific cut in some minicircles, linearizing the DNA.
3. Treatment with Topoisomerase II (20 units) overnight at 37° C. Topoisomerase II introduces double stranded transient breaks in DNA allowing decatenation of concatenated circles and reduction of supercoiling induced torsional strain in circular molecules, facilitating denaturation of the circular DNA.
4. Limited digestion with an inorganic Iron nuclease to introduce random double stranded breaks in all DNA present in the reaction.

5. Following treatments, each lysate was serially diluted 10 fold to give a range of 10 to 0.001 parasite equivalents, and subjected to PCR analysis as described.

#### Protocol 2 (tested for *L. tropica*)

Total DNA from *L. tropica* was prepared by extracting lysate of the parasites with phenol:chloroform and precipitation with ethanol.

1. Equal quantities of total DNA and cell lysates representing 1000 parasites were treated with 10 Units of Topoisomerase II (Topogen, Inc.) and 10-20 Units of restriction enzyme XhoI (this enzyme works better than other restriction enzymes and some times even better than Topoisomerase II for unrelated purified kDNA)
2. The samples treated with Topoisomerase were incubated for 60 min, 90 min and 120 minutes at 37° C and those treated with XhoI were incubated for 1-2 h at 37° C.
3. The reaction was stopped by heat treatment at 95° C for 5 minutes. Serial 10-fold dilutions (1000 - 0.1 parasites) were prepared and subjected for routine PCR amplification using JW 11i/12i primer set. Following amplification, the samples were detected using JW14-HRP probe as described.
4. Undigested DNA and lysate controls were also included with the assay.

#### **3.4.2 Results of the Decatenation Experiments**

Unfortunately, multiple experiments failed to show any significant difference in PCR product yield, and hence sensitivity of the reaction, when compared to untreated controls with the equivalent amounts of DNA or lysate in the reaction mix. The results of all tests were within +/- 20% of the signal produced by the untreated controls at each dilution point. A reproducible dose-response curve was not always obtained upon repeated testing to confirm the effect of decatenation (data not shown). Based on these results, we believe further experimentation along these lines is not currently warranted.

In view of the problems encountered in decatenation of kDNA, either from the total genomic DNA or from the specimen lysates, it was considered important to construct a copy control plasmid to test the sensitivity of the PCR assay developed for *Leishmania*. Such a copy number control not only would determine the analytical sensitivity of the assay, as copy number per reaction, rather than parasite number per reaction, but also would elucidate the meaning of weakly positive reactions. The best control would be derived from the strain(s) of interest, especially, *L. tropica*, where the sensitivity of the assay varies from 1 to 100 parasites. The primers bordering the constant regions, outside the JW 11/12 primer binding regions, were chosen for this purpose. The sequence of two such primers and their location in relation to JW 11/12 is presented below:

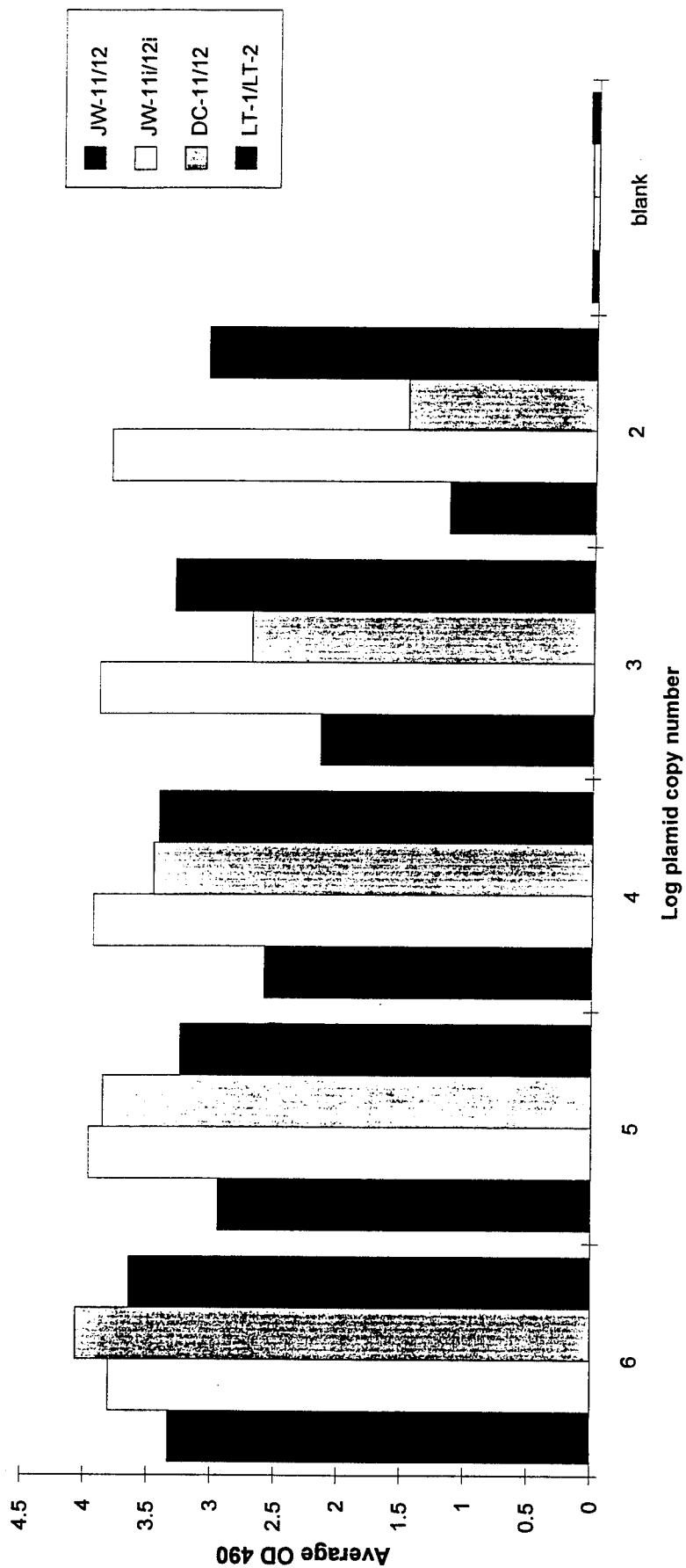
5' - CCTATTTTACACCAACCCC (C/T) AGTTT - 3' (JW 11)

5' -CGGGTAGGGGCGTTCTGCGAAA (A/T) T-3' (JW 12)

Primers DC-LT-1 and DC-LT-2 are up stream of JW 11 and JW 12 primers, respectively, but overlap 5-10 nucleotides towards their 3' end with the 5' ends of JW 11/12. Therefore, the amplicons generated with these primers are slightly larger than JW 11/12 amplicons and would carry the original JW 11/12 sequences for further amplification.

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Chart 12. Testing of L. tropica copy control plasmid with different primers



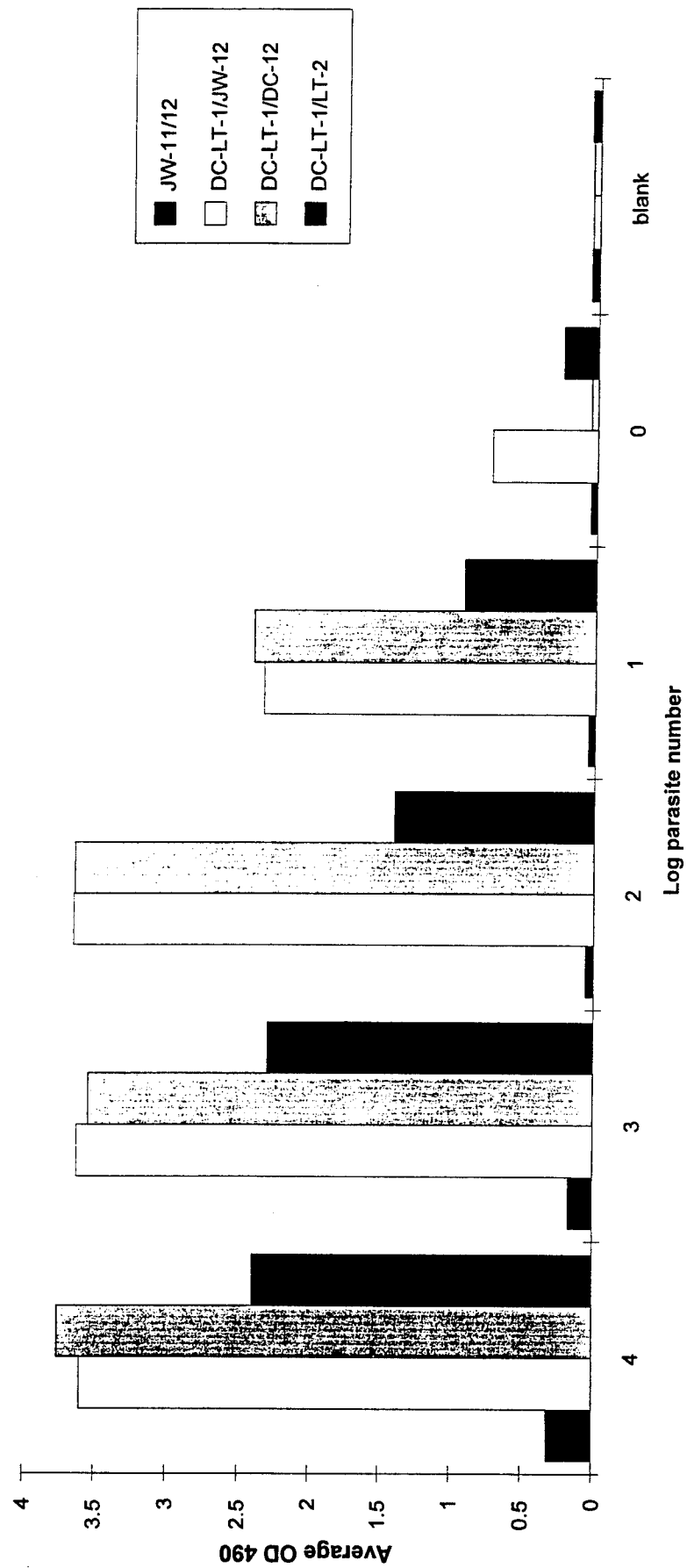
analytical sensitivity of PCR reactions for *L. tropica*. The result of one such test is presented in Chart 12. All primer sets tested (JW 11/12 and JW 11i/12i (the original primer sets); DC 11/12 (in-house primer set, similar to JW 11/12, with mixed base position matching to *L. tropica* sequence and DC-LT-1 and DC-LT-2 (the primer set used for cloning the PCR products), amplify the *L. tropica* copy control plasmid pDCL-3 very efficiently and the analytical sensitivity of detection is about 100 copies (lowest copy number used in the assay). The absolute signal strength with primer sets JW 11i/12i and DC-LT-1/LT-2 is about 2-fold higher compared with JW 11/12 and DC 11/12 indicating the sensitivities of assay may possibly be less than 100 copies (not tested).

The new primer set DC-LT-1/LT-2, although contains about 5-10 nucleotides from the 5' terminal regions of the original JW 11 and 12 primers, is unique from all other primer sets tested so far, in that, it completely lacks the mixed base sequences. The preliminary tests indicate that the sensitivity of this primer set and the combination of DC-LT-1 (sense primer) with JW 12 (antisense) provides a better sensitivity (detection of one parasite), compared to other primer sets used in the assay, in amplifying *L. tropica* sequences (Chart 13). It appears that the extreme degree of sequence variability present in *L. tropica*, prevents consistent amplification with JW 11/12 and other primers, which always include the mixed-base region; and primers outside this mixed base position seem to favour a reasonably good efficiency of amplification. Further tests are needed to confirm the superior performance of the new primer set, DC-LT-1/LT-2, before adopting it, over JW 11/12 or JW 11i/12i primer sets, for evaluation of clinical samples.

### 3.6 Heterologous DNA Testing

It was suggested in the Statement of Work and by the Leishmania Working Group that we test a variety of heterologous DNAs using our PCR detection technique to ensure specificity of the primers and

Chat 13. Testing of DC-LT-1 as the sense primer for L. tropica



probes being used. A list of possible organisms was provided by the Leishmania Working Group and included the following;

*Trypanosoma cruzi*, *T. gambiense*, *T. rhodesiense*, *T rangeli*.  
*Leptomone*, *Crithidia*, *Herpetomonas*, *Toxoplasma*, *Plasmodium falciparum*, *Babesia*, *Pneumocystis carinii*, *Herpes*, *Salmonella*, *Histoplasma capsulatum*, *Mycobacterium tuberculosis*, HIV, and Hepatitis B and C.

After receiving information from MAJ Grogl regarding the availability of certain heterologous DNAs from the ATCC, we obtained the necessary permit applications to purchase samples of these organisms as many of them are classified as Class II, III, and IV pathogens. Testing the DNA obtained by protocols appropriate for DNA isolation from each type of organism at 1  $\mu$ g concentrations in our standard PCR reactions did not produce any detectable signal from any of the organisms listed. Additional tests against blind negative control patients with Malaria and several other tropical diseases also produced no false positive reactions, indicating that the current PCR primers and conditions are specific for *Leishmania* kDNA and do not cross-react to any significant degree with the heterologous DNAs tested.

### 3.7 PCR Results on Patient Samples

During the course of the contract period, a number of patient samples have been received and tested using the PCR protocols described in this report. A complete listing of all samples tested to date is included in Appendix I of this report.

These samples partitioned into several categories including: (1) positive and negative control samples obtained from patients with known diseases including Kala-Azar, Malaria, HIV infection, etc; (2) canine samples used for potential animal model work; (3) spiked controls; and (4) a large number of "rule out" patient samples. The

latter category was comprised of samples (blood, bone marrow, and on occasion, spleen, liver, skin and other tissue specimens) from patients who present with clinical symptoms that may indicate *Leishmania* infection.

While the unknown patient samples represented the most important aspect of the assay development, they are also the most difficult to evaluate in terms of test accuracy. A large number of the unknown patient samples (1068) have tested negative while in comparison only a few (272) have tested positive. In most cases, positive results were confirmed by clinical data, such as, IFA, culture, etc (LTC E. Nuzum, personal communication). In contrast, some of the negative PCR results can be considered "biological false negatives". In some cases, very few or no circulating parasite-infected cells are present in the patient samples. This is particularly true with patients undergoing successful treatment or those exhibiting cutaneous lesions without visceralization of the disease. From the latter patients, aspirates of the lesion itself may test positive by PCR whereas peripheral blood samples may test negative.

This result is to be distinguished from a "technical false negative" which is defined as a sample that contains *Leishmania* DNA, but presents a false negative result when tested by PCR. These results are extremely rare and may be explained in several ways. As indicated previously (Charts 1, 2, 8, 9 and 10) different species of *Leishmania* exhibit differing limits of detection using our current PCR protocol. It is possible that in some cases, the particular species of *Leishmania* present in a given sample is at a level undetectable using our current primer sets. Another possible explanation is the presence of inhibitory material in the patient sample that prevents PCR amplification of the *Leishmania* DNA. This possibility was evaluated in all suspected "biological false negative" results seen to date by re-assaying the sample with a fixed level of *Leishmania* control DNA spiked into the sample. In all cases, the spiked DNA was detected with approximately the same signal

strength as seen from a parallel reaction containing the same level of *Leishmania* control DNA in reaction buffer, effectively ruling out the presence of significant inhibition in those samples.

During the course of Phase I of this contract, a number of blind negative samples have been assayed and results were clean (0/15 samples tested) with no false positives. Additionally, samples from patients with Malaria or other tropical diseases, prevalent in the region, where the *Leishmania* parasites are found, were also tested. In all cases, no positive reactions were seen. These results reflect the degree of care taken and the effectiveness of the containment procedures followed at our laboratory in preventing cross contamination.

In comparison, results obtained from a panel of 64 blinded control samples taken from patients with Kala-Azar disease at various stages, representing several geographical locations (Kenya, India, Brazil), the following statistics were obtained (provided by COL J. Berman); When samples come from samples prior to patient therapy,  $\geq 92\%$  are positive by PCR assay, therefore yielding a false negative rate of  $\leq 8\%$ , depending upon the source of the patient sample. If the patient has completed therapy by 2-6 months, 0-4 patients (0%) are positive, indicating the effectiveness of the therapy at removing detectable levels of *Leishmania* DNA from peripheral blood. Interestingly, the detection rate for purely cutaneous disease was approximately 11% (1 out of 9), indicating the absence of detectable *Leishmania* DNA in peripheral blood if the disease is confined to skin lesions. However, aspirates taken from cutaneous lesions of these patients do test positive by PCR, confirming the presence of parasitic DNA in an actively infected lesion.

#### 4. Conclusions

The need for sensitivity in detecting the very low levels of *Leishmania* present in peripheral blood suggests that the optimal

target sequence would be "pre-amplified", such as DNA present in multiple copies in each parasite. The kinetoplast DNA (kDNA) found in *Leishmania* and related organisms presents a good example of this kind of target. One inherent limitation this target sequence presents, however, is the genetic diversity of kDNA. Differences exist throughout this sequence, not only between different species of *Leishmania*, but also potentially within a given organism. The concatenated nature of the target, where some minicircles may not be available to the PCR primers and therefore amplification, presents yet another potential problem reducing sensitivity for the PCR reaction. Within these limitations, some regions of conserved DNA sequence have been observed across multiple *Leishmania* species, although there is more similarity within the New and Old World species than there is between these divisions.

Utilizing DNA sequence information and computer homology searches, multiple PCR primers have been designed within these kDNA sequences, and tested to produce specific PCR products with no significant cross-reaction with non-*Leishmania* DNA. The reaction conditions have been optimized to detect one parasite equivalents in peripheral blood and evaluated with blinded negative and positive control patient samples from various geographical locations. The testing procedures described reliably detect *Leishmania* in samples from most of the infected patients (> 90%) tested and resulted in no false positive diagnosis.

Based on the results obtained during this contract period, SRA Technologies has developed and validated a sensitive and specific PCR based diagnostic test for Leishmaniasis. While we received only 1420 patient samples (Appendix I) to screen for *Leishmania* infection, and there are certain biological factors regarding the low level of Leishmanial DNA present in peripheral blood in some cases, we are confident that the test can be applied successfully on a large scale basis.

Among the information gained as a result of our development and validation of the assay, are novel observations regarding the level of *Leishmania* parasites (as indicated by the presence of *Leishmania* DNA detectable by the PCR assay). It is evident even from our limited number of samples, that *Leishmania* are either absent, or present in extremely low levels (less than 1 parasite or infected cell in  $1.5 \times 10^6$  PBMCs or 1.5-2 mls of blood) in cases of cutaneous disease, or in patients during and after suitable anti-Leishmanial therapies. These biological effects may limit the use of any test that detects *Leishmania* in peripheral blood to cases of visceral disease prior to treatment. The test could be used, however, to monitor the success of therapy due to the observation that the parasites are largely cleared from circulation following successful therapy. Despite these limitations, the PCR based test described in this report exhibits sensitivity of one parasite equivalents or a single infected cell in 8 mls of blood, as demonstrated by spiking control experiments. Our attempts to develop a true second diagnostic primer set for *Leishmania* species was generally unsuccessful.

The progress of this work has been reported to the *Leishmania* working group and the COR in 15 reports and various memoranda (reporting results of sample analyses). One manuscript detailing the application of this technology for the detection and diagnosis of Leishmanial infections has been submitted for publication (Appendix II).

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## 6. Appendix I: List of *Leishmania* specimens tested

SRA Technologies has developed an in-house Relational Laboratory Information Manage (RLIMS), running in Oracle under UNIX, that is used to index and archive all patient information. This information includes patient ID numbers, sample type, date of r tested done, primers/probes used, date of analysis, interpretation of the test resu diagnostic report. As per the request of the *Leishmania* working group, all *Leishmani* data (both clinical as well as research) has been entered into our RLIMS system. He printout of all the *Leishmania* specimens analyzed by PCR during the course of this

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name               | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer              | Tube # 1 | Tube # 2 | Int | Comments | Final Result |
|---------|----------------------------|----------------|----------------------------|-------|------------------|-----------|---------------------|----------|----------|-----|----------|--------------|
| 11221   | GATES, JOHN                | -267-64-7560   | BL 04-FEB-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11346   | COLWELL, ROBERT            | 20-333-60-5644 | BM 11-FEB-92               | LM    | 90006            | 25-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11347   | COLWELL, ROBERT            | 20-333-60-5644 | BL 11-FEB-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11480   | OGDEN, RUSSEL W            | 20-467-45-9394 | BL 20-FEB-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11480.A | OGDEN, RUSSEL W            | 20-467-45-9394 | BM 20-FEB-92               | LM    | 90006            | 26-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11481   | MORRIS, TABITHA            | 20-491-78-6750 | BL 20-FEB-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11481.A | MORRIS, TABITHA            | 20-491-78-6750 | BM 20-FEB-92               | LM    | 90006            | 25-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11618   | PATIENT #1 03-MAR-92, LE - | -              | BL 03-MAR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11619   | PATIENT #2 03-MAR-92, LE - | -              | BL 03-MAR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11620   | PATIENT #3 03-MAR-92, LE - | -              | BL 03-MAR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11621   | PATIENT #4 03-MAR-92, LE - | -              | BL 03-MAR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11683   | SMITH, HERBERT J           | 20-264-56-5136 | BL 05-MAR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11684   | SERGOTT, PATRICK J         | 20-355-60-6724 | BL 05-MAR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11685   | SMITH, HERBERT J           | 20-264-56-5136 | BM 05-MAR-92               | LM    | 90006            | 25-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11686   | SERGOTT, PATRICK J         | 20-355-60-6724 | BM 05-MAR-92               | LM    | 90006            | 25-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11829   | PATIENT #1 12-MAR-92, LE - | -              | BM 12-MAR-92               | LM    | 90006            | 25-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11830   | PATIENT #2 12-MAR-92, LE - | -              | BL 12-MAR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11831   | PATIENT #3 12-MAR-92, LE - | -              | BL 12-MAR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11970   | KAPPLAN, LEISHMANIA        | -              | BL 19-MAR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11971   | KAPPLAN, LEISHMANIA        | -              | BM 19-MAR-92               | LM    | 90006            | 25-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11972   | PATIENT #2 19-MAR-92, LE - | -              | BL 19-MAR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11973   | SANTATERRARA, LEISHMANIA   | -              | BM 19-MAR-92               | LM    | 90006            | 25-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 11974   | PATIENT #3 19-MAR-92, LE - | -              | BL 19-MAR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12083   | ROBERTS, MICHAEL J         | -009-50-3733   | BL 30-MAR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name               | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer              | Tube # 1 | Tube # 2 | Int | Comments | Final Result |
|---------|----------------------------|----------------|----------------------------|-------|------------------|-----------|---------------------|----------|----------|-----|----------|--------------|
| 12129   | AMBORGI,                   | -              | BM 01-APR-92               | LM    | 90006            | 25-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12130   | ROBERTS, MICHAEL J         | -009-50-3733   | BM 01-APR-92               | LM    | 90006            | 25-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12131   | PATIENT #1 01-APR-92, LE - |                | BL 01-APR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12153   | PATIENT #2 03-APR-92, LE - |                | BL 03-APR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12154   | RILEY, SYLVIA              | 20-220-74-4985 | BM 03-APR-92               | LM    | 90006            | 25-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12155   | BATCHELDER, SHAWN          | 20-595-62-7906 | BM 03-APR-92               | LM    | 90006            | 25-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12156   | PATIENT #1 03-APR-92, LE - |                | BL 03-APR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12272   | PATIENT #1 10-APR-92, LI - |                | BL 10-APR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12273   | PATIENT #2 10-APR-92, LE - |                | BL 10-APR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12343   | PATIENT # 14-APR-92, LE -  |                | BL 14-APR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12379   | PATIENT #1 15-APR-92, LE - |                | BL 15-APR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12380   | PIRKEY, JASON              | 20-461-77-7490 | BL 15-APR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12381   | PIRKEY, JASON              | 20-461-77-7490 | BM 15-APR-92               | LM    | 90006            | 25-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12403   | PATIENT 17-APR-92, LEISH - |                | BL 17-APR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12404   | PATIENT 17-APR-92, LEISH - |                | BM 17-APR-92               | LM    | 90006            | 25-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12481   | PATIENT #B1 20-APR-92, L - |                | BL 20-APR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12482   | PATIENT 20-APR-92, LEISH - |                | BM 20-APR-92               | LM    | 90006            | 25-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12522   | DARLING, RALPH             | 20-324-40-5525 | BL 21-APR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12565   | PATIENT #1 23-APR-92, LE - |                | BL 23-APR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12566   | PATIENT #1 23-APR-92, LE - |                | BM 23-APR-92               | LM    | 90006            | 25-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12607   | PATIENT #2 27-APR-92, LE - |                | BL 27-APR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12608   | PATIENT #1 27-APR-92, LE - |                | BM 27-APR-92               | LM    | 90006            | 25-MAY-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 12668   | PATIENT 30-APR-92, LEISH - |                | BL 30-APR-92               | LM    | 90006            | 15-MAY-92 | LEISHMANIA JW 11/12 | +        | +        | RE  |          | POSITIVE     |
| 12669   | PATIENT 30-APR-92, LEISH - |                | BM 30-APR-92               | LM    | 90006            | 25-MAY-92 | LEISHMANIA JW 11/12 | +        | +        | RE  |          | POSITIVE     |

Report Date:

PCR Assay Results: LM  
Probe JW 14

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| Spec ID | Patient Name               | FPC + SSN | Spec Received<br>Type Date | Study | Panel Assay Date | Virus               | Primer | Tube Tube<br># 1 # 2 | Int | Comments | Final Result |
|---------|----------------------------|-----------|----------------------------|-------|------------------|---------------------|--------|----------------------|-----|----------|--------------|
| 12723   | PATIENT #1 04-MAY-92, LE - | -         | BM 04-MAY-92 LM            | LM    | 90006 25-MAY-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |
| 12724   | PATIENT #2 04-MAY-92, LE - | -         | BL 04-MAY-92 LM            | LM    | 90006 22-MAY-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |
| 12725   | PATIENT #3 04-MAY-92, LE - | -         | BM 04-MAY-92 LM            | LM    | 90006 25-MAY-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |
| 12726   | PATIENT #4 04-MAY-92, LE - | -         | BL 04-MAY-92 LM            | LM    | 90006 22-MAY-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |
| 12780   | PATIENT 05-MAY-92, LEISH - | -         | BM 05-MAY-92 LM            | LM    | 90006 22-MAY-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |
| 12781   | PATIENT 05-MAY-92, LEISH - | -         | BL 05-MAY-92 LM            | LM    | 90006 22-MAY-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |
| 12928   | STONE, LEISHMANIA          | -         | BL 12-MAY-92 LM            | LM    | 90006 22-MAY-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |
| 13403   | 701, LEISHMANIA            | -         | BL 08-JUN-92 LM            | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | + -                  | -   | IND      | NEGATIVE     |
| 13404   | 702, LEISHMANIA            | -         | BL 08-JUN-92 LM            | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |
| 13405   | 711, LEISHMANIA            | -         | BL 08-JUN-92 LM            | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | + -                  | +   | RE       | POSITIVE     |
| 13406   | 712, LEISHMANIA            | -         | BL 08-JUN-92 LM            | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |
| 13407   | 713, LEISHMANIA            | -         | BL 08-JUN-92 LM            | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |
| 13408   | 718, LEISHMANIA            | -         | BL 08-JUN-92 LM            | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |
| 13409   | 725, LEISHMANIA            | -         | BL 08-JUN-92 LM            | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |
| 13410   | 729, LEISHMANIA            | -         | BL 08-JUN-92 LM            | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |
| 13411   | 738, LEISHMANIA            | -         | BL 08-JUN-92 LM            | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | + -                  | -   | IND      | NEGATIVE     |
| 13412   | 740, LEISHMANIA            | -         | BL 08-JUN-92 LM            | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |
| 13413   | 761, LEISHMANIA            | -         | BL 08-JUN-92 LM            | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |
| 13414   | 767, LEISHMANIA            | -         | BL 08-JUN-92 LM            | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |
| 13415   | 768, LEISHMANIA            | -         | BL 08-JUN-92 LM            | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |
| 13416   | 778, LEISHMANIA            | -         | BL 08-JUN-92 LM            | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |
| 13417   | 779, LEISHMANIA            | -         | BL 08-JUN-92 LM            | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | - -                  | -   | NR       | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name     | FPC + SSN | Spec Received<br>Type Date | Study | Panel Assay Date | Virus               | Primer | Tube # 1 | Tube # 2 | Int | Comments | Final Result |
|---------|------------------|-----------|----------------------------|-------|------------------|---------------------|--------|----------|----------|-----|----------|--------------|
| 13418   | 780, LEISHMANIA  | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13419   | 789, LEISHMANIA  | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13420   | 790, LEISHMANIA  | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13421   | 795, LEISHMANIA  | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13422   | 796, LEISHMANIA  | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13423   | 803, LEISHMANIA  | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13424   | 806, LEISHMANIA  | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13425   | 807, LEISHMANIA  | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13426   | 815, LEISHMANIA  | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13427   | 816, LEISHMANIA  | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13428   | 817, LEISHMANIA  | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13429   | 955, LEISHMANIA  | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13430   | 960, LEISHMANIA  | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13431   | 962, LEISHMANIA  | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13432   | 963, LEISHMANIA  | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13433   | 967, LEISHMANIA  | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13434   | 1004, LEISHMANIA | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13435   | 1008, LEISHMANIA | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13436   | 1012, LEISHMANIA | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13437   | 1013, LEISHMANIA | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13438   | 1020, LEISHMANIA | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13439   | 1025, LEISHMANIA | -         | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name          | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus               | Primer | Tube # 1 | Tube # 2 | Int | Comments | Final Result |
|---------|-----------------------|----------------|----------------------------|-------|------------------|---------------------|--------|----------|----------|-----|----------|--------------|
| 13440   | 1026, LEISHMANIA      | -              | BL 08-JUN-92               | LM    | 90007 24-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
|         |                       |                |                            |       | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | +        | -        | IND |          | NEGATIVE     |
|         |                       |                |                            |       | 90007 09-JUL-92  | LEISHMANIA JW 11/12 |        | +        | -        | IND |          | NEGATIVE     |
| 13441   | 1033, LEISHMANIA      | -              | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13442   | 1037, LEISHMANIA      | -              | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13443   | 1038, LEISHMANIA      | -              | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13444   | 1045, LEISHMANIA      | -              | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13445   | 1051, LEISHMANIA      | -              | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13446   | 1055, LEISHMANIA      | -              | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13447   | 1058, LEISHMANIA      | -              | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13448   | 1064, LEISHMANIA      | -              | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13449   | 1073, LEISHMANIA      | -              | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13450   | 1076, LEISHMANIA      | -              | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13451   | 1089, LEISHMANIA      | -              | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13452   | 1092, LEISHMANIA      | -              | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13453   | 1095, LEISHMANIA      | -              | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 13454   | 1098, LEISHMANIA      | -              | BL 08-JUN-92               | LM    | 90007 10-JUN-92  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 23573   | LAROCHE,              | -              | BL 07-JUN-93               | LM    | 90008 08-JUN-93  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 23574   | HENDRICK, PETER       | -              | BL 07-JUN-93               | LM    | 90008 08-JUN-93  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 23575   | PATIENT #1 07-JUN-93, | -              | BL 07-JUN-93               | LM    | 90008 08-JUN-93  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 23576   | PATIENT #2 07-JUN-93, | -              | BM 07-JUN-93               | LM    | 90008 08-JUN-93  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 23677   | CHIPLEY, JOSHUA W     | 20-564-33-6887 | BL 11-JUN-93               | LM    | 90008 15-JUN-93  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| 23678   | CHIPLEY, JOSHUA W     | 20-564-33-6887 | BL 11-JUN-93               | LM    | 90008 15-JUN-93  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| Z000501 | 31601,                | -              | BL 09-JUN-93               | LM    | 90008 15-JUN-93  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |
| Z000502 | 31601,                | -              | BM 09-JUN-93               | LM    | 90008 15-JUN-93  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |          | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name      | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer              | Tube # 1 | Tube # 2 | Int | Comments | Final Result |
|---------|-------------------|----------------|----------------------------|-------|------------------|-----------|---------------------|----------|----------|-----|----------|--------------|
| Z000503 | 31621,            | -              | BL 11-JUN-93               | LM    | 90008            | 15-JUN-93 | LEISHMANIA JW 11/12 | +        | +        | RE  |          | POSITIVE     |
| Z000504 | 31622,            | -              | BL 14-JUN-93               | LM    | 90008            | 15-JUN-93 | LEISHMANIA JW 11/12 | +        | +        | RE  |          | POSITIVE     |
| Z000505 | 31623,            | -              | BL 11-JUN-93               | LM    | 90008            | 15-JUN-93 | LEISHMANIA JW 11/12 | +        | +        | RE  |          | POSITIVE     |
| Z000506 | 31624,            | -              | BL 11-JUN-93               | LM    | 90008            | 15-JUN-93 | LEISHMANIA JW 11/12 | +        | +        | RE  |          | POSITIVE     |
| Z000507 | CHIPLEY, JOSHUA W | 20-564-33-6887 | TE 11-JUN-93               | LM    | 90008            | 15-JUN-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000508 | CHIPLEY, JOSHUA W | 20-564-33-6887 | TE 11-JUN-93               | LM    | 90008            | 15-JUN-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 26642   | 54F,              | -              | BL 25-OCT-93               | LM    | 90010            | 29-OCT-93 | LEISHMANIA JW 11/12 | +        | +        | RE  |          | POSITIVE     |
| 26643   | 65F,              | -              | BL 25-OCT-93               | LM    | 90010            | 29-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 26644   | 71F,              | -              | BL 25-OCT-93               | LM    | 90010            | 29-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 26645   | 72F,              | -              | BL 25-OCT-93               | LM    | 90010            | 29-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 26646   | 73/74F,           | -              | BL 25-OCT-93               | LM    | 90010            | 29-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 26647   | 77F,              | -              | BL 25-OCT-93               | LM    | 90010            | 29-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 26648   | 102F,             | -              | BL 25-OCT-93               | LM    | 90010            | 29-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 26716   | VAUGHAN, GEORGE   | 20-543-62-6961 | OT 27-OCT-93               | LM    | 90010            | 29-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000692 | 328710,           | -              | BL 14-OCT-93               | LM    | 90010            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000693 | 328711,           | -              | BL 14-OCT-93               | LM    | 90010            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000694 | 328712,           | -              | BL 14-OCT-93               | LM    | 90010            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000695 | 32871,            | -              | BL 14-OCT-93               | LM    | 90010            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000696 | 32872,            | -              | BL 14-OCT-93               | LM    | 90010            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000697 | 32873,            | -              | BL 14-OCT-93               | LM    | 90010            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000698 | 32874,            | -              | BL 14-OCT-93               | LM    | 90010            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000699 | 32875,            | -              | BL 14-OCT-93               | LM    | 90010            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000704 | 32876,            | -              | BL 14-OCT-93               | LM    | 90010            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000705 | 32877,            | -              | BL 14-OCT-93               | LM    | 90010            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name     | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date Virus | Primer              | Tube # 1 | Tube # 2 | Int | Comments | Final Result |
|--------------------------|----------------|----------------------------|-------|------------------------|---------------------|----------|----------|-----|----------|--------------|
| Z000706 32878,           | -              | BL 14-OCT-93               | LM    | 90010 22-OCT-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000707 32879,           | -              | BL 14-OCT-93               | LM    | 90010 22-OCT-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000708 BACHMAN, JOHN    | 20-205-42-4875 | TC 20-OCT-93               | LM    | 90010 29-OCT-93        | LEISHMANIA JW 11/12 | +        | +        | RE  |          | POSITIVE     |
| Z000709 VAUGHAN, GEORGE  | 20-543-62-6961 | BL 21-OCT-93               | LM    | 90010 29-OCT-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000710 VAUGHAN, GEORGE  | 20-543-62-6961 | BM 21-OCT-93               | LM    | 90010 29-OCT-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000711 VAUGHAN, GEORGE  | 20-543-62-6961 | BM 21-OCT-93               | LM    | 90010 29-OCT-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 26879 NERONE, MARCUS A   | 20-257-17-3841 | BL 03-NOV-93               | LM    | 90011 08-NOV-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 26880 NERONE, MARCUS A   | 20-257-17-3841 | BM 03-NOV-93               | LM    | 90011 08-NOV-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000712 VAUGHAN, GEORGE  | 20-543-62-6961 | LI 01-NOV-93               | LM    | 90011 08-NOV-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 27156 BRANDES, RONALD    | 20-317-48-7871 | BL 17-NOV-93               | LM    | 90012 29-NOV-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 27157 BRANDES, RONALD    | 20-317-48-7871 | BM 17-NOV-93               | LM    | 90012 29-NOV-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 27165 VAUGHAN, GEORGE    | 20-543-62-6961 | TC 18-NOV-93               | LM    | 90012 29-NOV-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 27166 NERONE, MARCUS A   | 20-257-17-3841 | BL 18-NOV-93               | LM    | 90012 29-NOV-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000713 NERONE, MARCUS A | 20-257-17-3841 | TC 09-NOV-93               | LM    | 90012 29-NOV-94        | LEISHMANIA JW 11/12 | +        | -        | IND |          | INDETERMIN   |
| Z000714 BRANDES, RONALD  | 20-317-48-7871 | TC 17-NOV-93               | LM    | 90012 29-NOV-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000715 ELLIOTT, E       | -              | TC 17-NOV-93               | LM    | 90012 29-NOV-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000716 ELLIOTT, E       | -              | TC 17-NOV-93               | LM    | 90012 29-NOV-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000766 BRANDES, RONALD  | 20-317-48-7871 | LM 23-NOV-93               | LM    | 90012 02-DEC-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000767 BRANDES, RONALD  | 20-317-48-7871 | LI 23-NOV-93               | LM    | 90012 02-DEC-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000768 BRANDES, RONALD  | 20-317-48-7871 | TC 23-NOV-93               | LM    | 90012 02-DEC-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000769 BRANDES, RONALD  | 20-317-48-7871 | TC 23-NOV-93               | LM    | 90012 02-DEC-93        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 27882 HAYES, JANES       | -380-80-7107   | BL 21-JAN-94               | LM    | 90013 01-JAN-94        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 27883 HAYES, JANES       | -380-80-7107   | BM 21-JAN-94               | LM    | 90013 13-JAN-94        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| Z000771 40211,           | -              | BL 21-JAN-94               | LM    | 90013 26-JAN-94        | LEISHMANIA JW 11/12 | -        | -        | NR  |          | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name        | PFC + SSN    | Spec Received<br>Type Date | Study | Panel Assay Date | Virus               | Primer   | Tube # 1 | Tube # 2 | Int | Comments | Final Result |
|-----------------------------|--------------|----------------------------|-------|------------------|---------------------|----------|----------|----------|-----|----------|--------------|
| 2000772 40212,              | -            | BL 21-JAN-94               | LM    | 90013 26-JAN-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 2000773 40213,              | -            | BL 21-JAN-94               | LM    | 90013 26-JAN-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 2000774 40214,              | -            | BL 21-JAN-94               | LM    | 90013 26-JAN-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 2000775 40215,              | -            | BL 21-JAN-94               | LM    | 90013 26-JAN-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 2000776 40216,              | -            | BL 21-JAN-94               | LM    | 90013 26-JAN-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 2000777 40217,              | -            | BL 21-JAN-94               | LM    | 90013 26-JAN-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 2000778 40218,              | -            | BL 21-JAN-94               | LM    | 90013 26-JAN-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 2000779 40219,              | -            | BL 21-JAN-94               | LM    | 90013 26-JAN-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 2000780 402110,             | -            | BL 21-JAN-94               | LM    | 90013 26-JAN-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 2000781 402111,             | -            | BL 21-JAN-94               | LM    | 90013 26-JAN-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 2000782 402112,             | -            | BL 21-JAN-94               | LM    | 90013 26-JAN-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 2000783 402113,             | -            | BL 21-JAN-94               | LM    | 90013 26-JAN-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 2000784 402114,             | -            | BL 21-JAN-94               | LM    | 90013 26-JAN-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 2000785 ZUPEC, JEFFREY      | -            | BL 24-JAN-94               | LM    | 90013 26-JAN-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 2000786 ZUPEC, JEFFREY      | -            | BL 24-JAN-94               | LM    | 90013 26-JAN-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 2000787 HALLMAN, JAMES      | -419-62-6827 | LI 25-JAN-94               | LM    | 90013 27-JAN-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 28479 RAYNER, GREGORY       | -377-62-7801 | BL 28-JAN-94               | LM    | 90014 24-FEB-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 28480 RAYNER, GREGORY       | -377-62-7801 | BM 28-JAN-94               | LM    | 90014 24-FEB-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 28976 SCHUENEMAN, MATTHEW D | -            | BL 14-FEB-94               | LM    | 90014 01-MAR-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 28977 SCHUENEMAN, MATTHEW D | -            | BL 14-FEB-94               | LM    | 90014 01-MAR-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 29034 HAYNESWORTH, WILLIE   | -340-72-3179 | BM 22-FEB-94               | LM    | 90014 01-MAR-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 29141 LYNN, KENNY           | -465-35-8434 | OT 17-FEB-94               | LM    | 90014 01-MAR-94  | LEISHMANIA JW 11/12 | JW 11/12 | +        | +        | NR  |          | POSITIVE     |
| 29142 CRIBBS, JAMES         | -262-29-5975 | BM 17-FEB-94               | LM    | 90014 01-MAR-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |
| 2000788 40261,              | -            | BL 27-JAN-94               | LM    | 90014 24-FEB-94  | LEISHMANIA JW 11/12 | JW 11/12 | -        | -        | NR  |          | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name     | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date Virus | Primer              | Tube Tube<br># 1 # 2 | Int | Comments | Final Result |
|--------------------------|----------------|----------------------------|-------|------------------------|---------------------|----------------------|-----|----------|--------------|
| Z000789 40262,           | -              | BM 27-JAN-94               | LM    | 90014 24-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| Z000790 40271,           | -              | BM 28-JAN-94               | LM    | 90014 24-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| Z000791 40321,           | -              | TC 01-FEB-94               | LM    | 90014 24-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| Z000792 40331,           | -              | BL 02-FEB-94               | LM    | 90014 24-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| Z000793 40332,           | -              | BL 02-FEB-94               | LM    | 90014 24-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| Z000795 40341,           | -              | BL 04-FEB-94               | LM    | 90014 24-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| Z000796 40342,           | -              | BM 04-FEB-94               | LM    | 90014 24-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| Z000797 40343,           | -              | BM 04-FEB-94               | LM    | 90014 24-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| Z000798 BV4 DO,          | -              | SM 04-FEB-94               | LM    | 90014 24-FEB-94        | LEISHMANIA JW 11/12 | +                    | +   | RE       | POSITIVE     |
| Z000799 BP1 DO,          | -              | SM 04-FEB-94               | LM    | 90014 24-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| Z000800 JH,              | -              | SM 04-FEB-94               | LM    | 90014 24-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| Z000801 B5 DO,           | -              | SM 04-FEB-94               | LM    | 90014 24-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| Z000802 A5 DO,           | -              | SM 04-FEB-94               | LM    | 90014 24-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| Z000820 WILLIAMS, THOMAS | -441-46-2127   | OT 18-FEB-94               | LM    | 90014 01-MAR-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| Z000822 LYON, KENNY      | -465-35-8434   | OT 23-FEB-94               | LM    | 90014 01-MAR-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| 28852 WADDELL, DIRK      | 20-343-56-3223 | OT 08-FEB-94               | LM    | 90015 14-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| 28876 40401,             | -              | BL 09-FEB-94               | LM    | 90015 14-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| 28877 40402,             | -              | BM 09-FEB-94               | LM    | 90015 14-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| 28910 LYON, KENNY        | -465-35-8434   | BL 10-FEB-94               | LM    | 90015 14-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| Z000803 40391,           | -              | BL 07-FEB-94               | LM    | 90015 14-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| Z000804 40392,           | -              | BL 07-FEB-94               | LM    | 90015 14-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| Z000805 40393,           | -              | BL 07-FEB-94               | LM    | 90015 14-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| Z000806 40394,           | -              | BL 07-FEB-94               | LM    | 90015 14-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |
| Z000807 40395,           | -              | BL 07-FEB-94               | LM    | 90015 14-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR       | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name | FPC + SSN | Spec Received<br>Type Date | Study | Panel Assay Date Virus | Primer              | Tube Tube<br># 1 # 2 | Int | Comments            | Final Result |
|----------------------|-----------|----------------------------|-------|------------------------|---------------------|----------------------|-----|---------------------|--------------|
| Z000808 40396,       | -         | BL 07-FEB-94               | LM    | 90015 14-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| Z000809 40397,       | -         | BL 07-FEB-94               | LM    | 90015 14-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| Z000810 40398,       | -         | BL 07-FEB-94               | LM    | 90015 14-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| Z000811 40399,       | -         | BL 07-FEB-94               | LM    | 90015 14-APR-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| Z000812 403910,      | -         | BL 07-FEB-94               | LM    | 90015 14-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| Z000813 403911,      | -         | BL 07-FEB-94               | LM    | 90015 14-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| Z000814 403912,      | -         | BL 07-FEB-94               | LM    | 90015 14-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| Z000815 403913,      | -         | BL 07-FEB-94               | LM    | 90015 14-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| Z000816 403914,      | -         | BL 07-FEB-94               | LM    | 90015 14-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| Z000817 40411,       | -         | OT 10-FEB-94               | LM    | 90015 14-FEB-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| 29255 40551,         | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| 29256 40552,         | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| 29257 40553,         | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| 29258 40554,         | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| 29259 40555,         | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| 29260 40556,         | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| 29261 40557,         | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| 29262 40558,         | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| 29264 405510,        | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| 29265 405511,        | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| 29266 405512,        | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| 29267 405513,        | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| 29268 405514,        | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
| 29269 405515,        | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94        | LEISHMANIA JW 11/12 | -                    | -   | NR                  | NEGATIVE     |
|                      |           |                            |       |                        |                     |                      |     | 75% OF TOTAL VOLUME | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name | FPC + SSN | Spec Received<br>Type Date | Study | Panel Assay Date | Virus      | Primer   | Tube # 1 | Tube # 2 | Int | Comments            | Final Result |
|---------|--------------|-----------|----------------------------|-------|------------------|------------|----------|----------|----------|-----|---------------------|--------------|
| 29270   | 405516,      | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  | 70% OF TOTAL VOLUME | NEGATIVE     |
| 29271   | 405517,      | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  | 20% OF TOTAL VOLUME | NEGATIVE     |
| 29272   | 405518,      | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  | 31% OF TOTAL VOLUME | NEGATIVE     |
| 29273   | 405519,      | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  | 55% OF TOTAL VOLUME | NEGATIVE     |
| 29274   | 405520,      | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  | 27% OF TOTAL VOLUME | NEGATIVE     |
| 29275   | 405521,      | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  | 40% OF TOTAL VOLUME | NEGATIVE     |
| 29276   | 405522,      | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  |                     | NEGATIVE     |
| 29277   | 405523,      | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  | 20% OF TOTAL VOLUME | NEGATIVE     |
| 29278   | 405524,      | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  |                     | NEGATIVE     |
| 29279   | 405525,      | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  | 75% OF TOTAL VOLUME | NEGATIVE     |
| 29280   | 405526,      | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  |                     | NEGATIVE     |
| 29281   | 405527,      | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  |                     | NEGATIVE     |
| 29282   | 405528,      | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  |                     | NEGATIVE     |
| 29283   | 405529,      | -         | BL 25-FEB-94               | LM    | 90016 21-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  |                     | NEGATIVE     |
| 29284   | 405530,      | -         | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  |                     | NEGATIVE     |
| 29285   | 405531,      | -         | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  |                     | NEGATIVE     |
| 29286   | 405532,      | -         | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  | 25% OF TOTAL VOLUME | NEGATIVE     |
| 29287   | 405533,      | -         | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  | 30% OF TOTAL VOLUME | NEGATIVE     |
| 29288   | 405534,      | -         | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  | 50% OF TOTAL VOLUME | NEGATIVE     |
| 29289   | 405535,      | -         | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  |                     | NEGATIVE     |
| 29290   | 405536,      | -         | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  | 40% OF TOTAL VOLUME | NEGATIVE     |
| 29291   | 405537,      | -         | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  | 25% OF TOTAL VOLUME | NEGATIVE     |
| 29292   | 405538,      | -         | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  | 40% OF TOTAL VOLUME | NEGATIVE     |
| 29293   | 405539,      | -         | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA | JW 11/12 | -        | -        | NR  | 45% OF TOTAL VOLUME | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name          | FPC + SSN    | Spec Received<br>Type Date | Study | Panel Assay Date | Virus               | Primer | Tube Tube<br># 1 # 2 | Int | Comments             | Final Result |
|---------|-----------------------|--------------|----------------------------|-------|------------------|---------------------|--------|----------------------|-----|----------------------|--------------|
| 29294   | 405540,               | -            | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  | 55% OF TOTAL VOLUME  | NEGATIVE     |
| 29295   | 405541,               | -            | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  | 35% OF TOTAL VOLUME  | NEGATIVE     |
| 29296   | 405542,               | -            | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  | 35% OF TOTAL VOLUME  | NEGATIVE     |
| 29297   | 405543,               | -            | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  | 20% OF TOTAL VOLUME  | NEGATIVE     |
| 29298   | 405544,               | -            | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  | 45% OF TOTAL VOLUME  | NEGATIVE     |
| 29299   | 405545,               | -            | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  | 35% OF TOTAL VOLUME  | NEGATIVE     |
| 29300   | 405546,               | -            | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  | 25% OF RTOTAL VOLUME | NEGATIVE     |
| 29301   | 405547,               | -            | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  |                      | NEGATIVE     |
| 29302   | 405548,               | -            | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  |                      | NEGATIVE     |
| 29303   | 405549,               | -            | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  |                      | NEGATIVE     |
| 29304   | MALDONADO, TONY       | -            | BL 25-FEB-94               | LM    | 90016 24-MAR-94  | LEISHMANIA JW 11/12 |        | +                    | RE  |                      | POSITIVE     |
| 29384   | CHEPSERY, CHENNGETICH | -            | OT 03-MAR-94               | LM    | 90016 24-MAR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  |                      | NEGATIVE     |
| 29385   | PORONJO, MARY         | -            | OT 04-MAR-94               | LM    | 90016 24-MAR-94  | LEISHMANIA JW 11/12 |        | +/-                  | RE  |                      | POSITIVE     |
| 29387   | SAMOSN, GRACE         | -            | OT 04-MAR-94               | LM    | 90016 24-MAR-94  | LEISHMANIA JW 11/12 |        | +                    | RE  |                      | POSITIVE     |
| 29388   | KIPKEMEI, CHEROP      | -            | OT 04-MAR-94               | LM    | 90016 24-MAR-94  | LEISHMANIA JW 11/12 |        | +                    | RE  |                      | POSITIVE     |
| 29389   | BARTENJO, CHRISTINE   | -            | OT 04-MAR-94               | LM    | 90016 24-MAR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  |                      | NEGATIVE     |
| 29390   | KANDAGOR, PERIS       | -            | OT 04-MAR-94               | LM    | 90016 24-MAR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  |                      | NEGATIVE     |
| 29375   | GOULD, MICHAEL        | -227-74-3133 | BM 04-MAR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  |                      | NEGATIVE     |
| 29386   | GOULD, MICHAEL        | -227-74-3133 | BL 04-MAR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  |                      | NEGATIVE     |
| 29391   | MAHER, JEROME         | -361-42-2793 | BL 04-MAR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  |                      | NEGATIVE     |
| 29392   | MAHER, JEROME         | -361-42-2793 | BM 04-MAR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  |                      | NEGATIVE     |
| 29393   | WOOD, STEVE W         | -594-20-7800 | BL 04-MAR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  |                      | NEGATIVE     |
| 29394   | WOOD, STEVE W         | -594-20-7800 | BM 04-MAR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  |                      | NEGATIVE     |
| 29823   | TOOLE, JAMES          | -274-40-7928 | BL 14-MAR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -                    | NR  |                      | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name         | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus               | Primer | Tube # 1 | Tube # 2 | Int | Comments            | Final Result |
|---------|----------------------|----------------|----------------------------|-------|------------------|---------------------|--------|----------|----------|-----|---------------------|--------------|
| 29824   | TOOLE, JAMES         | -274-40-7928   | BL 14-MAR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |                     | NEGATIVE     |
| 29897   | WADDELL, DIRK        | 20-343-56-3223 | BL 21-MAR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |                     | NEGATIVE     |
| 29919   | LIBERTY, SCHANEN     | -450-47-5981   | BL 22-MAR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  | 45% OF TOTAL VOLUME | NEGATIVE     |
| 29948   | LIBERTY, SCHANEN     | -450-47-5981   | BM 22-MAR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |                     | NEGATIVE     |
| 29959   | 40831,               | -              | BL 25-MAR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | +        | +        | RE  | 15% OF TOTAL VOLUME | POSITIVE     |
| 29960   | 40832,               | -              | BL 25-MAR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | +        | +        | RE  |                     | POSITIVE     |
| 29961   | 40833,               | -              | BL 25-MAR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  | 50% OF TOTAL VOLUME | NEGATIVE     |
| 29962   | 40834,               | -              | BL 25-MAR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  | 5% OF TOTAL VOLUME  | NEGATIVE     |
| 29985   | SCHVERMAN, KRISTIE L | -569-53-0637   | BL 29-MAR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |                     | NEGATIVE     |
| 30105   | SCHVERMAN, KRISTIE L | -569-53-0637   | BL 29-MAR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |                     | NEGATIVE     |
| 30201   | 40901,               | -              | BL 01-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |                     | NEGATIVE     |
| 30202   | 40902,               | -              | BL 01-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |                     | NEGATIVE     |
| 30203   | 40903,               | -              | BL 01-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |                     | NEGATIVE     |
| 30204   | 40904,               | -              | BL 01-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |                     | NEGATIVE     |
| 30205   | 40905,               | -              | BL 01-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |                     | NEGATIVE     |
| 30206   | 40906,               | -              | BL 01-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  |                     | NEGATIVE     |
| 30207   | 40908,               | -              | BL 01-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  | 25% OF TOTAL VOLUME | NEGATIVE     |
| 30208   | 40908,               | -              | BL 01-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  | 25% OF TOTAL VOLUME | NEGATIVE     |
| 30209   | 40909,               | -              | BL 01-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  | 15% OF TOTAL VOLUME | NEGATIVE     |
| 30210   | 409010,              | -              | BL 01-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  | 15% OF TOTAL VOLUME | NEGATIVE     |
| 30211   | 409011,              | -              | BL 01-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  | 30% OF TOTAL VOLUME | NEGATIVE     |
| 30212   | 409012,              | -              | BL 01-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  | 80% OF TOTAL VOLUME | NEGATIVE     |
| 30213   | 409013,              | -              | BL 01-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  | 20% OF TOTAL VOLUME | NEGATIVE     |
| 30214   | 409014,              | -              | BL 01-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -        | -        | NR  | 25% OF TOTAL VOLUME | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name        | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus               | Primer | Tube # | Tube # 2 | Int | Comments            | Final Result |
|---------|---------------------|----------------|----------------------------|-------|------------------|---------------------|--------|--------|----------|-----|---------------------|--------------|
| 30263   | PAULSEN, KIM        | -571-35-8120   | BL 06-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  |                     | NEGATIVE     |
| 30264   | PAULSEN, KIM        | -571-35-8120   | BL 06-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  |                     | NEGATIVE     |
| 30265   | CVC 39 EDTA,        | -              | BL 06-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | +      | +        | RE  |                     | POSITIVE     |
| 30266   | CVC 37 EDTA,        | -              | BL 06-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  |                     | NEGATIVE     |
| 30267   | CVC 39 HEPARIN,     | -              | BL 06-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  | 5% OF TOTAL VOLUME  | NEGATIVE     |
| 30268   | CVC 40 HEPARIN,     | -              | BL 06-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  | 20% OF TOTAL VOLUME | NEGATIVE     |
| 30269   | CVC 37 HEPARIN,     | -              | BL 06-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  | 10% OF TOTAL VOLUME | NEGATIVE     |
| 30270   | CVC 40 EDTA,        | -              | BL 06-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  |                     | NEGATIVE     |
| 30271   | CVC 36 HEPARIN,     | -              | BL 06-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  | 10% OF TOTAL VOLUME | NEGATIVE     |
| 30272   | CVC 34 HEPARIN,     | -              | BL 06-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  |                     | NEGATIVE     |
| 30273   | CVC 36 EDTA,        | -              | BL 06-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  |                     | NEGATIVE     |
| 30274   | CVC 34 EDTA,        | -              | BL 06-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  |                     | NEGATIVE     |
| 30289   | BALOGH, STEPHEN     | -160-58-7391   | BL 06-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  |                     | NEGATIVE     |
| 30290   | BALOGH, STEPHEN     | -160-58-7391   | BM 06-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  |                     | NEGATIVE     |
| 30423   | 40981,              | -              | BL 08-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | +      | +        | RE  |                     | POSITIVE     |
| 30424   | 40982,              | -              | BL 08-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | +      | +        | RE  |                     | POSITIVE     |
| 30425   | KRAMER, GARY        | -178-42-7910   | BL 08-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  |                     | NEGATIVE     |
| 30450   | NORTHEY, DUWAYNE F  | 20-395-84-5069 | BL 13-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  |                     | NEGATIVE     |
| 30451   | NORTHEY, DUWAYNE F  | 20-395-84-5069 | BM 13-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  |                     | NEGATIVE     |
| 30467   | STECKBECK, DEAN     | 20-179-54-7414 | LI 13-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  |                     | NEGATIVE     |
| 30468   | CHOVERI, ROY        | 20-215-70-5824 | BL 13-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  |                     | NEGATIVE     |
| 30469   | CHOVERI, ROY        | 20-215-70-5824 | BM 13-APR-94               | LM    | 90017 19-APR-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  |                     | NEGATIVE     |
| 28911   | LYON, KENNY         | -465-35-8434   | BM 10-FEB-94               | LM    | 90018 02-MAY-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  |                     | NEGATIVE     |
| 29033   | HAYNESWORTH, WILLIE | -340-72-3179   | BL 22-FEB-94               | LM    | 90018 02-MAY-94  | LEISHMANIA JW 11/12 |        | -      | -        | NR  |                     | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name    | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus                 | Primer | Tube Tube<br># 1 # 2 | Int | Comments | Final Result |
|---------|-----------------|----------------|----------------------------|-------|------------------|-----------------------|--------|----------------------|-----|----------|--------------|
| 29263   | 40559,          | -              | BL 25-FEB-94               | LM    | 90018 02-MAY-94  | LEISHMANIA JW 11/12   |        | -                    | -   | NR       | NEGATIVE     |
| 30621   | LATHAM, TRAVIS  | 20-006-82-9838 | SK 19-APR-94               | LM    | 90018 02-MAY-94  | LEISHMANIA JW 11/12   |        | +                    | +   | RE       | POSITIVE     |
| 30622   | CROFT, JOSHUA   | 20-402-08-9845 | SK 20-APR-94               | LM    | 90018 02-MAY-94  | LEISHMANIA JW 11/12   |        | +                    | +   | RE       | POSITIVE     |
| 30660   | BOWLES, HERBERT | 20-138-50-6441 | LI 19-APR-94               | LM    | 90018 02-MAY-94  | LEISHMANIA JW 11/12   |        | -                    | -   | NR       | NEGATIVE     |
| 30802   | 41161,          | -              | BL 26-APR-94               | LM    | 90018 02-MAY-94  | LEISHMANIA JW 11/12   |        | +                    | +   | RE       | POSITIVE     |
| 30803   | 41162,          | -              | BL 26-APR-94               | LM    | 90018 02-MAY-94  | LEISHMANIA JW 11/12   |        | +                    | +   | RE       | POSITIVE     |
| 30804   | 41163,          | -              | BL 26-APR-94               | LM    | 90018 02-MAY-94  | LEISHMANIA JW 11/12   |        | +                    | +   | RE       | POSITIVE     |
| 30805   | 41164,          | -              | BL 26-APR-94               | LM    | 90018 02-MAY-94  | LEISHMANIA JW 11/12   |        | -                    | -   | NR       | NEGATIVE     |
| 30806   | PLUMMER, SCOTT  | 20-018-48-7159 | SK 26-APR-94               | LM    | 90018 02-MAY-94  | LEISHMANIA JW 11/12   |        | +                    | +   | RE       | POSITIVE     |
| Z000795 | 40341,          | -              | BL 04-FEB-94               | LM    | 90018 02-MAY-94  | LEISHMANIA JW 11/12   |        | -                    | -   | NR       | NEGATIVE     |
| Z000821 | KAPPLAN, BARRY  | 20-206-52-6668 | LN 18-FEB-94               | LM    | 90018 02-MAY-94  | LEISHMANIA JW 11/12   |        | -                    | -   | NR       | NEGATIVE     |
| 30911   | 41221,          | -              | BL 02-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | +                    | +   | RE       | POSITIVE     |
| 30912   | 41222,          | -              | BL 02-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | +                    | +   | RE       | POSITIVE     |
| 30913   | 41223,          | -              | BL 02-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 30914   | 41224,          | -              | BL 02-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 30915   | 41225,          | -              | BL 02-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | +                    | +   | RE       | POSITIVE     |
| 30916   | 41226,          | -              | BL 02-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | +                    | +   | RE       | POSITIVE     |
| 30917   | 41227,          | -              | BL 02-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | +                    | +   | RE       | POSITIVE     |
| 30918   | 41228,          | -              | BL 02-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 30919   | 41229,          | -              | BL 02-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 30920   | 412210,         | -              | BL 02-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | +                    | +   | RE       | POSITIVE     |
| 30959   | PLUMMER, SCOTT  | 20-018-48-7159 | SK 05-MAY-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | +                    | +   | RE       | POSITIVE     |
| 30990   | 41261,          | 20-            | BL 06-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 30991   | 41262,          | 20-            | BL 06-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | +                    | +   | RE       | POSITIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name          | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus                 | Primer | Tube Tube |     | Int | Comments            | Final Result |
|---------|-----------------------|----------------|----------------------------|-------|------------------|-----------------------|--------|-----------|-----|-----|---------------------|--------------|
|         |                       |                |                            |       |                  |                       |        | # 1       | # 2 |     |                     |              |
| 30992   | 41263,                | 20-            | BL 06-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | +         | +   | RE  |                     | POSITIVE     |
| 30993   | 41264,                | 20-            | BL 06-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | +         | +   | RE  |                     | POSITIVE     |
| 30994   | 41265,                | 20-            | BL 06-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | +         | +   | RE  | SEE REPORT          | POSITIVE     |
| 31015   | BOYLE, SEAN P         | 20-226-19-1869 | BL 08-MAY-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR  | SEE REPORT          | NEGATIVE     |
| 31016   | BOYLE, SEAN P         | 20-226-19-1869 | BM 08-MAY-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR  | SEE REPORT          | NEGATIVE     |
| 31017   | SANTAFERRARA, JAMES P | 20-057-50-1286 | SP 08-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR  |                     | NEGATIVE     |
| 31018   | SANTAFERRARA, JAMES P | 20-057-50-1286 | SP 08-MAY-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR  |                     | NEGATIVE     |
| 31019   | BACHMAN, JOHN         | 20-205-42-4875 | LN 10-MAY-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR  |                     | NEGATIVE     |
| 31047   | ABDALLAH, BASSAM K    | 20-            | SK 11-MAY-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR  | SEE REPORT          | NEGATIVE     |
| 31048   | LOCKETT, NORMA        | 20-450-53-3473 | BL 11-MAY-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | ND  | SEE REPORT          | NON-DIAG     |
| 31049   | LOCKETT, NORMA        | 20-450-53-3473 | BM 11-MAY-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | ND  | SEE REPORT          | NON-DIAG     |
| 31051   | 41301,                | 20-            | BL 10-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR  | 30% OF SUGG. NUMBER | NEGATIVE     |
| 31052   | 41302,                | 20-            | BL 10-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | +         | +   | RE  |                     | POSITIVE     |
| 31053   | CROSSMAN, STEWART     | 20-025-48-6417 | BL 10-MAY-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR  |                     | NEGATIVE     |
| 31054   | CROSSMAN, STEWART     | 20-025-48-6417 | BM 10-MAY-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR  |                     | NEGATIVE     |
| 31085   | BOYLE, SEAN P         | 20-226-19-1869 | LN 16-MAY-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR  |                     | NEGATIVE     |
| 31356   | WILLIAM, FRADY D      | -239-37-0026   | BL 20-MAY-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR  |                     | NEGATIVE     |
| 31357   | WILLIAM, FRADY D      | -239-37-0026   | BM 20-MAY-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR  |                     | NEGATIVE     |
| 31379   | 41401,                | -              | BL 23-MAY-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | +         | +   | RE  |                     | POSITIVE     |
| 31380   | 41402,                | -              | BL 23-MAY-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | +         | +   | RE  |                     | POSITIVE     |
| 31381   | 41404,                | -              | BL 23-MAY-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR  |                     | NEGATIVE     |
| 31382   | 41405,                | -              | BL 23-MAY-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR  |                     | NEGATIVE     |
| 31505   | CHIPLEY, JOSHUA W     | 20-564-33-6887 | BL 31-MAY-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR  |                     | NEGATIVE     |
| 31506   | CHIPLEY, JOSHUA W     | 20-564-33-6887 | BM 31-MAY-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR  |                     | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name        | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus                 | Primer | Tube Tube |     | Comments | Final Result |
|---------|---------------------|----------------|----------------------------|-------|------------------|-----------------------|--------|-----------|-----|----------|--------------|
|         |                     |                |                            |       |                  |                       |        | # 1       | # 2 |          |              |
| 31529   | MALLOY, VICTOR      | 20-308-60-0996 | BL 01-JUN-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR       | NEGATIVE     |
| 31530   | MALLOY, VICTOR      | 20-308-60-0996 | BM 02-JUN-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR       | NEGATIVE     |
| 31553   | COSNER, BRUCE       | -              | BM 02-JUN-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR       | NEGATIVE     |
| 31554   | CHIPLEY, JOSHUA W   | 20-564-33-6887 | SK 02-JUN-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR       | NEGATIVE     |
| 31731   | MARTINEZ, ARGUELIO  | -581-06-2752   | BM 08-JUN-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR       | NEGATIVE     |
| 31749   | HAMDEN, CHARLES     | -381-58-4162   | BL 09-JUN-94               | LM    | 90019 12-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR       | NEGATIVE     |
| 31750   | HAMDEN, CHARLES     | -381-58-4162   | BM 09-JUN-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR       | NEGATIVE     |
| 31751   | JEMIOLA, RICHARD    | -156-40-2576   | LI 09-JUN-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR       | NEGATIVE     |
| 31797   | MAZUR, JOACHIM      | -416-86-2154   | BL 16-JUN-94               | LM    | 90019 08-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR       | NEGATIVE     |
| 29033   | HAYNESWORTH, WILLIE | -340-72-3179   | BL 22-FEB-94               | LM    | 90020 28-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR       | NEGATIVE     |
| 30802   | 41161,              | -              | BL 26-APR-94               | LM    | 90020 28-JUL-94  | LEISHMANIA JW 11i/12i |        | +         | +   | RE       | POSITIVE     |
| 30803   | 41162,              | -              | BL 26-APR-94               | LM    | 90020 28-JUL-94  | LEISHMANIA JW 11i/12i |        | +         | +   | RE       | POSITIVE     |
| 30804   | 41163,              | -              | BL 26-APR-94               | LM    | 90020 28-JUL-94  | LEISHMANIA JW 11i/12i |        | +         | +   | RE       | POSITIVE     |
| 30806   | PLUMMER, SCOTT      | 20-018-48-7159 | SK 26-APR-94               | LM    | 90020 28-JUL-94  | LEISHMANIA JW 11i/12i |        | +         | +   | RE       | POSITIVE     |
| 30995   | 41266,              | 20-            | BL 06-MAY-94               | LM    | 90020 01-AUG-94  | LEISHMANIA JW 11i/12i |        | +         | +   | RE       | POSITIVE     |
| 31753   | TAYLOR, JAMES       | -512-82-7559   | LI 10-JUN-94               | LM    | 90020 01-AUG-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR       | NEGATIVE     |
| 32325   | 54F,                | -              | BL 13-JUL-94               | LM    | 90020 28-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR       | NEGATIVE     |
| 32326   | 53F,                | -              | BL 13-JUL-94               | LM    | 90020 28-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR       | NEGATIVE     |
| 32350   | ZUPEC, JEFFREY      | -              | BM 12-JUL-94               | LM    | 90020 28-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR       | NEGATIVE     |
| 32351   | GEIGER, MITCH       | -529-63-0706   | SK 13-JUL-94               | LM    | 90020 28-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR       | NEGATIVE     |
| 32352   | GEIGER, MITCH       | -529-63-0706   | BL 13-JUL-94               | LM    | 90020 28-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR       | NEGATIVE     |
| 32354   | PERDUE, JAMES       | -256-92-2772   | BL 13-JUL-94               | LM    | 90020 28-JUL-94  | LEISHMANIA JW 11i/12i |        | -         | -   | NR       | NEGATIVE     |
| 32376   | 419433,             | -              | SBL 13-JUL-94              | LM    | 90020 01-AUG-94  | LEISHMANIA JW 11i/12i |        | +         | -   | IND      | INDETERMIN   |
| 32377   | 419432,             | -              | SBL 13-JUL-94              | LM    | 90020 01-AUG-94  | LEISHMANIA JW 11i/12i |        | +         | -   | IND      | INDETERMIN   |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name | FPC + SSN | Spec Received<br>Type Date | Study | Panel Assay Date | Virus                 | Primer     | Tube # 1 | Tube # 2 | Int | Comments | Final Result |
|---------|--------------|-----------|----------------------------|-------|------------------|-----------------------|------------|----------|----------|-----|----------|--------------|
| 32378   | 419435,      | -         | SBL 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | +        | +        | RE  |          | POSITIVE     |
| 32379   | 419431,      | -         | SBL 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 32380   | 419436,      | -         | SBL 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 32380   | 419436,      | -         | SBL 13-JUL-94              | LM    | 90020 05-AUG-94  | LEISHMANIA JW 11/12   | JW 11/12   | -        | -        | NR  |          | NEGATIVE     |
| 32381   | 419434,      | -         | SBL 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 32381   | 419434,      | -         | SBL 13-JUL-94              | LM    | 90020 05-AUG-94  | LEISHMANIA JW 11/12   | JW 11/12   | -        | -        | NR  |          | NEGATIVE     |
| 32382   | 419430,      | -         | SBL 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | +        | +        | RE  |          | POSITIVE     |
| 32383   | 419429,      | -         | SBL 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 32383   | 419429,      | -         | SBL 13-JUL-94              | LM    | 90020 05-AUG-94  | LEISHMANIA JW 11/12   | JW 11/12   | -        | -        | NR  |          | NEGATIVE     |
| 32384   | 419428,      | -         | SBL 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 32384   | 419428,      | -         | SBL 13-JUL-94              | LM    | 90020 05-AUG-94  | LEISHMANIA JW 11/12   | JW 11/12   | -        | -        | NR  |          | NEGATIVE     |
| 32385   | 419427,      | -         | SBL 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 32385   | 419427,      | -         | SBL 13-JUL-94              | LM    | 90020 05-AUG-94  | LEISHMANIA JW 11/12   | JW 11/12   | -        | -        | NR  |          | NEGATIVE     |
| 32386   | 419426,      | -         | SBL 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | +        | +        | RE  |          | POSITIVE     |
| 32386   | 419426,      | -         | SBL 13-JUL-94              | LM    | 90020 05-AUG-94  | LEISHMANIA JW 11/12   | JW 11/12   | -        | -        | NR  |          | NEGATIVE     |
| 32387   | 419425,      | -         | SBL 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 32387   | 419425,      | -         | SBL 13-JUL-94              | LM    | 90020 05-AUG-94  | LEISHMANIA JW 11/12   | JW 11/12   | -        | -        | NR  |          | NEGATIVE     |
| 32388   | 419423,      | -         | SBL 13-JUL-94              | LM    | 90020 01-AUG-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | +        | -        | IND |          | INDETERMIN   |
| 32389   | 419422,      | -         | SBL 13-JUL-94              | LM    | 90020 01-AUG-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | +        | +        | RE  |          | POSITIVE     |
| 32390   | 419424,      | -         | SBL 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 32390   | 419424,      | -         | SBL 13-JUL-94              | LM    | 90020 05-AUG-94  | LEISHMANIA JW 11/12   | JW 11/12   | -        | -        | NR  |          | NEGATIVE     |
| 32391   | 419421,      | -         | SBL 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 32391   | 419421,      | -         | SBL 13-JUL-94              | LM    | 90020 05-AUG-94  | LEISHMANIA JW 11/12   | JW 11/12   | +        | +        | RE  |          | POSITIVE     |
| 32392   | 419419,      | -         | SBL 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name | FPC + SSN | Spec Received<br>Type Date | Study | Panel Assay Date | Virus                 | Primer | Tube # 1 | Tube # 2 | Int | Comments | Final Result |
|---------|--------------|-----------|----------------------------|-------|------------------|-----------------------|--------|----------|----------|-----|----------|--------------|
| 32392   | 419419,      | -         | SBL 13-JUL-94              | LM    | 90020 05-AUG-94  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          | NEGATIVE     |
| 32393   | 419420,      | -         | SBL 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |          | NEGATIVE     |
| 32394   | 41941,       | -         | SCU 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |          | NEGATIVE     |
| 32394   | 41941,       | -         | SCU 13-JUL-94              | LM    | 90020 05-AUG-94  | LEISHMANIA JW 11/12   |        | +        | +        | RE  |          | POSITIVE     |
| 32395   | 41942,       | -         | SCU 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i |        | +        | +        | RE  |          | POSITIVE     |
| 32396   | 41943,       | -         | SCU 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i |        | +        | +        | RE  |          | POSITIVE     |
| 32397   | 41944,       | -         | SCU 13-JUL-94              | LM    | 90020 01-AUG-94  | LEISHMANIA JW 11i/12i |        | +        | +        | RE  |          | POSITIVE     |
| 32398   | 41945,       | -         | SCU 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i |        | +        | +        | RE  |          | POSITIVE     |
| 32399   | 41946,       | -         | SCU 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |          | NEGATIVE     |
| 32399   | 41946,       | -         | SCU 13-JUL-94              | LM    | 90020 05-AUG-94  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          | NEGATIVE     |
| 32400   | 41948,       | -         | SCU 13-JUL-94              | LM    | 90020 05-AUG-94  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          | NEGATIVE     |
| 32400   | 41948,       | -         | SCU 13-JUL-94              | LM    | 90020 01-AUG-94  | LEISHMANIA JW 11i/12i |        | +        | -        | IND |          | INDETERMIN   |
| 32501   | 41948,       | -         | SCU 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i |        | +        | +        | RE  |          | POSITIVE     |
| 32502   | 41949,       | -         | SCU 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i |        | +        | +        | RE  |          | POSITIVE     |
| 32503   | 419410,      | -         | SCU 13-JUL-94              | LM    | 90020 27-JUL-94  | LEISHMANIA JW 11i/12i |        | +        | +        | RE  |          | POSITIVE     |
| 32504   | 419411,      | -         | SCU 13-JUL-94              | LM    | 90020 01-AUG-94  | LEISHMANIA JW 11i/12i |        | +        | +        | RE  |          | POSITIVE     |
| 32505   | 419412,      | -         | SCU 13-JUL-94              | LM    | 90020 01-AUG-94  | LEISHMANIA JW 11i/12i |        | +        | +        | RE  |          | POSITIVE     |
| 32505   | 419412,      | -         | SCU 13-JUL-94              | LM    | 90020 05-AUG-94  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          | NEGATIVE     |
| 32506   | 419413,      | -         | SCU 13-JUL-94              | LM    | 90020 01-AUG-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |          | NEGATIVE     |
| 32506   | 419413,      | -         | SCU 13-JUL-94              | LM    | 90020 05-AUG-94  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          | NEGATIVE     |
| 32507   | 419414,      | -         | SCU 13-JUL-94              | LM    | 90020 28-JUL-94  | LEISHMANIA JW 11i/12i |        | +        | +        | RE  |          | POSITIVE     |
| 32508   | 419415,      | -         | SCU 13-JUL-94              | LM    | 90020 01-AUG-94  | LEISHMANIA JW 11i/12i |        | +        | +        | RE  |          | POSITIVE     |
| 32509   | 419416,      | -         | SCU 13-JUL-94              | LM    | 90020 28-JUL-94  | LEISHMANIA JW 11i/12i |        | +        | +        | RE  |          | POSITIVE     |
| 32510   | 419417,      | -         | SCU 13-JUL-94              | LM    | 90020 28-JUL-94  | LEISHMANIA JW 11i/12i |        | +        | +        | RE  |          | POSITIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name       | FPC + SN       | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer                | Tube # | Tube Int | Comments | Final Result                  |
|---------|--------------------|----------------|----------------------------|-------|------------------|-----------|-----------------------|--------|----------|----------|-------------------------------|
|         |                    |                |                            |       |                  |           |                       | 1      | 2        |          |                               |
| 32511   | 419418,            | -              | SCU 13-JUL-94              | LM    | 90020            | 28-JUL-94 | LEISHMANIA JW 11i/12i | +      | +        | RE       | POSITIVE                      |
| 2000795 | 40341,             | -              | BL 04-FEB-94               | LM    | 90020            | 28-JUL-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 2000821 | KAPLAN, BARRY      | 20-206-52-6668 | LN 18-FEB-94               | LM    | 90020            | 28-JUL-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 28911   | LYON, KENNY        | -465-35-8434   | BM 10-FEB-94               | LM    | 90021            | 05-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 30805   | 41164,             | -              | BL 26-APR-94               | LM    | 90021            | 05-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 31796   | MAZUR, JOACHIM     | -416-86-2154   | LN 16-JUN-94               | LM    | 90021            | 05-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 31841   | FRANKS, JOHNNIE    | -509-82-2357   | LN 22-JUN-94               | LM    | 90021            | 05-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 31854   | SMITH, HERBERT J   | 20-264-56-5136 | BM 23-JUN-94               | LM    | 90021            | 05-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 31943   | MCKEE, THEODORE    | -473-72-0140   | BL 28-JUN-94               | LM    | 90021            | 05-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 31944   | MCKEE, THEODORE    | -473-72-0140   | BM 28-JUN-94               | LM    | 90021            | 05-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 31945   | FRANKS, JOHNNIE    | -509-82-2357   | BL 28-JUN-94               | LM    | 90021            | 05-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 31946   | FRANKS, JOHNNIE    | -509-82-2357   | BM 28-JUN-94               | LM    | 90021            | 05-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 32349   | ZUPEC, JEFFREY     | -              | BL 12-JUL-94               | LM    | 90021            | 05-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 32353   | PERDUE, JAMES      | -256-92-2772   | SK 13-JUL-94               | LM    | 90021            | 05-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 32869   | COOKE, THOMAS      | -101-46-8509   | LI 03-AUG-94               | LM    | 90022            | 18-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 32993   | NORTHEY, DUWAYNE F | 20-395-84-5069 | BL 12-AUG-94               | LM    | 90022            | 18-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 32994   | MARTEN, JOSEPH     | -043-48-9687   | BL 11-AUG-94               | LM    | 90022            | 18-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 32995   | MARTEN, JOSEPH     | -043-48-9687   | BM 11-AUG-94               | LM    | 90022            | 18-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 32996   | EDWARD, JAMES      | -158-50-4154   | BL 11-AUG-94               | LM    | 90022            | 18-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 32997   | EDWARD, JAMES      | -158-50-4154   | LI 11-AUG-94               | LM    | 90022            | 18-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 32998   | ROVNAN, MATTHEW    | -209-58-7439   | BL 11-AUG-94               | LM    | 90022            | 18-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |
| 32999   | ROVNAN, MATTHEW    | -209-58-7439   | SK 11-AUG-94               | LM    | 90022            | 18-AUG-94 | LEISHMANIA JW 11i/12i | +      | +        | RE       | POSITIVE                      |
| 33000   | ROVNAN, MATTHEW    | -209-58-7439   | ASP 11-AUG-94              | LM    | 90022            | 18-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | 1.5% OF TOTAL VOLUME NEGATIVE |
| 33101   | NORTHEY, DUWAYNE F | 20-395-84-5069 | LI 12-AUG-94               | LM    | 90022            | 18-AUG-94 | LEISHMANIA JW 11i/12i | -      | -        | NR       | NEGATIVE                      |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name      | FPC + SSN    | Spec Received<br>Type Date | Study | Panel | Assay Date | Virus                 | Primer | Tube # 1 | Tube # 2 | Int | Comments            | Final Result |
|---------|-------------------|--------------|----------------------------|-------|-------|------------|-----------------------|--------|----------|----------|-----|---------------------|--------------|
| 33111   | ROVNAV, MATTHEW   | -209-58-7439 | ASP 17-AUG-94              | LM    | 90023 | 25-AUG-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  | 20% OF TOTAL VOLUME | NEGATIVE     |
| 33193   | RITTER, BARBARA K | -404-60-5363 | BL 25-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33194   | RITTER, BARBARA K | -404-60-5363 | BM 25-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33195   | LALITA, DEVI      | -            | BL 25-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  | ABLC-19             | NEGATIVE     |
| 33196   | RAKESH, KV        | -            | BL 25-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | +        | +        | RE  | ABLC-018            | POSITIVE     |
| 33197   | RITTER, BARBARA K | -404-60-5363 | LI 25-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33198   | 42381,            | -            | BL 26-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33199   | 42382,            | -            | BL 26-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33200   | 42383,            | -            | BL 26-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33301   | 42384,            | -            | BL 26-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33302   | 42385,            | -            | BL 26-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33303   | 42386,            | -            | BL 26-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33304   | 42387,            | -            | BL 26-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33305   | 42388,            | -            | BL 26-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33306   | 42389,            | -            | BL 26-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33307   | 423810,           | -            | BL 26-AUG-94               | LM    | 90024 | 19-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33308   | 423811,           | -            | BL 26-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33309   | 423812,           | -            | BL 26-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33310   | 423813,           | -            | BL 26-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33311   | 423814,           | -            | BL 26-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33312   | 423815,           | -            | BL 26-AUG-94               | LM    | 90024 | 19-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33313   | 423816,           | -            | BL 26-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33314   | 423817,           | -            | BL 26-AUG-94               | LM    | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |
| 33315   | 423818,           | -            | BL 26-AUG-94               | LM    | 90024 | 19-SEP-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |                     | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name | FPC + SSN | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer                | Tube # 1 | Tube # 2 | Int | Comments | Final Result |
|---------|--------------|-----------|----------------------------|-------|------------------|-----------|-----------------------|----------|----------|-----|----------|--------------|
| 33316   | 423819,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33317   | 423820,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33318   | 423821,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33319   | 423822,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33320   | 423823,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33321   | 423824,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33322   | 423825,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33323   | 423826,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33324   | 423827,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33325   | 423828,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33326   | 423829,      | -         | BL 26-AUG-94               | LM    | 90024            | 19-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33327   | 423830,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33328   | 423831,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33329   | 423832,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33330   | 423833,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33331   | 423834,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33332   | 423835,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33333   | 423836,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33334   | 423837,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33335   | 423838,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33336   | 423839,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33337   | 423840,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33338   | 423841,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33339   | 423842,      | -         | BL 26-AUG-94               | LM    | 90024            | 13-SEP-94 | LEISHMANIA JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name     | FPC + SSN      | Spec Received<br>Type Date | Study    | Panel | Assay Date | Virus                 | Primer     | Tube # 1 | Tube # 2 | Int | Comments | Final Result |
|---------|------------------|----------------|----------------------------|----------|-------|------------|-----------------------|------------|----------|----------|-----|----------|--------------|
| 33340   | 423843,          | -              | BL 26-AUG-94               | LM       | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33341   | 423844,          | -              | BL 26-AUG-94               | LM       | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33342   | 423845,          | -              | BL 26-AUG-94               | LM       | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33343   | 423846,          | -              | BL 26-AUG-94               | LM       | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33344   | 423847,          | -              | BL 26-AUG-94               | LM       | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33409   | BACHMAN, JOHN    | 20-205-42-4875 | CO 31-AUG-94               | LM       | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33411   | HORNER, BRENT A  | -440-70-8754   | BL 31-AUG-94               | LM       | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33412   | HORNER, BRENT A  | -440-70-8754   | LI 31-AUG-94               | LM       | 90024 | 13-SEP-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33616   | 425202,          | -              | BL 09-SEP-94               | LM       | 90025 | 06-OCT-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | +        | +        | RE  |          | POSITIVE     |
| 33617   | 425203,          | -              | BL 09-SEP-94               | LM       | 90025 | 06-OCT-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33618   | 425204,          | -              | BL 09-SEP-94               | LM       | 90025 | 06-OCT-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | +        | +        | RE  |          | POSITIVE     |
| 33619   | 425205,          | -              | BL 09-SEP-94               | LM       | 90025 | 06-OCT-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | +        | +        | RE  |          | POSITIVE     |
| 33620   | 425206,          | -              | BL 09-SEP-94               | LM       | 90025 | 06-OCT-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | +        | +        | RE  |          | POSITIVE     |
| 33621   | JOHNSON, JANET L | -417-80-7607   | BL 09-SEP-94               | LM       | 90025 | 06-OCT-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33622   | JOHNSON, JANET L | -417-80-7607   | BM 09-SEP-94               | LM       | 90025 | 06-OCT-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33649   | 42551,           | -              | BL 12-SEP-94               | LM       | 90025 | 06-OCT-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | +        | +        | RE  |          | POSITIVE     |
| 33650   | 42552,           | -              | BL 12-SEP-94               | LM       | 90025 | 06-OCT-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | +        | +        | RE  |          | POSITIVE     |
| 33673   | PRIEM, RICHARD G | -452-88-8338   | BL 13-SEP-94               | LM       | 90025 | 06-OCT-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33728   | MARABLE, GARY L  | -254-51-6352   | BL 16-SEP-94               | LM       | 90025 | 06-OCT-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33729   | MARABLE, GARY L  | -254-51-6352   | BL 16-SEP-94               | LM       | 90025 | 06-OCT-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | +        | +        | RE  |          | POSITIVE     |
| 33733   | HUMPHREY, MARK   | 20-339-68-9001 | BL 19-SEP-94               | RV21 IIB | 90025 | 06-OCT-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33744   | 426201,          | -              | BL 19-SEP-94               | LM       | 90025 | 06-OCT-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33745   | 426202,          | -              | BL 19-SEP-94               | LM       | 90025 | 06-OCT-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |
| 33746   | 426203,          | -              | BL 19-SEP-94               | LM       | 90025 | 06-OCT-94  | LEISHMANIA JW 11i/12i | JW 11i/12i | -        | -        | NR  |          | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name | FPC + SSN | Spec Received<br>Type Date | Study | Panel Assay Date | Virus                 | Primer | Tube Tube<br># 1 # 2 | Int | Comments | Final Result |
|---------|--------------|-----------|----------------------------|-------|------------------|-----------------------|--------|----------------------|-----|----------|--------------|
| 33747   | 426204,      | -         | BL 19-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33748   | 426205,      | -         | BL 19-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33749   | 426206,      | -         | BL 19-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33750   | 426207,      | -         | BL 19-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33752   | 426209,      | -         | BL 19-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33754   | 426211,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33755   | 426212,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33757   | 426214,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33758   | 426215,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33759   | 426216,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33760   | 426217,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33761   | 426218,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33762   | 426219,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33763   | 426220,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33764   | 426221,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33765   | 426222,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33766   | 426223,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33767   | 426224,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33768   | 426225,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33769   | 426226,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33770   | 426227,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33771   | 426228,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33772   | 426229,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |
| 33773   | 426230,      | -         | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -                    | -   | NR       | NEGATIVE     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name     | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus                 | Primer | Tube # 1 | Tube # 2 | Int | Comments | Final Result |
|---------|------------------|----------------|----------------------------|-------|------------------|-----------------------|--------|----------|----------|-----|----------|--------------|
| 33774   | 426231,          | -              | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |          | NEGATIVE     |
| 33775   | 426232,          | -              | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |          | NEGATIVE     |
| 33776   | 426233,          | -              | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |          | NEGATIVE     |
| 33777   | 426234,          | -              | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |          | NEGATIVE     |
| 33778   | 426235,          | -              | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |          | NEGATIVE     |
| 33779   | 426236,          | -              | BL 16-SEP-94               | LM    | 90025 06-OCT-94  | LEISHMANIA JW 11i/12i |        | -        | -        | NR  |          | NEGATIVE     |
| Spec ID | Patient Name     | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus                 | Primer | Tube # 1 | Tube # 2 | Int | Comments |              |
| 20027   | BACHMAN, JOHN    | 20-205-42-4875 | BL 13-JAN-93               | LM    | 90009 03-FEB-92  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          |              |
| 20028   | BACHMAN, JOHN    | 20-205-42-4875 | BM 13-JAN-93               | LM    | 90009 03-FEB-93  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          |              |
| 20278   | CLARK, ALVIN     | 20-249-13-9659 | BL 26-JAN-93               | LM    | 90009 03-FEB-93  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          |              |
| 20279   | CLARK, ALVIN     | 20-249-13-9659 | BM 26-JAN-93               | LM    | 90009 03-FEB-93  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          |              |
| 20280   | RICO, JUAN N     | 20-447-40-4827 | BL 26-JAN-93               | LM    | 90009 03-FEB-93  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          |              |
| 20281   | RICO, JUAN N     | 20-447-40-4827 | BM 26-JAN-93               | LM    | 90009 03-FEB-93  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          |              |
| 20344   | HAMMACK, WENDELL | 20-427-49-8157 | BL 27-JAN-93               | LM    | 90009 03-FEB-93  | LEISHMANIA JW 11/12   |        | -        | +        | IND |          |              |
| 20345   | HAMMACK, WENDELL | 20-427-49-8157 | BM 27-JAN-93               | LM    | 90009 03-FEB-93  | LEISHMANIA JW 11/12   |        | -        | -        | IND |          |              |
| 20366   | DUNSON, DAVID    | 20-250-53-3477 | BL 28-JAN-93               | LM    | 90009 03-FEB-93  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          |              |
| 20367   | DUNSON, DAVID    | 20-250-53-3477 | BM 28-JAN-93               | LM    | 90009 03-FEB-93  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          |              |
| 20401   | GOODMAN, JONH J  | 20-187-50-9316 | BL 01-FEB-93               | LM    | 90009 19-FEB-93  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          |              |
| 20402   | GOODMAN, JONH J  | 20-187-50-9316 | BM 01-FEB-93               | LM    | 90009 19-FEB-93  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          |              |
| 21243   | HAMMACK, WENDELL | 20-427-49-8157 | BL 03-MAR-93               | LM    | 90009 09-MAR-93  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          |              |
| 21244   | HAMMACK, WENDELL | 20-427-49-8157 | BM 03-MAR-93               | LM    | 90009 09-MAR-93  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          |              |
| 21459   | REIGLE, KERRY J  | 20-180-48-6847 | BL 10-MAR-93               | LM    | 90009 15-MAR-93  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          |              |
| 21460   | REIGLE, KERRY J  | 20-180-48-6847 | BM 10-MAR-93               | LM    | 90009 15-MAR-93  | LEISHMANIA JW 11/12   |        | -        | -        | NR  |          |              |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name         | FPC + SSN      | Spec Received<br>Type Date | Study    | Panel Assay Date | Virus     | Primer              | Tube # | Tube #2 | Int | Comments |
|---------|----------------------|----------------|----------------------------|----------|------------------|-----------|---------------------|--------|---------|-----|----------|
| 21551   | SCHONEBOOM, BRAD L   | 20-367-76-1421 | BL 15-MAR-93               | LM       | 90009            | 25-MAR-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| 21551   | SCHONEBOOM, BRAD L   | 20-367-76-1421 | BL 15-MAR-93               | LM       | 90009            | 05-APR-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| 21552   | SCHONEBOOM, BRAD L   | 20-367-76-1421 | BM 15-MAR-93               | LM       | 90009            | 25-MAR-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| 21552   | SCHONEBOOM, BRAD L   | 20-367-76-1421 | BM 15-MAR-93               | LM       | 90009            | 05-APR-93 | LEISHMANIA JW 11/12 | +      | +       | RE  |          |
| 22756   | WILSON, PHILLIP R    | 20-246-72-7869 | BL 05-MAY-93               | RV2      | 90009            | 13-MAY-93 | LEISHMANIA JW 11/12 | +      | -       | IND |          |
| 22955   | DARBY, GREGORY S     | 20-505-06-2923 | BL 12-MAY-93               | LM       | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | -      | +/-     | IND | +REP     |
| 23056   | WADDELL, DIRK        | 20-343-56-3223 | BM 18-MAY-93               | LM       | 90009            | 20-MAY-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| 23250   | CHIPLEY, JOSHUA W    | 20-564-33-6887 | BL 21-MAY-93               | LM       | 90009            | 01-JUL-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| 23250   | CHIPLEY, JOSHUA W    | 20-564-33-6887 | BL 21-MAY-93               | LM       | 90009            | 25-MAY-93 | LEISHMANIA JW 11/12 | +      | +       | RE  |          |
| 23251   | JOHNSON, ROBERT      | -              | BL 21-MAY-93               | LM       | 90009            | 01-JUL-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| 23251   | JOHNSON, ROBERT      | -              | BL 21-MAY-93               | LM       | 90009            | 25-MAY-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| 23324   | MYKUT, STEVEN        | -              | BL 26-MAY-93               | LM       | 90009            | 27-MAY-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| 23328   | J4,                  | -              | BL 26-MAY-93               | LM       | 90009            | 27-MAY-93 | LEISHMANIA JW 11/12 | +      | +       | RE  |          |
| 23328   | J4,                  | -              | BL 26-MAY-93               | LM       | 90009            | 30-MAY-93 | LEISHMANIA JW 11/12 | *      |         | IND |          |
| 23328   | J4,                  | -              | BL 26-MAY-93               | LM       | 90009            | 01-JUL-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| 23328   | J4,                  | -              | BL 26-MAY-93               | LM       | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | -      | -       | NR  | +REP     |
| 23328   | J4,                  | -              | BL 26-MAY-93               | LM       | 90009            | 27-MAY-93 | LEISHMANIA JW 11/12 | +/-    | -       | IND |          |
| 23328   | J4,                  | -              | BL 26-MAY-93               | LM       | 90009            | 30-MAY-93 | LEISHMANIA JW 11/12 | *      |         | IND |          |
| 23344   | GHEBREMESCHEL, TADDE | 20-049-34-1541 | BL 27-MAY-93               | LM       | 90009            | 01-JUL-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| 23344   | GHEBREMESCHEL, TADDE | 20-049-34-1541 | BL 27-MAY-93               | LM       | 90009            | 09-JUL-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| 23384   | LILLY, SCOTT D       | 20-496-78-0235 | SM 01-JUN-93               | RV43     | 90009            | 02-JUL-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| 23384   | LILLY, SCOTT D       | 20-496-78-0235 | SM 01-JUN-93               | RV43     | 90009            | 02-JUL-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| 23385   | CRADDOCK, ANNETTE    | 30-455-02-0568 | BL 01-JUN-93               | RV21 IIB | 90009            | 02-JUL-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name         | FPC + SSN      | Spec Received<br>Type Date | Study | Panel | Assay Date | Virus      | Primer   | Tube # | Tube # | Int | Comments |
|---------|----------------------|----------------|----------------------------|-------|-------|------------|------------|----------|--------|--------|-----|----------|
| 23573   | LAROCHE,             | -              | BL 07-JUN-93               | LM    | 90009 | 08-JUN-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23574   | HENDRICK, PETER      | -              | BL 07-JUN-93               | LM    | 90009 | 08-JUN-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23575   | PATIENT #1 07-JUN-93 | -              | BL 07-JUN-93               | LM    | 90009 | 08-JUN-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23576   | PATIENT #2 07-JUN-93 | -              | BM 07-JUN-93               | LM    | 90009 | 08-JUN-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23677   | CHIPLEY, JOSHUA W    | 20-564-33-6887 | BL 11-JUN-93               | LM    | 90009 | 15-JUN-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23678   | CHIPLEY, JOSHUA W    | 20-564-33-6887 | BL 11-JUN-93               | LM    | 90009 | 15-JUN-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23757   | SMITH, TRACY L       | -095-50-9074   | BM 15-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23757   | SMITH, TRACY L       | -095-50-9074   | BM 15-JUN-93               | LM    | 90009 | 02-JUL-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23758   | SMITH, TRACY L       | -095-50-9074   | BL 15-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23758   | SMITH, TRACY L       | -095-50-9074   | BL 15-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23758   | SMITH, TRACY L       | -095-50-9074   | BL 15-JUN-93               | LM    | 90009 | 02-JUL-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23827   | INGALLS, J           | -056-48-8347   | BL 17-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23827   | INGALLS, J           | -056-48-8347   | BL 17-JUN-93               | LM    | 90009 | 02-JUL-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  | DIL_1:2  |
| 23828   | INGALLS, J           | -056-48-8347   | BM 17-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23828   | INGALLS, J           | -056-48-8347   | BM 17-JUN-93               | LM    | 90009 | 02-JUL-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23877   | SIMON, MICHAEL       | 20-304-82-0936 | BL 21-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23877   | SIMON, MICHAEL       | 20-304-82-0936 | BL 21-JUN-93               | LM    | 90009 | 02-JUL-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23878   | SIMON, MICHAEL       | 20-304-82-0936 | BM 21-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23878   | SIMON, MICHAEL       | 20-304-82-0936 | BM 21-JUN-93               | LM    | 90009 | 02-JUL-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23879   | YOUNG, RYAN          | 20-027-68-3250 | BL 21-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23879   | YOUNG, RYAN          | 20-027-68-3250 | BL 21-JUN-93               | LM    | 90009 | 02-JUL-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23879   | YOUNG, RYAN          | 20-027-68-3250 | BL 21-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |
| 23879   | YOUNG, RYAN          | 20-027-68-3250 | BL 21-JUN-93               | LM    | 90009 | 02-JUL-93  | LEISHMANIA | JW 11/12 | -      | -      | NR  |          |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name         | FPC + SSN      | Spec Received<br>Type Date | Study | Panel | Assay Date | Virus      | Primer   | Tube # 1 | Tube # 2 | Int | Comments  |
|---------|----------------------|----------------|----------------------------|-------|-------|------------|------------|----------|----------|----------|-----|-----------|
| 23879   | YOUNG, RYAN          | 20-027-68-3250 | BL 21-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23879   | YOUNG, RYAN          | 20-027-68-3250 | BL 21-JUN-93               | LM    | 90009 | 02-JUL-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  | DIL 1:2   |
| 23880   | JOHNSON, DAVID L     | 20-232-31-6597 | BL 21-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23880   | JOHNSON, DAVID L     | 20-232-31-6597 | BL 21-JUN-93               | LM    | 90009 | 02-JUL-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23881   | LABONTE, KEVIN P     | 20-003-64-2509 | BL 21-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23881   | LABONTE, KEVIN P     | 20-003-64-2509 | BL 21-JUN-93               | LM    | 90009 | 02-JUL-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  | NEG. CONT |
| 23882   | ZIMMERLEE, MICHAEL K | 20-465-45-8835 | BL 21-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23882   | ZIMMERLEE, MICHAEL K | 20-465-45-8835 | BL 21-JUN-93               | LM    | 90009 | 02-JUL-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23883   | FERGUSON, MARCUS W   | 20-220-84-2859 | BL 21-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23883   | FERGUSON, MARCUS W   | 20-220-84-2859 | BL 21-JUN-93               | LM    | 90009 | 02-JUL-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23884   | ROWAN, THOMAS        | 20-301-68-8561 | BL 21-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23884   | ROWAN, THOMAS        | 20-301-68-8561 | BL 21-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23885   | JOHNSON, CRAIG T     | 20-449-91-1471 | BL 21-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23886   | STONER, JOHN P       | 20-335-68-3772 | BL 21-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23886   | STONER, JOHN P       | 20-335-68-3772 | BL 21-JUN-93               | LM    | 90009 | 02-JUL-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23887   | ERICKSON, PATRICK G  | 20-553-95-8555 | BL 21-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23887   | ERICKSON, PATRICK G  | 20-553-95-8555 | BL 21-JUN-93               | LM    | 90009 | 02-JUL-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23888   | SMITHERS, STEVE      | -403-08-2991   | BL 21-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23888   | SMITHERS, STEVE      | -403-08-2991   | BL 21-JUN-93               | LM    | 90009 | 02-JUL-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23889   | CROSBY, PAUL N       | 20-082-58-3233 | BL 21-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23889   | CROSBY, PAUL N       | 20-082-58-3233 | BL 21-JUN-93               | LM    | 90009 | 02-JUL-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23890   | WILT, JIM            | 20-265-55-8545 | BL 21-JUN-93               | LM    | 90009 | 24-JUN-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |
| 23890   | WILT, JIM            | 20-265-55-8545 | BL 21-JUN-93               | LM    | 90009 | 02-JUL-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |           |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name         | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer              | Tube # 1 | Tube # 2 | Int | Comments |
|---------|----------------------|----------------|----------------------------|-------|------------------|-----------|---------------------|----------|----------|-----|----------|
| 24068   | WILLIAMS, MICHAEL    | 20-412-37-5674 | BL 25-JUN-93               | LM    | 90009            | 07-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24069   | BEST, JAMIE          | 20-237-23-1335 | BL 25-JUN-93               | LM    | 90009            | 07-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24070   | HAMILTON, WAYNE      | 20-101-72-4824 | BL 25-JUN-93               | LM    | 90009            | 07-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24071   | ORTIZ, JOSE V        | -583-70-9171   | BL 25-JUN-93               | LM    | 90009            | 07-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24072   | WALY, GLEN           | -020-64-1508   | BL 25-JUN-93               | LM    | 90009            | 07-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24073   | ANDERSON, JEFFREY J  | -561-67-7471   | BL 25-JUN-93               | LM    | 90009            | 07-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24074   | MORGAN, MARK J       | -419-19-7233   | BL 25-JUN-93               | LM    | 90009            | 07-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24075   | FERNANDEZ, EDWARD W  | -141-70-1400   | BL 25-JUN-93               | LM    | 90009            | 07-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24102   | TONEY, ANGELA        | 20-231-88-9557 | BL 28-JUN-93               | LM    | 90009            | 07-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24125   | NICELY, SCOTT        | -595-01-8826   | BM 29-JUN-93               | LM    | 90009            | 07-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24126   | NICELY, SCOTT        | -595-01-8826   | BL 29-JUN-93               | LM    | 90009            | 07-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24127   | TONEY, ANGELA        | 20-231-88-9557 | BM 29-JUN-93               | LM    | 90009            | 07-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24175   | STEWART, KRYSTAL     | 20-456-29-6993 | BL 02-JUL-93               | LM    | 90009            | 09-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24176   | ABLE, MARSHALL       | 20-215-11-2232 | BL 02-JUL-93               | LM    | 90009            | 09-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24177   | KAYS, WILLIAM        | 20-050-58-3509 | BL 02-JUL-93               | LM    | 90009            | 09-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24178   | JENNESS, BRENT       | 20-350-58-6774 | BL 02-JUL-93               | LM    | 90009            | 09-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24223   | RUSH, JAMES          | 20-256-21-0213 | BL 06-JUL-93               | LM    | 90009            | 09-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24224   | RUSH, JAMES          | 20-256-21-0213 | BM 06-JUL-93               | LM    | 90009            | 09-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24454   | PATIENT 14-JUL-93, L | -              | BL 14-JUL-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| 24454   | PATIENT 14-JUL-93, L | -              | BL 14-JUL-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| Z000101 | 2A125-A,             | -              | BL 09-DEC-92               | LM    | 90009            | 16-DEC-93 | LEISHMANIA JW 11/12 | +        | +        | RE  |          |
| Z000101 | 2A125-A,             | -              | BL 09-DEC-92               | LM    | 90009            | 10-DEC-92 | LEISHMANIA JW 11/12 | +        | +        | RE  |          |
| Z000102 | 2A125-B,             | -              | BL 09-DEC-92               | LM    | 90009            | 16-DEC-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |

PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name | PPC + SSN | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer              | Tube # 1 | Tube # 2 | Int | Comments |
|----------------------|-----------|----------------------------|-------|------------------|-----------|---------------------|----------|----------|-----|----------|
| Z000102 2A125-B,     | -         | BL 09-DEC-92               | LM    | 90009            | 10-DEC-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| Z000103 2A125-C,     | -         | BL 09-DEC-92               | LM    | 90009            | 16-DEC-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| Z000103 2A125-C,     | -         | BL 09-DEC-92               | LM    | 90009            | 10-DEC-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| Z000104 2A125-D,     | -         | BL 09-DEC-92               | LM    | 90009            | 16-DEC-93 | LEISHMANIA JW 11/12 | +        | +        | RE  |          |
| Z000104 2A125-D,     | -         | BL 09-DEC-92               | LM    | 90009            | 10-DEC-92 | LEISHMANIA JW 11/12 | +        | +        | RE  |          |
| Z000105 2A125-E,     | -         | BL 09-DEC-92               | LM    | 90009            | 16-DEC-93 | LEISHMANIA JW 11/12 | +        | +        | RE  |          |
| Z000105 2A125-E,     | -         | BL 09-DEC-92               | LM    | 90009            | 10-DEC-92 | LEISHMANIA JW 11/12 | +        | +        | RE  |          |
| Z000106 2A125-F,     | -         | BL 09-DEC-92               | LM    | 90009            | 16-DEC-93 | LEISHMANIA JW 11/12 | +        | +        | RE  |          |
| Z000106 2A125-F,     | -         | BL 09-DEC-92               | LM    | 90009            | 10-DEC-92 | LEISHMANIA JW 11/12 | +        | +        | RE  |          |
| Z000107 2A125-1,     | -         | BL 09-DEC-92               | LM    | 90009            | 16-DEC-93 | LEISHMANIA JW 11/12 | +        | +        | RE  |          |
| Z000107 2A125-1,     | -         | BL 09-DEC-92               | LM    | 90009            | 10-DEC-92 | LEISHMANIA JW 11/12 | +        | +        | RE  |          |
| Z000108 2A125-2,     | -         | BL 09-DEC-92               | LM    | 90009            | 16-DEC-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| Z000108 2A125-2,     | -         | BL 09-DEC-92               | LM    | 90009            | 10-DEC-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| Z000109 2A125-3,     | -         | BL 09-DEC-92               | LM    | 90009            | 16-DEC-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| Z000109 2A125-3,     | -         | BL 09-DEC-92               | LM    | 90009            | 10-DEC-92 | LEISHMANIA JW 11/12 | -        | -        | NR  |          |
| Z000110 2A125-4,     | -         | BL 09-DEC-92               | LM    | 90009            | 16-DEC-93 | LEISHMANIA JW 11/12 | +        | +        | RE  |          |
| Z000110 2A125-4,     | -         | BL 09-DEC-92               | LM    | 90009            | 10-DEC-92 | LEISHMANIA JW 11/12 | +        | +        | RE  |          |
| Z000111 2A125-5,     | -         | BL 09-DEC-92               | LM    | 90009            | 16-DEC-93 | LEISHMANIA JW 11/12 | -        | +        | IND |          |
| Z000111 2A125-5,     | -         | BL 09-DEC-92               | LM    | 90009            | 10-DEC-92 | LEISHMANIA JW 11/12 | +        | +        | RE  |          |
| Z000112 2A125-6,     | -         | BL 09-DEC-92               | LM    | 90009            | 16-DEC-93 | LEISHMANIA JW 11/12 | +        | +        | RE  |          |
| Z000112 2A125-6,     | -         | BL 09-DEC-92               | LM    | 90009            | 10-DEC-92 | LEISHMANIA JW 11/12 | +        | +        | RE  |          |
| Z000117 2A125-A,     | -         | BL 16-DEC-92               | LM    | 90009            | 21-DEC-93 | LEISHMANIA JW 11/12 | +        | +        | RE  |          |
| Z000118 2A125-B,     | -         | BL 16-DEC-92               | LM    | 90009            | 21-DEC-93 | LEISHMANIA JW 11/12 | +        | +        | RE  |          |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name  | FPC + SSN | Spec Received<br>Type Date | Study | Panel Assay Date Virus     | Primer   | Tube<br># 1 | Tube<br># 2 | Int | Comments |
|-----------------------|-----------|----------------------------|-------|----------------------------|----------|-------------|-------------|-----|----------|
| Z000119 2A125-C,      | -         | BL 16-DEC-92 LM            | LM    | 90009 21-DEC-93 LEISHMANIA | JW 11/12 | -           | -           | NR  |          |
| Z000120 2A125-D,      | -         | BL 16-DEC-92 LM            | LM    | 90009 21-DEC-93 LEISHMANIA | JW 11/12 | +           | +           | RE  |          |
| Z000121 ANTI GP160/2, | -         | CE 09-DEC-92 FLOW          | FLOW  | 90009 21-DEC-93 LEISHMANIA | JW 11/12 | +           | +           | RE  |          |
| Z000122 ANTI GP160/2, | -         | CE 09-DEC-92 FLOW          | FLOW  | 90009 21-DEC-93 LEISHMANIA | JW 11/12 | -           | -           | NR  |          |
| Z000161 A,            | -         | BL 30-DEC-92 LM            | LM    | 90009 06-JAN-93 LEISHMANIA | JW 11/12 | -           | -           | NR  |          |
| Z000162 C,            | -         | BL 30-DEC-92 LM            | LM    | 90009 06-JAN-93 LEISHMANIA | JW 11/12 | +           | +           | RE  |          |
| Z000162 C,            | -         | BL 30-DEC-92 LM            | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +           | +           | RE  | +REP     |
| Z000163 E,            | -         | BL 30-DEC-92 LM            | LM    | 90009 06-JAN-93 LEISHMANIA | JW 11/12 | +           | +           | RE  |          |
| Z000163 E,            | -         | BL 30-DEC-92 LM            | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +           | +           | RE  | +REP     |
| Z000164 F,            | -         | BL 30-DEC-92 LM            | LM    | 90009 06-JAN-93 LEISHMANIA | JW 11/12 | -           | -           | NR  |          |
| Z000164 F,            | -         | BL 30-DEC-92 LM            | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +           | +           | RE  | +REP     |
| Z000165 H,            | -         | BL 30-DEC-92 LM            | LM    | 90009 06-JAN-93 LEISHMANIA | JW 11/12 | +           | +           | RE  |          |
| Z000165 H,            | -         | BL 30-DEC-92 LM            | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +           | +           | RE  | +REP     |
| Z000166 J,            | -         | BL 30-DEC-92 LM            | LM    | 90009 06-JAN-93 LEISHMANIA | JW 11/12 | +           | +           | RE  |          |
| Z000166 J,            | -         | BL 30-DEC-92 LM            | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +           | +           | RE  | +REP     |
| Z000167 MARY,         | -         | BL 11-JAN-93 LM            | LM    | 90009 11-JAN-93 LEISHMANIA | JW 11/12 | +           | +           | RE  |          |
| Z000167 MARY,         | -         | BL 11-JAN-93 LM            | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +           | +           | RE  | +REP     |
| Z000168 BABLU HEUL,   | -         | BL 11-JAN-93 LM            | LM    | 90009 11-JAN-93 LEISHMANIA | JW 11/12 | +           | +           | RE  |          |
| Z000168 BABLU HEUL,   | -         | BL 11-JAN-93 LM            | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +           | +           | RE  | +REP     |
| Z000169 LAUZSHMI,     | -         | BL 11-JAN-93 LM            | LM    | 90009 11-JAN-93 LEISHMANIA | JW 11/12 | +           | +           | RE  |          |
| Z000169 LAUZSHMI,     | -         | BL 11-JAN-93 LM            | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +           | +           | RE  | +REP     |
| Z000170 KOMESHCOAR,   | -         | BL 11-JAN-93 LM            | LM    | 90009 11-JAN-93 LEISHMANIA | JW 11/12 | +           | +           | RE  |          |
| Z000170 KOMESHCOAR,   | -         | BL 11-JAN-93 LM            | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +           | -           | IND | +REP     |

PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name    | FPC + SSN | Spec Received<br>Type Date | Study | Panel Assay Date Virus     | Primer   | Tube<br># 1 | Tube<br># 2 | Int<br>Comments |
|-------------------------|-----------|----------------------------|-------|----------------------------|----------|-------------|-------------|-----------------|
| Z000171 BIMALDEY,       | -         | BL 11-JAN-93               | LM    | 90009 11-JAN-93 LEISHMANIA | JW 11/12 | +           | +           | RE              |
| Z000171 BIMALDEY,       | -         | BL 11-JAN-93               | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +           | +           | RE +REP         |
| Z000172 JAINAI,         | -         | BL 11-JAN-93               | LM    | 90009 11-JAN-93 LEISHMANIA | JW 11/12 | +           | +           | RE              |
| Z000172 JAINAI,         | -         | BL 11-JAN-93               | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +           | -           | IND +REP        |
| Z000173 ALOR-RAYAR,     | -         | BL 11-JAN-93               | LM    | 90009 11-JAN-93 LEISHMANIA | JW 11/12 | +/-         | +/-         | IND             |
| Z000173 ALOR-RAYAR,     | -         | BL 11-JAN-93               | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | -           | -           | NR +REP         |
| Z000174 ARMIN-KUMANE,   | -         | BL 11-JAN-93               | LM    | 90009 11-JAN-93 LEISHMANIA | JW 11/12 | +           | +           | RE              |
| Z000174 ARMIN-KUMANE,   | -         | BL 11-JAN-93               | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +           | +           | RE +REP         |
| Z000175 AMIL KUMAR,     | -         | BL 11-JAN-93               | LM    | 90009 11-JAN-93 LEISHMANIA | JW 11/12 | -           | -           | NR              |
| Z000176 HASINER-KHAFIN, | -         | BL 11-JAN-93               | LM    | 90009 11-JAN-93 LEISHMANIA | JW 11/12 | +           | +           | RE              |
| Z000176 HASINER-KHAFIN, | -         | BL 11-JAN-93               | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +           | -           | IND +REP        |
| Z000177 SUMAY KUMAR,    | -         | BL 11-JAN-93               | LM    | 90009 11-JAN-93 LEISHMANIA | JW 11/12 | -           | -           | NR              |
| Z000178 RULI-KUMAR,     | -         | BL 11-JAN-93               | LM    | 90009 11-JAN-93 LEISHMANIA | JW 11/12 | +           | +           | RE              |
| Z000178 RULI-KUMAR,     | -         | BL 11-JAN-93               | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +           | +           | RE +REP         |
| Z000257 30491,          | -         | BL 18-FEB-93               | LM    | 90009 19-FEB-93 LEISHMANIA | JW 11/12 | +           | +           | RE              |
| Z000257 30491,          | -         | BL 18-FEB-93               | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +           | +           | RE +REP         |
| Z000258 30492,          | -         | BL 18-FEB-93               | LM    | 90009 19-FEB-93 LEISHMANIA | JW 11/12 | +           | +           | RE              |
| Z000258 30492,          | -         | BL 18-FEB-93               | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +           | +           | RE +REP         |
| Z000259 30493,          | -         | BL 19-FEB-93               | LM    | 90009 19-FEB-93 LEISHMANIA | JW 11/12 | +           | +           | RE              |
| Z000259 30493,          | -         | BL 19-FEB-93               | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +           | +           | RE +REP         |
| Z000260 30494,          | -         | BL 18-FEB-93               | LM    | 90009 19-FEB-93 LEISHMANIA | JW 11/12 | +           | +           | RE              |
| Z000260 30494,          | -         | BL 18-FEB-93               | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +           | +           | RE +REP         |
| Z000261 30495,          | -         | BL 18-FEB-93               | LM    | 90009 19-FEB-93 LEISHMANIA | JW 11/12 | -           | -           | NR              |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name    | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer              | Tube # | Tube #2 | Int | Comments |
|---------|-----------------|----------------|----------------------------|-------|------------------|-----------|---------------------|--------|---------|-----|----------|
| Z000262 | 30496,          | -              | BL 18-FEB-93               | LM    | 90009            | 19-FEB-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| Z000263 | 30497,          | -              | BL 18-FEB-93               | LM    | 90009            | 19-FEB-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| Z000264 | 30481,          | -              | BL 18-FEB-93               | LM    | 90009            | 19-FEB-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| Z000308 | 30691A,         | -              | BL 11-MAR-93               | LM    | 90009            | 15-MAR-93 | LEISHMANIA JW 11/12 | +      | +       | RE  |          |
| Z000308 | 30691A,         | -              | BL 11-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +      | +       | RE  | +REP     |
| Z000309 | 30692A,         | -              | BL 11-MAR-93               | LM    | 90009            | 15-MAR-93 | LEISHMANIA JW 11/12 | +      | +       | RE  |          |
| Z000309 | 30692A,         | -              | BL 11-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +      | +       | RE  | +REP     |
| Z000310 | 30693A,         | -              | BL 11-MAR-93               | LM    | 90009            | 15-MAR-93 | LEISHMANIA JW 11/12 | +      | +       | RE  |          |
| Z000310 | 30693A,         | -              | BL 11-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +      | -       | IND | +REP     |
| Z000311 | 30694A,         | -              | BL 11-MAR-93               | LM    | 90009            | 15-MAR-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| Z000312 | 30695A,         | -              | BL 11-MAR-93               | LM    | 90009            | 15-MAR-93 | LEISHMANIA JW 11/12 | +      | +       | RE  |          |
| Z000312 | 30695A,         | -              | BL 11-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +      | +       | RE  | +REP     |
| Z000313 | WILT, TIMOTHY L | 03-205-21-9045 | BL 12-MAR-93               | LM    | 90009            | 15-MAR-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| Z000320 | PT. PLC,        | -              | BL 23-MAR-93               | LM    | 90009            | 25-MAR-93 | LEISHMANIA JW 11/12 | +      | +       | RE  |          |
| Z000320 | PT. PLC,        | -              | BL 23-MAR-93               | LM    | 90009            | 05-APR-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| Z000320 | PT. PLC,        | -              | BL 23-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | -      | +/-     | IND | +REP     |
| Z000321 | PT. C.A.N.,     | -              | BL 23-MAR-93               | LM    | 90009            | 05-APR-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| Z000321 | PT. C.A.N.,     | -              | BL 23-MAR-93               | LM    | 90009            | 25-MAR-93 | LEISHMANIA JW 11/12 | -      | -       | NR  |          |
| Z000336 | 1,              | -              | BL 26-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +      | +       | RE  | +REP     |
| Z000339 | 4,              | -              | BL 26-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +      | +       | RE  | +REP     |
| Z000340 | 5,              | -              | BL 26-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | -      | -       | NR  | +REP     |
| Z000342 | 7,              | -              | BL 26-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +      | +       | RE  | +REP     |
| Z000343 | 8,              | -              | BL 26-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +      | +       | RE  | +REP     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name      | FPC + SSN | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer              | Tube # 1 | Tube # 2 | Int | Comments   |
|---------------------------|-----------|----------------------------|-------|------------------|-----------|---------------------|----------|----------|-----|------------|
| Z000344 9,                | -         | BL 26-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +        | +        | RE  | +REP       |
| Z000345 10,               | -         | BL 26-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +        | +        | RE  | +REP, -CON |
| Z000346 11,               | -         | BL 26-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +        | +        | RE  | +REP       |
| Z000347 12,               | -         | BL 26-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +        | +        | RE  | +REP       |
| Z000349 14,               | -         | BL 26-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +        | +        | RE  | +REP       |
| Z000351 16,               | -         | BL 26-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +        | +        | RE  | +REP       |
| Z000352 17,               | -         | BL 26-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +        | +        | RE  | +REP       |
| Z000353 20,               | -         | BL 26-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +        | +        | RE  | +REP       |
| Z000354 21,               | -         | BL 26-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +        | +        | RE  | +REP       |
| Z000355 22,               | -         | BL 26-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +        | +        | RE  | +REP       |
| Z000356 23,               | -         | BL 26-MAR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | +        | +        | RE  | +REP       |
| Z000367 30911,            | -         | BL 01-APR-93               | LM    | 90009            | 05-APR-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000368 30912,            | -         | BL 01-APR-93               | LM    | 90009            | 05-APR-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000369 30913,            | -         | BL 01-APR-93               | LM    | 90009            | 05-APR-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000370 30914,            | -         | BL 01-APR-93               | LM    | 90009            | 05-APR-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000379 31022,            | -         | BL 12-APR-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | +REP       |
| Z000413 GABORONE, JB      | -         | BL 23-APR-93               | LM    | 90009            | 27-APR-93 | LEISHMANIA JW 11/12 | -        | -        | IND |            |
| Z000414 JASON, DAVID      | -         | BL 23-APR-93               | LM    | 90009            | 27-APR-93 | LEISHMANIA JW 11/12 | -        | -        | IND |            |
| Z000460 RUBENSTEIN, GRAIG | -         | BM 21-MAY-93               | LM    | 90009            | 01-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000460 RUBENSTEIN, GRAIG | -         | BM 21-MAY-93               | LM    | 90009            | 25-MAY-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000461 RUBENSTEIN, GRAIG | -         | BL 21-MAY-93               | LM    | 90009            | 01-JUL-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000461 RUBENSTEIN, GRAIG | -         | BL 21-MAY-93               | LM    | 90009            | 25-MAY-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000473 J40,              | -         | BL 26-MAY-93               | LM    | 90009            | 27-MAY-93 | LEISHMANIA JW 11/12 | +        | +        | RE  |            |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name      | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer              | Tube # | Tube Int | Comments |
|---------------------------|----------------|----------------------------|-------|------------------|-----------|---------------------|--------|----------|----------|
| Z000473 J40,              | -              | BL 26-MAY-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | -      | -        | NR +REP  |
| Z000474 PBS AIL,          | -              | BL 26-MAY-93               | LM    | 90009            | 27-MAY-93 | LEISHMANIA JW 11/12 | +      | +        | RE       |
| Z000474 PBS AIL,          | -              | BL 26-MAY-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | -      | -        | NR +REP  |
| Z000475 J45,              | -              | BL 26-MAY-93               | LM    | 90009            | 27-MAY-93 | LEISHMANIA JW 11/12 | +      | +        | RE       |
| Z000475 J45,              | -              | BL 26-MAY-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | -      | -        | NR +REP  |
| Z000476 J4N,              | -              | BL 26-MAY-93               | LM    | 90009            | 27-MAY-93 | LEISHMANIA JW 11/12 | +      | +        | RE       |
| Z000476 J4N,              | -              | BL 26-MAY-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | -      | -        | NR +REP  |
| Z000501 31601,            | -              | BL 09-JUN-93               | LM    | 90009            | 15-JUN-93 | LEISHMANIA JW 11/12 | -      | -        | NR       |
| Z000502 31601,            | -              | BM 09-JUN-93               | LM    | 90009            | 15-JUN-93 | LEISHMANIA JW 11/12 | -      | -        | NR       |
| Z000503 31621,            | -              | BL 11-JUN-93               | LM    | 90009            | 15-JUN-93 | LEISHMANIA JW 11/12 | +      | +        | RE       |
| Z000503 31621,            | -              | BL 11-JUN-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | -      | +/-      | IND +REP |
| Z000504 31622,            | -              | BL 14-JUN-93               | LM    | 90009            | 15-JUN-93 | LEISHMANIA JW 11/12 | +      | +        | RE       |
| Z000504 31622,            | -              | BL 14-JUN-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | -      | -        | NR +REP  |
| Z000505 31623,            | -              | BL 11-JUN-93               | LM    | 90009            | 15-JUN-93 | LEISHMANIA JW 11/12 | +      | +        | RE       |
| Z000505 31623,            | -              | BL 11-JUN-93               | LM    | 90009            | 29-JUL-93 | LEISHMANIA JW 11/12 | -      | -        | NR +REP  |
| Z000506 31624,            | -              | BL 11-JUN-93               | LM    | 90009            | 15-JUN-93 | LEISHMANIA JW 11/12 | +      | +        | RE       |
| Z000507 CHIPLEY, JOSHUA W | 20-564-33-6887 | TE 11-JUN-93               | LM    | 90009            | 15-JUN-93 | LEISHMANIA JW 11/12 | -      | -        | NR       |
| Z000508 CHIPLEY, JOSHUA W | 20-564-33-6887 | TE 11-JUN-93               | LM    | 90009            | 15-JUN-93 | LEISHMANIA JW 11/12 | -      | -        | NR       |
| Z000511 WILLIAMS, MARQUET | -              | PL 24-JUN-93               | LM    | 90009            | 07-JUL-93 | LEISHMANIA JW 11/12 | -      | -        | NR       |
| Z000517 31881,            | -              | BL 07-JUL-93               | LM    | 90009            | 09-JUL-93 | LEISHMANIA JW 11/12 | -      | -        | NR       |
| Z000518 31882,            | -              | BL 07-JUL-93               | LM    | 90009            | 09-JUL-93 | LEISHMANIA JW 11/12 | -      | -        | NR       |
| Z000519 31883,            | -              | BL 07-JUL-93               | LM    | 90009            | 09-JUL-93 | LEISHMANIA JW 11/12 | +      | +        | RE       |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name       | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date Virus     | Primer   | Tube Tube<br># 1 # 2 |   | Int | Comments |
|----------------------------|----------------|----------------------------|-------|----------------------------|----------|----------------------|---|-----|----------|
| Z000520 31884,             | -              | BL 07-JUL-93               | LM    | 90009 09-JUL-93 LEISHMANIA | JW 11/12 | +                    | + | RE  |          |
| Z000541 31931,             | -              | BL 12-JUL-93               | LM    | 90009 16-JUL-93 LEISHMANIA | JW 11/12 | +                    | + | RE  |          |
| Z000541 31931,             | -              | BL 12-JUL-93               | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +                    | + | RE  |          |
| Z000542 31932,             | -              | BL 12-JUL-93               | LM    | 90009 16-JUL-93 LEISHMANIA | JW 11/12 | +                    | + | RE  |          |
| Z000542 31932,             | -              | BL 12-JUL-93               | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +                    | + | RE  |          |
| Z000543 31933,             | -              | BL 12-JUL-93               | LM    | 90009 16-JUL-93 LEISHMANIA | JW 11/12 | +                    | + | RE  |          |
| Z000543 31933,             | -              | BL 12-JUL-93               | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | +                    | + | RE  |          |
| Z000550 31971,             | -              | BL 16-JUL-93               | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | -                    | + | IND |          |
| Z000551 31972,             | -              | BL 16-JUL-93               | LM    | 90009 29-JUL-93 LEISHMANIA | JW 11/12 | -                    | - | NR  |          |
| 20345 HAMWACK, WENDELL     | 20-427-49-8157 | BM 27-JAN-93               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | +                    | - | IND | +REP     |
| 20344 HAMWACK, WENDELL     | 20-427-49-8157 | BL 27-JAN-93               | LM    | 90009 03-AUG-93 LEISHMANIA | JW 11/12 | -                    | - | NR  | +REP     |
| 23345 GHEBREMESCHEL, TADDE | 20-049-34-1541 | BM 27-MAY-93               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | +                    | - | IND | +REP     |
| Z000101 2A125-A,           | -              | BL 09-DEC-92               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | +                    | + | RE  | +REP     |
| Z000104 2A125-D,           | -              | BL 09-DEC-92               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | +                    | + | RE  | +REP     |
| Z000105 2A125-E,           | -              | BL 09-DEC-92               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | +                    | + | RE  | +REP     |
| Z000112 2A125-6,           | -              | BL 09-DEC-92               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | +                    | + | RE  | +REP     |
| Z000117 2A125-A,           | -              | BL 16-DEC-92               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | +                    | + | RE  | +REP     |
| Z000145 2A125-5,           | -              | BL 16-DEC-92               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | -                    | - | NR  | +REP     |
| Z000310 30693A,            | -              | BL 11-MAR-93               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | +                    | + | RE  | +REP     |
| Z000340 5,                 | -              | BL 26-MAR-93               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | -                    | - | NR  | +REP     |
| Z000379 31022,             | -              | BL 12-APR-93               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | +                    | + | RE  | +REP     |
| Z000439 1,                 | -              | BL 30-APR-93               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | -                    | - | NR  | +REP     |
| Z000440 2,                 | -              | BL 30-APR-93               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | -                    | + | IND | +REP     |

PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name       | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date Virus     | Primer   | Tube Tube<br># 1 # 2 |   | Int | Comments        |
|----------------------------|----------------|----------------------------|-------|----------------------------|----------|----------------------|---|-----|-----------------|
| Z000466 GRAVES, ERIC       | 20-003-60-3564 | LI 25-MAY-93               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | +                    | + | RE  | +REP            |
| Z000473 J40,               | -              | BL 26-MAY-93               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | -                    | + | IND | +REP            |
| Z000474 PBS AIL,           | -              | BL 26-MAY-93               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | +                    | + | RE  | +REP            |
| Z000475 J45,               | -              | BL 26-MAY-93               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | +                    | - | IND | +REP            |
| Z000476 J4N,               | -              | BL 26-MAY-93               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | +                    | + | RE  | +REP            |
| Z000503 31621,             | -              | BL 11-JUN-93               | LM    | 90009 04-AUG-93 LEISHMANIA | JW 11/12 | +                    | + | RE  | +REP            |
| 20345 HAMMACK, WENDELL     | 20-427-49-8157 | BM 27-JAN-93               | LM    | 90009 03-AUG-93 LEISHMANIA | JW 11/12 | -                    | - | NR  | +REP, NEG. CONT |
| 21552 SCHONEBOOM, BRAD L   | 20-367-76-1421 | BM 15-MAR-93               | LM    | 90009 03-AUG-93 LEISHMANIA | JW 11/12 | -                    | - | NR  | +REP            |
| 22745 WADDELL, DIRK        | 20-343-56-3223 | BL 04-MAY-93               | LM    | 90009 03-AUG-93 LEISHMANIA | JW 11/12 | -                    | - | NR  | +REP            |
| 23046 WADDELL, DIRK        | 20-343-56-3223 | BL 17-MAY-93               | LM    | 90009 03-AUG-93 LEISHMANIA | JW 11/12 | -                    | - | NR  | +REP            |
| 23250 CHIPLEY, JOSHUA W    | 20-564-33-6887 | BL 21-MAY-93               | LM    | 90009 03-AUG-93 LEISHMANIA | JW 11/12 | -                    | + | IND | +REP            |
| 23345 GHEBREMESCHEL, TADDE | 20-049-34-1541 | BM 27-MAY-93               | LM    | 90009 03-AUG-93 LEISHMANIA | JW 11/12 | -                    | - | NR  | +REP            |
| Z000101 2A125-A,           | -              | BL 09-DEC-92               | LM    | 90009 03-AUG-93 LEISHMANIA | JW 11/12 | -                    | - | NR  | +REP            |
| Z000104 2A125-D,           | -              | BL 09-DEC-92               | LM    | 90009 03-AUG-93 LEISHMANIA | JW 11/12 | -                    | - | NR  | +REP            |
| Z000105 2A125-E,           | -              | BL 09-DEC-92               | LM    | 90009 03-AUG-93 LEISHMANIA | JW 11/12 | -                    | + | IND | +REP            |
| Z000106 2A125-F,           | -              | BL 09-DEC-92               | LM    | 90009 03-AUG-93 LEISHMANIA | JW 11/12 | +                    | + | RE  | +REP            |
| Z000107 2A125-1,           | -              | BL 09-DEC-92               | LM    | 90009 03-AUG-93 LEISHMANIA | JW 11/12 | +                    | + | RE  | +REP            |
| Z000110 2A125-4,           | -              | BL 09-DEC-92               | LM    | 90009 03-AUG-93 LEISHMANIA | JW 11/12 | +                    | + | RE  | +REP            |
| Z000111 2A125-5,           | -              | BL 09-DEC-92               | LM    | 90009 03-AUG-93 LEISHMANIA | JW 11/12 | +                    | + | RE  | +REP            |
| Z000112 2A125-6,           | -              | BL 09-DEC-92               | LM    | 90009 03-AUG-93 LEISHMANIA | JW 11/12 | +                    | + | RE  | +REP            |
| Z000117 2A125-A,           | -              | BL 16-DEC-92               | LM    | 90009 03-AUG-93 LEISHMANIA | JW 11/12 | -                    | - | NR  | +REP            |
| Z000118 2A125-B,           | -              | BL 16-DEC-92               | LM    | 90009 03-AUG-93 LEISHMANIA | JW 11/12 | -                    | - | NR  | +REP            |
| Z000120 2A125-D,           | -              | BL 16-DEC-92               | LM    | 90009 03-AUG-93 LEISHMANIA | JW 11/12 | +                    | + | RE  | +REP            |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name    | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer     | Tube # 1 | Tube # 2 | Int | Comments      |
|-------------------------|----------------|----------------------------|-------|------------------|-----------|------------|----------|----------|-----|---------------|
| Z000144 2A125-4,        | -              | BL 16-DEC-92               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | +        | +   | RE +REP       |
| Z000145 2A125-5,        | -              | BL 16-DEC-92               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | -        | -   | NR +REP       |
| Z000146 2A125-6,        | -              | BL 16-DEC-92               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | -        | -   | NR +REO       |
| Z000440 2,              | -              | BL 30-APR-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | -        | -   | NR +REP       |
| Z000350 15,             | -              | BL 26-MAR-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | +        | +   | RE +REP       |
| Z000439 1,              | -              | BL 30-APR-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | -        | -   | NR +REP       |
| Z000466 GRAVES, ERIC    | 20-003-60-3564 | LI 25-MAY-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | -        | -   | NR +REP       |
| 22955 DAREY, GREGORY S  | 20-505-06-2923 | BL 12-MAY-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | +        | +   | RE +REP       |
| 23328 J4,               | -              | BL 26-MAY-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | +        | +   | RE +REP       |
| 23328 J4,               | -              | BL 26-MAY-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | +        | +   | RE +REP       |
| Z000170 KOMESHCOAR,     | -              | BL 11-JAN-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | +        | +   | RE +REP,CONT  |
| Z000172 JAINAI,         | -              | BL 11-JAN-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | +        | +   | RE +REP       |
| Z000173 ALOR-RAVAR,     | -              | BL 11-JAN-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | -        | -   | NR +REP       |
| Z000176 HASINER-KHAFIN, | -              | BL 11-JAN-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | +        | +   | RE +REP       |
| Z000310 30693A,         | -              | BL 11-MAR-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | +        | +   | RE +REP,CONT  |
| Z000320 PT. PLC,        | -              | BL 23-MAR-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | +        | +   | RE +REP       |
| Z000379 31022,          | -              | BL 12-APR-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | -        | -   | NR +REP       |
| Z000473 J40,            | -              | BL 26-MAY-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | -        | -   | NR +REP       |
| Z000474 PBS AIL,        | -              | BL 26-MAY-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | -        | +   | IND +REP,CONT |
| Z000475 J45,            | -              | BL 26-MAY-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | +        | -   | IND +REP      |
| Z000476 J4N,            | -              | BL 26-MAY-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | +        | -   | IND +REP      |
| Z000503 31621,          | -              | BL 11-JUN-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | -        | +   | IND +REP      |
| Z000504 31622,          | -              | BL 14-JUN-93               | LM    | 90009            | 03-AUG-93 | LEISHMANIA | JW 11/12 | +        | +   | RE +REP       |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name         | FPC + SSN      | Spec Received<br>Type Date | Study | Panel | Assay Date | Virus      | Primer   | Tube # 1 | Tube # 2 | Int | Comments    |
|------------------------------|----------------|----------------------------|-------|-------|------------|------------|----------|----------|----------|-----|-------------|
| Z000505 31623,               | -              | BL 11-JUN-93               | LM    | 90009 | 03-AUG-93  | LEISHMANIA | JW 11/12 | +        | +        | RE  | +REP        |
| Z000170 KOMESHCOAR,          | -              | BL 11-JAN-93               | LM    | 90009 | 03-AUG-93  | LEISHMANIA | JW 11/12 | +        | -        | IND | REP.PL.     |
| Z000170 KOMESHCOAR,          | -              | BL 11-JAN-93               | LM    | 90009 | 03-AUG-93  | LEISHMANIA | JW 11/12 | +        | -        | IND | REP.PL.     |
| Z000310 30693A,              | -              | BL 11-MAR-93               | LM    | 90009 | 03-AUG-93  | LEISHMANIA | JW 11/12 | +        | -        | IND | REP.PL.     |
| Z000310 30693A,              | -              | BL 11-MAR-93               | LM    | 90009 | 03-AUG-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  | REP.PL      |
| Z000503 31621,               | -              | BL 11-JUN-93               | LM    | 90009 | 03-AUG-93  | LEISHMANIA | JW 11/12 | +        | +        | RE  | REP.PL.     |
| Z000504 31622,               | -              | BL 14-JUN-93               | LM    | 90009 | 03-AUG-93  | LEISHMANIA | JW 11/12 | +        | -        | IND | REP.PL.     |
| 23250 CHIPLEY, JOSHUA W      | 20-564-33-6887 | BL 21-MAY-93               | LM    | 90009 | 03-AUG-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  | REP.PL.,DIL |
| Z000105 2A125-E,             | -              | BL 09-DEC-92               | LM    | 90009 | 03-AUG-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  | REP.PL      |
| 24930 32211,                 | -              | BL 09-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |             |
| 24931 32212,                 | -              | BL 09-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |             |
| 24932 32213,                 | -              | BL 09-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |             |
| Z000550 31971,               | -              | BL 16-JUL-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |             |
| Z000591 LEH1663,             | -              | BL 05-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |             |
| Z000592 LEH1664,             | -              | BL 05-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |             |
| Z000593 LEH1665,             | -              | BL 05-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |             |
| Z000594 LEH1666,             | -              | BL 05-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | -        | -        | NR  |             |
| Z000595 LEH1667,             | -              | BL 05-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | +        | +        | RE  |             |
| Z000596 LEH1668,             | -              | BL 05-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | +        | +        | RE  |             |
| Z000597 BLUSAU-MIZBSA, SHASH | -              | BL 10-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | +        | +        | RE  |             |
| Z000598 BLUSAU-MIZBSA, SHASH | -              | BL 10-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | +        | +        | RE  |             |
| Z000599 CPT-2,               | -              | BL 10-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | +        | +        | RE  |             |
| Z000600 KERRY, SUDHRI        | -              | BL 10-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | +        | +        | RE  |             |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name         | FPC + SSN | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer              | Tube Tube<br># 1 # 2 | Int | Comments |
|------------------------------|-----------|----------------------------|-------|------------------|-----------|---------------------|----------------------|-----|----------|
| Z000601 RAJUARA, SUTHER-KWAR | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | +                    | +   | RE       |
| Z000602 DEIS, GEETA          | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | +                    | +   | RE       |
| Z000603 DEIS, GEETA          | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | +                    | +   | RE       |
| Z000604 THAFCEP, SURON       | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | +                    | +   | RE       |
| Z000605 SHUYARRU, KERROZ     | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | +                    | +   | RE       |
| Z000606 REEJU, DAS           | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | +                    | +   | RE       |
| Z000607 SHAUMARTI, CHEWHY    | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000608 RAKESH, KUMAR        | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000609 MIRTHELESWAR, PRASAD | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000610 NARESH, PASWAN       | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000611 RAJESWAR, PD         | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000612 RUBY, KUMARI         | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000613 RAM, JINISH          | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000614 MANOJ, RAM           | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000615 RUBI, KUMARIS        | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000616 RAITHA, MAHAU        | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000617 GANESH, MALRBO       | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | +                    | +   | RE       |
| Z000618 SUMIL, KUMAR         | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | +                    | +   | RE       |
| Z000619 UMESH, SAH           | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | +                    | +   | RE       |
| Z000620 SARISI,              | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | +                    | +   | RE       |
| Z000625 RHARAUBIR,           | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | +                    | +   | RE       |
| Z000626 MANAJ, RAUU          | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000627 RAMJINEESH, PANDIT   | -         | BL 10-AUG-93               | LM    | 90009            | 16-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name         | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date Virus     | Primer   | Tube Tube<br># 1 # 2 | Int<br>Comments |
|------------------------------|----------------|----------------------------|-------|----------------------------|----------|----------------------|-----------------|
| Z000628 ANUJ, RANJAN         | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | -                    | NR              |
| Z000629 MITHILESHWAR, PD SIR | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +                    | RE              |
| Z000630 RAKESH, KUMAR        | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +                    | RE              |
| Z000631 NARESH, PASWAN       | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +                    | RE              |
| Z000632 GHAMANDI, CHAUDHARY  | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +                    | RE              |
| Z000633 RAJU, DAS            | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +                    | RE              |
| Z000634 SHYAM, SAH K         | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +                    | RE              |
| Z000635 RAMAKANKA, RAI       | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +                    | RE              |
| Z000636 SITAWAR, DEVI        | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | -                    | NR              |
| Z000637 SAMOD, KUMAR         | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | -                    | NR              |
| Z000638 RAJESH, KUMAR        | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | -                    | NR              |
| Z000639 CHAUDSA, CHEVZ       | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +                    | RE              |
| Z000640 RAYETH, LAMB         | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | -                    | NR              |
| Z000641 SUJAFEI, LAMB        | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +                    | RE              |
| Z000642 SAMUR, KUNG          | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | -                    | NR              |
| Z000643 AREY, RAYAN          | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | -                    | NR              |
| Z000644 DHARAURLIY,          | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +                    | RE              |
| Z000645 VMESH, SCH           | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +                    | RE              |
| Z000646 SARITA, KUMARI       | -              | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +                    | RE              |
| 23084 VEGA, EDUARDO A        | 20-584-54-8581 | BL 18-MAY-93               | RV2   | 90009 20-MAY-93 LEISHMANIA | JW 11/12 | -                    | NR              |
| 23085 GRAVES, ERIC           | 20-003-60-3564 | BM 18-MAY-93               | LM    | 90009 20-MAY-93 LEISHMANIA | JW 11/12 | -                    | NR              |
| 22977 274D,                  | -              | BL 12-MAY-93               | LM    | 90009 20-MAY-93 LEISHMANIA | JW 11/12 | -                    | NR              |
| 23012 WADDELL, DIRK          | 20-343-56-3223 | BL 14-MAY-93               | LM    | 90009 20-MAY-93 LEISHMANIA | JW 11/12 | -                    | +/- IND         |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name       | FPC + SSN      | Spec Received<br>Type Date | Study | Panel | Assay Date | Virus      | Primer   | Tube Tube |     | Int | Comments |
|---------|--------------------|----------------|----------------------------|-------|-------|------------|------------|----------|-----------|-----|-----|----------|
|         |                    |                |                            |       |       |            |            |          | # 1       | # 2 |     |          |
| 23046   | WADDELL, DIRK      | 20-343-56-3223 | BL 17-MAY-93               | LM    | 90009 | 20-MAY-93  | LEISHMANIA | JW 11/12 | +         | +   | RE  |          |
| Z000647 | RUSHA, MOHAN SINGH | -              | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -         | -   | NR  | -REP     |
| 22970   | A346,              | -              | BL 12-MAY-93               | LM    | 90009 | 20-MAY-93  | LEISHMANIA | JW 11/12 | +         | -   | IND |          |
| Z000650 | RAMESH, KW         | -              | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | +         | -   | IND | -REP     |
| Z000651 | RAMAKAWT, RAI      | -              | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -         | -   | NR  | -REP     |
| Z000652 | BUDHAN, MAHTO      | -              | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | +         | +   | RE  | -REP     |
| Z000653 | SILAWAR, DEVI      | -              | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -         | -   | NR  | -REP     |
| Z000654 | SUDHIR, KUMAR      | -              | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | +         | +   | RE  | -REP     |
| Z000655 | SARRAULLAH,        | -              | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -         | -   | NR  | -REP     |
| Z000657 | MO, SONAWAE        | -              | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -         | -   | NR  | -REP     |
| Z000658 | SWUGH, THAKUR      | -              | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | +         | +   | RE  | -REP     |
| Z000659 | 32251,             | -              | BL 13-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -         | -   | NR  | -REP     |
| Z000660 | 32252,             | -              | BM 13-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -         | -   | NR  | -REP     |
| Z000668 | 32253,             | -              | BL 13-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -         | -   | NR  | -REP     |
| Z000669 | 32254,             | -              | BL 13-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -         | +   | IND | -REP     |
| 24930   | 32211,             | -              | BL 09-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | +/        | -   | IND | -REP     |
| 24931   | 32212,             | -              | BL 09-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -         | -   | NR  | -REP     |
| 24932   | 32213,             | -              | BL 09-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -         | -   | NR  | -REP     |
| Z000591 | LEH1663,           | -              | BL 05-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | +         | -   | IND | -REP     |
| Z000592 | LEH1664,           | -              | BL 05-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -         | -   | NR  | -REP     |
| Z000593 | LEH1665,           | -              | BL 05-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -         | -   | NR  | -REP     |
| Z000594 | LEH1666,           | -              | BL 05-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -         | -   | NR  | -REP     |
| Z000607 | SHAUMARTI, CHEWHY  | -              | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | +         | +   | RE  | -REP     |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name         | FPC + SSN | Spec Received<br>Type Date | Study | Panel | Assay Date | Virus      | Primer   | Tube # | Tube | Int | Comments |
|---------|----------------------|-----------|----------------------------|-------|-------|------------|------------|----------|--------|------|-----|----------|
| Z000608 | RAKESH, KUMAR        | -         | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | +      | +    | RE  | -REP     |
| Z000609 | MIRTHELESWAR, PRASAD | -         | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | +      | +    | RE  | -REP     |
| Z000610 | NARESH, PASWAN       | -         | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | +      | +    | RE  | -REP     |
| Z000611 | RAJESWAR, PD         | -         | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  | -REP     |
| Z000612 | RUBY, KUMARI         | -         | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  | -REP     |
| Z000613 | RAM, JINISH          | -         | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  | -REP     |
| Z000614 | MANOJ, RAM           | -         | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  | -REP     |
| Z000615 | RUBI, KUMARIS        | -         | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  | -REP     |
| Z000616 | RAITHA, MAHAU        | -         | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  | -REP     |
| Z000626 | MANAJ, RAU           | -         | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  | -REP     |
| Z000627 | RAMJINEESH, PANDIT   | -         | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  | -REP     |
| Z000638 | RAJESH, KUMAR        | -         | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  | -REP     |
| Z000640 | RAYETH, LAMB         | -         | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  | -REP     |
| Z000642 | SAMUR, KUNG          | -         | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  | -REP     |
| Z000643 | AREY, RAYAN          | -         | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  | -REP     |
| 24930   | 32211,               | -         | BL 09-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  |          |
| 24931   | 32212,               | -         | BL 09-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  |          |
| 24932   | 32213,               | -         | BL 09-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  |          |
| Z000550 | 31971,               | -         | BL 16-JUL-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  |          |
| Z000591 | LEH1663,             | -         | BL 05-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  |          |
| Z000592 | LEH1664,             | -         | BL 05-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  |          |
| Z000593 | LEH1665,             | -         | BL 05-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  |          |
| Z000594 | LEH1666,             | -         | BL 05-AUG-93               | LM    | 90009 | 16-AUG-93  | LEISHMANIA | JW 11/12 | -      | -    | NR  |          |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name         | PFC + SSN | Spec Received<br>Type Date | Study | Panel Assay Date Virus     | Primer   | Tube # 1 | Tube # 2 | Int | Comments |
|------------------------------|-----------|----------------------------|-------|----------------------------|----------|----------|----------|-----|----------|
| Z000595 LEH1667,             | -         | BL 05-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +        | +        | RE  |          |
| Z000596 LEH1668,             | -         | BL 05-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +        | +        | RE  |          |
| Z000597 BLUSAU-MIZBSA, SHASH | -         | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +        | +        | RE  |          |
| Z000598 BLUSAU-MIZBSA, SHASH | -         | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +        | +        | RE  |          |
| Z000600 KERRY, SUDHRI        | -         | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +        | +        | RE  |          |
| Z000601 RAJUARA, SUTHER-KWAR | -         | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +        | +        | RE  |          |
| Z000602 DEIS, GEETA          | -         | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +        | +        | RE  |          |
| Z000603 DEIS, GEETA          | -         | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +        | +        | RE  |          |
| Z000604 THAFER, SURON        | -         | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +        | +        | RE  |          |
| Z000605 SHUYARU, KERROZ      | -         | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +        | +        | RE  |          |
| Z000606 REEJU, DAS           | -         | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | +        | +        | RE  |          |
| Z000607 SHAUMARTI, CHEWHY    | -         | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | -        | -        | NR  |          |
| Z000608 RAKESH, KUMAR        | -         | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | -        | -        | NR  |          |
| Z000609 MIRTHELESWAR, PRASAD | -         | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | -        | -        | NR  |          |
| Z000610 NARESH, PASWAN       | -         | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | -        | -        | NR  |          |
| Z000611 RAJESWAR, PD         | -         | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | -        | -        | NR  |          |
| Z000612 RUBY, KUMARI         | -         | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | -        | -        | NR  |          |
| Z000613 RAM, JINISH          | -         | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | -        | -        | NR  |          |
| Z000614 MANOJ, RAM           | -         | BL 10-AUG-93               | LM    | 90009 16-AUG-93 LEISHMANIA | JW 11/12 | -        | -        | NR  |          |
| Z000647 RUSHA, MOHAN SINGH   | -         | BL 10-AUG-93               | LM    | 90009 23-AUG-93 LEISHMANIA | JW 11/12 | -        | -        | NR  |          |
| Z000648 SUNIL, KUMAR         | -         | BL 10-AUG-93               | LM    | 90009 23-AUG-93 LEISHMANIA | JW 11/12 | +        | +        | RE  |          |
| Z000649 AMRENDON, KUMA       | -         | BL 10-AUG-93               | LM    | 90009 23-AUG-93 LEISHMANIA | JW 11/12 | +        | +        | RE  |          |
| Z000650 RAMESH, KW           | -         | BL 10-AUG-93               | LM    | 90009 23-AUG-93 LEISHMANIA | JW 11/12 | +        | -        | IND |          |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name       | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer              | Tube Tube<br># 1 # 2 | Int | Comments |
|----------------------------|----------------|----------------------------|-------|------------------|-----------|---------------------|----------------------|-----|----------|
| Z000651 RAWAKAWT, RAI      | -              | BL 10-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000652 BUDHAN, MAHTO      | -              | BL 10-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000653 SILAWAR, DEVI      | -              | BL 10-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000654 SUDHIR, KUMAR      | -              | BL 10-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000655 SARRAULLAH,        | -              | BL 10-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000656 YANESH, MAHITA     | -              | BL 10-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | +                    | +   | RE       |
| Z000657 MO, SONAWAE        | -              | BL 10-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000658 SWUGH, THAKUR      | -              | BL 10-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | +   | IND      |
| Z000659 32251,             | -              | BL 13-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000660 32252,             | -              | BM 13-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | +                    | -   | IND      |
| Z000668 32253,             | -              | BL 13-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000669 32254,             | -              | BL 13-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000673 WADDELL, DIRK      | 20-343-56-3223 | BL 17-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000674 WADDELL, DIRK      | 20-343-56-3223 | BM 17-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| Z000675 WADDELL, DIRK      | 20-343-56-3223 | LI 18-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| 25229 LEISHMANIA, 19-AUG-9 | -              | BL 19-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| 25230 LEISHMANIA, 19-AUG-9 | -              | BL 19-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| 25231 LEISHMANIA, 19-AUG-9 | -              | BL 19-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| 25232 LEISHMANIA, 19-AUG-9 | -              | BL 19-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| 25233 LEISHMANIA, 19-AUG-9 | -              | BL 19-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| 25234 LEISHMANIA, 19-AUG-9 | -              | BL 19-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| 25235 LEISHMANIA, 19-AUG-9 | -              | BL 19-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |
| 25236 LEISHMANIA, 19-AUG-9 | -              | BL 19-AUG-93               | LM    | 90009            | 23-AUG-93 | LEISHMANIA JW 11/12 | -                    | -   | NR       |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name   | FPC + SSN      | Spec Received<br>Type Date | Study | Panel | Assay Date | Virus      | Primer   | Tube # | Tube #2 | Int | Comments    |
|---------|----------------|----------------|----------------------------|-------|-------|------------|------------|----------|--------|---------|-----|-------------|
| 2000643 | AREY, RAYAN    | -              | BL 10-AUG-93               | LM    | 90009 | 24-AUG-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  | -REP        |
| 25286   | WADDELL, DIRK  | 20-343-56-3223 | BL 24-AUG-93               | LM    | 90009 | 01-SEP-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  | SPLEEN ASP. |
| 25391   | LYON, JOAN     | -234-88-3551   | BL 30-AUG-93               | LM    | 90009 | 08-SEP-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  |             |
| 25474   | LAKNER, GEORGE | -156-59-8935   | BL 03-SEP-93               | LM    | 90009 | 08-SEP-93  | LEISHMANIA | JW 11/12 | -      | +       | IND | IND         |
| 25475   | LAKNER, GEORGE | -156-59-8935   | BM 03-SEP-93               | LM    | 90009 | 08-SEP-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  |             |
| 25609   | 53F,           | -              | BL 08-SEP-93               | LM    | 90009 | 14-SEP-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  |             |
| 25610   | 54F,           | -              | BL 08-SEP-93               | LM    | 90009 | 14-SEP-93  | LEISHMANIA | JW 11/12 | -      | +       | IND |             |
| 25611   | 65F,           | -              | BL 08-SEP-93               | LM    | 90009 | 14-SEP-93  | LEISHMANIA | JW 11/12 | +      | +       | RE  | NEG. CONT.  |
| 25612   | 67F,           | -              | BL 08-SEP-93               | LM    | 90009 | 14-SEP-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  |             |
| 25613   | 70F,           | -              | BL 08-SEP-93               | LM    | 90009 | 14-SEP-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  |             |
| 25614   | 71F,           | -              | BL 08-SEP-93               | LM    | 90009 | 14-SEP-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  |             |
| 25615   | 72F,           | -              | BL 08-SEP-93               | LM    | 90009 | 14-SEP-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  |             |
| 25616   | 73/74F,        | -              | BL 08-SEP-93               | LM    | 90009 | 14-SEP-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  |             |
| 25617   | 77F,           | -              | BL 08-SEP-93               | LM    | 90009 | 14-SEP-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  |             |
| 25618   | 78F,           | -              | BL 08-SEP-93               | LM    | 90009 | 14-SEP-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  |             |
| 25619   | 80F,           | -              | BL 08-SEP-93               | LM    | 90009 | 14-SEP-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  |             |
| 25620   | 81F,           | -              | BL 08-SEP-93               | LM    | 90009 | 14-SEP-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  |             |
| 25646   | MYKUT, STEVEN  | -              | BL 09-SEP-93               | LM    | 90009 | 14-SEP-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  |             |
| 25647   | MYKUT, STEVEN  | -              | BM 09-SEP-93               | LM    | 90009 | 14-SEP-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  |             |
| 25474   | LAKNER, GEORGE | -156-59-8935   | BL 03-SEP-93               | LM    | 90009 | 14-SEP-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  |             |
| 25931   | MALLOY, VICTOR | 20-308-60-0996 | BL 23-SEP-93               | LM    | 90009 | 05-OCT-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  | REP. PLATE  |
| 25932   | MALLOY, VICTOR | 20-308-60-0996 | OT 23-SEP-93               | LM    | 90009 | 05-OCT-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  | REP. PLATE  |
| Z000703 | MALLOY, VICTOR | 20-308-60-0996 | OT 23-SEP-93               | LM    | 90009 | 05-OCT-93  | LEISHMANIA | JW 11/12 | -      | -       | NR  | REP. PLATE  |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name   | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer              | Tube # 1 | Tube # 2 | Int | Comments   |
|---------|----------------|----------------|----------------------------|-------|------------------|-----------|---------------------|----------|----------|-----|------------|
| 25976   | 53,            | -              | BL 27-SEP-93               | LM    | 90009            | 05-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | REP. PLATE |
| 25977   | 54,            | -              | BM 27-SEP-93               | LM    | 90009            | 05-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | REP. PLATE |
| 25978   | 65,            | -              | BM 27-SEP-93               | LM    | 90009            | 05-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | REP. PLATE |
| 25979   | 67,            | -              | BL 27-SEP-93               | LM    | 90009            | 05-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | REP. PLATE |
| 25980   | 70,            | -              | BL 27-SEP-93               | LM    | 90009            | 05-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | REP. PLATE |
| 25981   | 71,            | -              | BM 27-SEP-93               | LM    | 90009            | 05-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | REP. PLATE |
| 25982   | 72,            | -              | BM 27-SEP-93               | LM    | 90009            | 05-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | REP. PLATE |
| 25983   | 73/74,         | -              | BL 27-SEP-93               | LM    | 90009            | 05-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | REP. PLATE |
| 25984   | 77,            | -              | BM 27-SEP-93               | LM    | 90009            | 05-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | REP. PLATE |
| 25985   | 78,            | -              | BL 27-SEP-93               | LM    | 90009            | 05-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | REP. PLATE |
| 25986   | 80,            | -              | BL 27-SEP-93               | LM    | 90009            | 05-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | REP. PLATE |
| 25987   | 81,            | -              | BL 27-SEP-93               | LM    | 90009            | 05-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | REP. PLATE |
| 25931   | MALLOY, VICTOR | 20-308-60-0996 | BL 23-SEP-93               | LM    | 90009            | 30-SEP-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| 25932   | MALLOY, VICTOR | 20-308-60-0996 | OT 23-SEP-93               | LM    | 90009            | 30-SEP-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| 2000703 | MALLOY, VICTOR | 20-308-60-0996 | OT 23-SEP-93               | LM    | 90009            | 30-SEP-93 | LEISHMANIA JW 11/12 | +        | -        | IND |            |
| 25976   | 53,            | -              | BL 27-SEP-93               | LM    | 90009            | 30-SEP-93 | LEISHMANIA JW 11/12 | -        | +        | IND |            |
| 25977   | 54,            | -              | BM 27-SEP-93               | LM    | 90009            | 30-SEP-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| 25978   | 65,            | -              | BM 27-SEP-93               | LM    | 90009            | 30-SEP-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| 25979   | 67,            | -              | BL 27-SEP-93               | LM    | 90009            | 30-SEP-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| 25980   | 70,            | -              | BL 27-SEP-93               | LM    | 90009            | 30-SEP-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| 25981   | 71,            | -              | BM 27-SEP-93               | LM    | 90009            | 30-SEP-93 | LEISHMANIA JW 11/12 | -        | +        | IND |            |
| 25982   | 72,            | -              | BM 27-SEP-93               | LM    | 90009            | 30-SEP-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| 25983   | 73/74,         | -              | BL 27-SEP-93               | LM    | 90009            | 30-SEP-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name   | FPC + SSN    | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer              | Tube Tube<br># 1 # 2 |   | Int | Comments   |
|---------|----------------|--------------|----------------------------|-------|------------------|-----------|---------------------|----------------------|---|-----|------------|
| 25984   | 77,            | -            | BM 27-SEP-93               | LM    | 90009            | 30-SEP-93 | LEISHMANIA JW 11/12 | -                    | + | IND |            |
| 25985   | 78,            | -            | BL 27-SEP-93               | LM    | 90009            | 30-SEP-93 | LEISHMANIA JW 11/12 | -                    | - | NR  |            |
| 25986   | 80,            | -            | BL 27-SEP-93               | LM    | 90009            | 30-SEP-93 | LEISHMANIA JW 11/12 | -                    | + | IND |            |
| 25987   | 81,            | -            | BL 27-SEP-93               | LM    | 90009            | 30-SEP-93 | LEISHMANIA JW 11/12 | -                    | - | NR  |            |
| 25474   | LAKNER, GEORGE | -156-59-8935 | BL 03-SEP-93               | LM    | 90009            | 30-SEP-93 | LEISHMANIA JW 11/12 | -                    | - | NR  |            |
| 25610   | 54F,           | -            | BL 08-SEP-93               | LM    | 90009            | 30-SEP-93 | LEISHMANIA JW 11/12 | -                    | - | NR  |            |
| 25611   | 65F,           | -            | BL 08-SEP-93               | LM    | 90009            | 30-SEP-93 | LEISHMANIA JW 11/12 | +                    | + | RE  |            |
| Z000720 | 32731,         | -            | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -                    | - | NR  | REP. PLATE |
| Z000721 | 32732,         | -            | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -                    | - | NR  | REP. PLATE |
| Z000722 | 32733,         | -            | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -                    | - | NR  | REP. PLATE |
| Z000723 | 32734,         | -            | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -                    | - | NR  | REP. PLATE |
| Z000724 | 32735,         | -            | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -                    | - | NR  | REP. PLATE |
| Z000725 | 32736,         | -            | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -                    | - | NR  | REP. PLATE |
| Z000726 | 32737,         | -            | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -                    | - | NR  | REP. PLATE |
| Z000727 | 32738,         | -            | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -                    | - | NR  | REP. PLATE |
| Z000728 | 32739,         | -            | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -                    | - | NR  | REP. PLATE |
| Z000729 | 327310,        | -            | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -                    | - | NR  | REP. PLATE |
| Z000730 | 327311,        | -            | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -                    | - | NR  | REP. PLATE |
| Z000731 | 327312,        | -            | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -                    | - | NR  | REP. PLATE |
| Z000732 | 327313,        | -            | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -                    | - | NR  | REP. PLATE |
| Z000733 | 327314,        | -            | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -                    | - | NR  | REP. PLATE |
| Z000734 | 327315,        | -            | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -                    | - | NR  | REP. PLATE |
| Z000735 | 327316,        | -            | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -                    | - | NR  | REP. PLATE |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name | FPC + SSN | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer              | Tube # 1 | Tube # 2 | Int | Comments   |
|----------------------|-----------|----------------------------|-------|------------------|-----------|---------------------|----------|----------|-----|------------|
| Z000736 327317,      | -         | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | REP. PLATE |
| Z000737 327318,      | -         | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | REP. PLATE |
| Z000738 327319,      | -         | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | REP. PLATE |
| Z000739 327320,      | -         | BL 30-SEP-93               | LM    | 90009            | 14-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | REP. PLATE |
| Z000720 32731,       | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | +        | -        | IND |            |
| Z000721 32732,       | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000722 32733,       | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000723 32734,       | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000724 32735,       | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000725 32736,       | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000726 32737,       | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000727 32738,       | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000728 32739,       | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000729 327310,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000730 327311,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000731 327312,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000732 327313,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000733 327314,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000734 327315,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000735 327316,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000736 327317,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000737 327318,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |
| Z000738 327319,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |            |

Report Date:

PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name | FPC + SSN | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer     | Tube # 1 | Tube # 2 | Int | Comments |
|----------------------|-----------|----------------------------|-------|------------------|-----------|------------|----------|----------|-----|----------|
| Z000739 327320,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000740 327321,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000741 327322,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000742 327323,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000743 327324,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000744 327325,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000745 327326,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000746 327327,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000747 327328,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000748 327329,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000749 327330,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000750 327331,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000751 327332,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000752 327333,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000753 327334,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000754 327335,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000755 327336,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000756 327337,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000757 327338,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000758 327339,      | -         | BL 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| 26137 KISH, JOHN     | -         | OT 30-SEP-93               | LM    | 90009            | 13-OCT-93 | LEISHMANIA | JW 11/12 | +        | +   | RE       |
| Z000740 327321,      | -         | BL 30-SEP-93               | LM    | 90009            | 15-OCT-93 | LEISHMANIA | JW 11/12 | -        | -   | NR       |
| Z000741 327322,      | -         | BL 30-SEP-93               | LM    | 90009            | 15-OCT-93 | LEISHMANIA | JW 11/12 | +        | +   | RE       |

REP. PLATE

REP. PLATE, NEG CONT.

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name  | FPC + SSN      | Spec Received<br>Type Date | Study | Panel | Assay Date | Virus      | Primer   | Tube<br># 1 | Tube<br># 2 | Int | Comments               |
|-----------------------|----------------|----------------------------|-------|-------|------------|------------|----------|-------------|-------------|-----|------------------------|
| Z000742 327323,       | -              | BL 30-SEP-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | +           | +           | RE  | REP. PLATE, NEG. CONT. |
| Z000743 327324,       | -              | BL 30-SEP-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | +           | +           | RE  | REP. PLATE, NEG. CONT. |
| Z000744 327325,       | -              | BL 30-SEP-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | REP. PLATE             |
| Z000745 327326,       | -              | BL 30-SEP-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | REP. PLATE             |
| Z000746 327327,       | -              | BL 30-SEP-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | REP. PLATE             |
| Z000747 327328,       | -              | BL 30-SEP-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | REP. PLATE             |
| Z000748 327329,       | -              | BL 30-SEP-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | REP. PLATE             |
| Z000749 327330,       | -              | BL 30-SEP-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | REP. PLATE             |
| Z000750 327331,       | -              | BL 30-SEP-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | +           | -           | IND | REP. PLATE             |
| Z000751 327332,       | -              | BL 30-SEP-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | REP. PLATE             |
| Z000752 327333,       | -              | BL 30-SEP-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | REP. PLATE             |
| Z000753 327334,       | -              | BL 30-SEP-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | REP. PLATE             |
| Z000754 327335,       | -              | BL 30-SEP-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | REP. PLATE             |
| Z000755 327336,       | -              | BL 30-SEP-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | REP. PLATE             |
| Z000756 327337,       | -              | BL 30-SEP-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | REP. PLATE             |
| Z000757 327338,       | -              | BL 30-SEP-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | REP. PLATE             |
| Z000758 327339,       | -              | BL 30-SEP-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | REP. PLATE             |
| 26137 KISH, JOHN      | -              | OT 30-SEP-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | PLATE                  |
| 26305 VAUGHAN, GEORGE | 20-543-62-6961 | BL 08-OCT-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | PLATE                  |
| 26306 VAUGHAN, GEORGE | 20-543-62-6961 | BM 08-OCT-93               | LM    | 90009 | 15-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | PLATE                  |
| Z000720 32731,        | -              | BL 30-SEP-93               | LM    | 90009 | 21-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | 2X. VOL                |
| Z000721 32732,        | -              | BL 30-SEP-93               | LM    | 90009 | 21-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | 2X. VOL                |
| Z000722 32733,        | -              | BL 30-SEP-93               | LM    | 90009 | 21-OCT-93  | LEISHMANIA | JW 11/12 | -           | -           | NR  | 2X. VOL                |

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name | FPC + SSN | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer              | Tube Tube<br># 1 # 2 | Int | Comments |
|----------------------|-----------|----------------------------|-------|------------------|-----------|---------------------|----------------------|-----|----------|
| Z000723 32734,       | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000724 32735,       | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000725 32736,       | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000726 32737,       | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000727 32738,       | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000728 32739,       | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000729 327310,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000730 327311,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000731 327312,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000732 327313,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000733 327314,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000734 327315,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000735 327316,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000736 327317,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000737 327318,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000738 327319,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000739 327320,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000740 327321,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000741 327322,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000742 327323,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000743 327324,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000744 327325,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |
| Z000745 327326,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -                    | NR  | 2X.VOL   |

Report Date:

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name | FPC + SSN | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer              | Tube # 1 | Tube # 2 | Int | Comments       |
|----------------------|-----------|----------------------------|-------|------------------|-----------|---------------------|----------|----------|-----|----------------|
| Z000746 327327,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | 2X VOL         |
| Z000747 327328,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | 2X VOL         |
| Z000720 32731,       | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000721 32732,       | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000722 32733,       | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000723 32734,       | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000724 32735,       | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000725 32736,       | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000726 32737,       | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000727 32738,       | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000728 32739,       | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000729 327310,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000730 327311,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000731 327312,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000732 327313,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000733 327314,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000734 327315,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000735 327316,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000736 327317,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000737 327318,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000738 327319,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000739 327320,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000740 327321,      | -         | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |

Report Date:

PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name    | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer              | Tube # 1 | Tube # 2 | Int | Comments       |
|-------------------------|----------------|----------------------------|-------|------------------|-----------|---------------------|----------|----------|-----|----------------|
| Z000741 327322,         | -              | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000742 327323,         | -              | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000743 327324,         | -              | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000744 327325,         | -              | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000745 327326,         | -              | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000746 327327,         | -              | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000747 327328,         | -              | BL 30-SEP-93               | LM    | 90009            | 21-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000692 328710,         | -              | BL 14-OCT-93               | LM    | 90009            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000693 328711,         | -              | BL 14-OCT-93               | LM    | 90009            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000694 328712,         | -              | BL 14-OCT-93               | LM    | 90009            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000695 32871,          | -              | BL 14-OCT-93               | LM    | 90009            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000696 32872,          | -              | BL 14-OCT-93               | LM    | 90009            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000697 32873,          | -              | BL 14-OCT-93               | LM    | 90009            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000698 32874,          | -              | BL 14-OCT-93               | LM    | 90009            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000699 32875,          | -              | BL 14-OCT-93               | LM    | 90009            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000704 32876,          | -              | BL 14-OCT-93               | LM    | 90009            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000705 32877,          | -              | BL 14-OCT-93               | LM    | 90009            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000706 32878,          | -              | BL 14-OCT-93               | LM    | 90009            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000707 32879,          | -              | BL 14-OCT-93               | LM    | 90009            | 22-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  | JW11-3, JW12-1 |
| Z000708 BACHMAN, JOHN   | 20-205-42-4875 | TC 20-OCT-93               | LM    | 90009            | 29-OCT-93 | LEISHMANIA JW 11/12 | +        | +        | RE  |                |
| Z000709 VAUGHAN, GEORGE | 20-543-62-6961 | BL 21-OCT-93               | LM    | 90009            | 29-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |                |
| Z000710 VAUGHAN, GEORGE | 20-543-62-6961 | BM 21-OCT-93               | LM    | 90009            | 29-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |                |
| Z000711 VAUGHAN, GEORGE | 20-543-62-6961 | BM 21-OCT-93               | LM    | 90009            | 29-OCT-93 | LEISHMANIA JW 11/12 | -        | -        | NR  |                |

Report Date:

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PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name     | FPC + SSN      | Spec Received<br>Type Date | Study | Panel | Assay Date | Virus      | Primer   | Tube |     | Int | Comments      |
|---------|------------------|----------------|----------------------------|-------|-------|------------|------------|----------|------|-----|-----|---------------|
|         |                  |                |                            |       |       |            |            |          | # 1  | # 2 |     |               |
| 26642   | 54F,             | -              | BL 25-OCT-93               | LM    | 90009 | 29-OCT-93  | LEISHMANIA | JW 11/12 | +    | +   | RE  |               |
| 26643   | 65F,             | -              | BL 25-OCT-93               | LM    | 90009 | 29-OCT-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  |               |
| 26644   | 71F,             | -              | BL 25-OCT-93               | LM    | 90009 | 29-OCT-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  |               |
| 26645   | 72F,             | -              | BL 25-OCT-93               | LM    | 90009 | 29-OCT-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  |               |
| 26646   | 73/74F,          | -              | BL 25-OCT-93               | LM    | 90009 | 29-OCT-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  |               |
| 26647   | 77F,             | -              | BL 25-OCT-93               | LM    | 90009 | 29-OCT-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  |               |
| 26648   | 102F,            | -              | BL 25-OCT-93               | LM    | 90009 | 29-OCT-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  |               |
| 26716   | VAUGHAN, GEORGE  | 20-543-62-6961 | OT 27-OCT-93               | LM    | 90009 | 08-NOV-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  | INSUFF. CELLS |
| Z000712 | VAUGHAN, GEORGE  | 20-543-62-6961 | LI 01-NOV-93               | LM    | 90009 | 08-NOV-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  |               |
| 26879   | NERONE, MARCUS A | 20-257-17-3841 | BL 03-NOV-93               | LM    | 90009 | 08-NOV-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  |               |
| 26880   | NERONE, MARCUS A | 20-257-17-3841 | BM 03-NOV-93               | LM    | 90009 | 08-NOV-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  |               |
| Z000714 | BRANDES, RONALD  | 20-317-48-7871 | TC 17-NOV-93               | LM    | 90009 | 22-NOV-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  |               |
| Z000715 | ELLIOTT, E       | -              | TC 17-NOV-93               | LM    | 90009 | 22-NOV-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  |               |
| Z000716 | ELLIOTT, E       | -              | TC 17-NOV-93               | LM    | 90009 | 22-NOV-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  |               |
| 27156   | BRANDES, RONALD  | 20-317-48-7871 | BL 17-NOV-93               | LM    | 90009 | 22-NOV-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  |               |
| 27165   | VAUGHAN, GEORGE  | 20-543-62-6961 | TC 18-NOV-93               | LM    | 90009 | 22-NOV-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  |               |
| 27166   | NERONE, MARCUS A | 20-257-17-3841 | BL 18-NOV-93               | LM    | 90009 | 22-NOV-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  |               |
| Z000714 | BRANDES, RONALD  | 20-317-48-7871 | TC 17-NOV-93               | LM    | 90009 | 24-NOV-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  | PL1           |
| Z000715 | ELLIOTT, E       | -              | TC 17-NOV-93               | LM    | 90009 | 24-NOV-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  | PL1           |
| Z000716 | ELLIOTT, E       | -              | TC 17-NOV-93               | LM    | 90009 | 24-NOV-93  | LEISHMANIA | JW 11/12 | +    | -   | IND | PL1           |
| 27165   | VAUGHAN, GEORGE  | 20-543-62-6961 | TC 18-NOV-93               | LM    | 90009 | 24-NOV-93  | LEISHMANIA | JW 11/12 | +    | -   | IND | PL1, -CONT    |
| 27166   | NERONE, MARCUS A | 20-257-17-3841 | BL 18-NOV-93               | LM    | 90009 | 24-NOV-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  | PL1, -CONT    |
| Z000714 | BRANDES, RONALD  | 20-317-48-7871 | TC 17-NOV-93               | LM    | 90009 | 29-NOV-93  | LEISHMANIA | JW 11/12 | -    | -   | NR  | PL2           |

Report Date:

PCR Assay Results: LM  
Probe JW 14

| Spec ID | Patient Name     | FPC + SSN      | Spec Received<br>Type Date | Study | Panel Assay Date | Virus     | Primer              | Tube # | Tube # | Int | Comments |
|---------|------------------|----------------|----------------------------|-------|------------------|-----------|---------------------|--------|--------|-----|----------|
| Z000715 | ELLIOTT, E       | -              | TC 17-NOV-93               | LM    | 90009            | 29-NOV-93 | LEISHMANIA JW 11/12 | -      | -      | NR  | PL2      |
| Z000716 | ELLIOTT, E       | -              | TC 17-NOV-93               | LM    | 90009            | 29-NOV-93 | LEISHMANIA JW 11/12 | -      | -      | NR  | PL2      |
| 27165   | VAUGHAN, GEORGE  | 20-543-62-6961 | TC 18-NOV-93               | LM    | 90009            | 29-NOV-93 | LEISHMANIA JW 11/12 | -      | -      | NR  | PL2      |
| 27166   | NERONE, MARCUS A | 20-257-17-3841 | BL 18-NOV-93               | LM    | 90009            | 29-NOV-93 | LEISHMANIA JW 11/12 | -      | -      | NR  | PL2      |
| Z000714 | BRANDES, RONALD  | 20-317-48-7871 | TC 17-NOV-93               | LM    | 90009            | 29-NOV-93 | LEISHMANIA JW 11/12 | -      | -      | NR  | PL3      |
| Z000715 | ELLIOTT, E       | -              | TC 17-NOV-93               | LM    | 90009            | 29-NOV-93 | LEISHMANIA JW 11/12 | +/-    | +      | IND | PL3      |
| Z000716 | ELLIOTT, E       | -              | TC 17-NOV-93               | LM    | 90009            | 29-NOV-93 | LEISHMANIA JW 11/12 | -      | -      | NR  | PL3      |
| 27165   | VAUGHAN, GEORGE  | 20-543-62-6961 | TC 18-NOV-93               | LM    | 90009            | 29-NOV-93 | LEISHMANIA JW 11/12 | -      | -      | IND | PL3      |
| 27166   | NERONE, MARCUS A | 20-257-17-3841 | BL 18-NOV-93               | LM    | 90009            | 29-NOV-93 | LEISHMANIA JW 11/12 | -      | -      | NR  | PL3      |
| Z000766 | BRANDES, RONALD  | 20-317-48-7871 | LM 23-NOV-93               | LM    | 90009            | 02-DEC-93 | LEISHMANIA JW 11/12 | -      | -      | NR  |          |
| Z000767 | BRANDES, RONALD  | 20-317-48-7871 | LI 23-NOV-93               | LM    | 90009            | 02-DEC-93 | LEISHMANIA JW 11/12 | -      | -      | NR  |          |
| Z000768 | BRANDES, RONALD  | 20-317-48-7871 | TC 23-NOV-93               | LM    | 90009            | 02-DEC-93 | LEISHMANIA JW 11/12 | -      | -      | NR  |          |
| Z000769 | BRANDES, RONALD  | 20-317-48-7871 | TC 23-NOV-93               | LM    | 90009            | 02-DEC-93 | LEISHMANIA JW 11/12 | -      | -      | NR  |          |
| 27882   | HAYES, JAMES     | -380-80-7107   | BL 21-JAN-94               | LM    | 90009            | 13-JAN-94 | LEISHMANIA JW 11/12 | -      | -      | NR  |          |
| 27883   | HAYES, JAMES     | -380-80-7107   | BM 21-JAN-94               | LM    | 90009            | 13-JAN-94 | LEISHMANIA JW 11/12 | -      | -      | NR  |          |
| Z000771 | 40211,           | -              | BL 21-JAN-94               | LM    | 90009            | 26-JAN-94 | LEISHMANIA JW 11/12 | -      | -      | NR  |          |
| Z000772 | 40212,           | -              | BL 21-JAN-94               | LM    | 90009            | 26-JAN-94 | LEISHMANIA JW 11/12 | -      | -      | NR  |          |
| Z000773 | 40213,           | -              | BL 21-JAN-94               | LM    | 90009            | 26-JAN-94 | LEISHMANIA JW 11/12 | -      | -      | NR  |          |
| Z000774 | 40214,           | -              | BL 21-JAN-94               | LM    | 90009            | 26-JAN-94 | LEISHMANIA JW 11/12 | -      | -      | NR  |          |
| Z000775 | 40215,           | -              | BL 21-JAN-94               | LM    | 90009            | 26-JAN-94 | LEISHMANIA JW 11/12 | -      | -      | NR  |          |
| Z000776 | 40216,           | -              | BL 21-JAN-94               | LM    | 90009            | 26-JAN-94 | LEISHMANIA JW 11/12 | -      | -      | NR  |          |
| Z000777 | 40217,           | -              | BL 21-JAN-94               | LM    | 90009            | 26-JAN-94 | LEISHMANIA JW 11/12 | -      | -      | NR  |          |
| Z000778 | 40218,           | -              | BL 21-JAN-94               | LM    | 90009            | 26-JAN-94 | LEISHMANIA JW 11/12 | -      | -      | NR  |          |

Report Date:

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PCR Assay Results: LM  
Probe JW 14

| Spec ID Patient Name   | FPC + SSN    | Spec Received<br>Type Date | Study | Panel | Assay Date | Virus      | Primer   | Tube Tube<br># 1 # 2 | Int | Comments |
|------------------------|--------------|----------------------------|-------|-------|------------|------------|----------|----------------------|-----|----------|
| Z000779 40219,         | -            | BL 21-JAN-94               | LM    | 90009 | 26-JAN-94  | LEISHMANIA | JW 11/12 | -                    | -   | NR       |
| Z000780 402110,        | -            | BL 21-JAN-94               | LM    | 90009 | 26-JAN-94  | LEISHMANIA | JW 11/12 | -                    | -   | NR       |
| Z000781 402111,        | -            | BL 21-JAN-94               | LM    | 90009 | 26-JAN-94  | LEISHMANIA | JW 11/12 | -                    | -   | NR       |
| Z000782 402112,        | -            | BL 21-JAN-94               | LM    | 90009 | 26-JAN-94  | LEISHMANIA | JW 11/12 | -                    | -   | NR       |
| Z000783 402113,        | -            | BL 21-JAN-94               | LM    | 90009 | 26-JAN-94  | LEISHMANIA | JW 11/12 | -                    | -   | NR       |
| Z000784 402114,        | -            | BL 21-JAN-94               | LM    | 90009 | 26-JAN-94  | LEISHMANIA | JW 11/12 | -                    | -   | NR       |
| Z000785 ZUPEC, JEFFREY | -            | BL 24-JAN-94               | LM    | 90009 | 26-JAN-94  | LEISHMANIA | JW 11/12 | -                    | -   | NR       |
| Z000786 ZUPEC, JEFFREY | -            | BL 24-JAN-94               | LM    | 90009 | 26-JAN-94  | LEISHMANIA | JW 11/12 | -                    | -   | NR       |
| Z000787 HALLMAN, JAMES | -419-62-6827 | LI 25-JAN-94               | LM    | 90009 | 27-JAN-94  | LEISHMANIA | JW 11/12 | -                    | -   | NR       |

| Interpretation Key  | Specimen Type    |
|---------------------|------------------|
| RE = Reactive       | BL = Blood       |
| NR = Non-Reactive   | SK = Skin        |
| ND = Non-Diagnostic | LI = Liver       |
|                     | CO = Colon       |
|                     | LN = Lymphnode   |
|                     | BM = Bone Marrow |
|                     | ASP = Aspirate   |

**Appendix II. Manuscript submitted for publication**

Nuzum, E., White III, F., Thakur, C., Dietze, R., Wages, J.,  
Grogl, M. and Berman, J. 1994. Diagnosis of visceral  
leishmaniasis from patient blood using the polymerase chain  
reaction. (Submitted to Journal of Infectious Diseases)

FOR: DR. SURYANARAYANA

SRA Technologies

## TITLE:

Diagnosis of visceral leishmaniasis from patient blood using the  
polymerase chain reaction

Fax: 301-424-8501

## RUNNING TITLE:

Leishmania PCR

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**ABSTRACT**

To diagnose visceral leishmaniasis (kala-azar) using peripheral blood rather than tissue aspirates, a PCR technique was developed for which the detection limit is one Leishmania-infected macrophage in 8 mls blood. For Indian, Kenyan, or Brazilian patients with parasitologically proven kala-azar, 57 of 63 cases prior to treatment had blood that was PCR positive (90% sensitivity). None of 40 uninfected persons had PCR positive blood (100% specificity). 12 of 13 Indian patients successfully treated had negative PCR reactions on their blood 1-6 months post treatment (92%). This PCR procedure is capable of parasitologically diagnosing the vast majority of kala-azar cases pre-therapy, may identify patients who have been successfully treated by chemotherapy, and should substantially obviate the need for invasive tests to diagnose kala-azar.

## INTRODUCTION

Visceral leishmaniasis (kala-azar), infection of the liver, spleen, and bone marrow with the Leishmania donovani complex, is a significant parasitic problem in the developing world and, with increased international travel and AIDS, in the developed world [1,2]. The diagnosis of visceral leishmaniasis requires parasitological identification of Leishmania from lesion material. The ability to diagnose visceral leishmaniasis from easily obtained material such as patients' blood would be a major advance. Because of our interest in diagnosing visceralizing Leishmania (tropica) infection in Operation Desert Storm personnel, we devised a polymerase chain reaction (PCR) method to diagnose Leishmania in patients' blood, using L tropica minicircle kDNA as the basis for the PCR primers. We validated the test with blood from patients with the most prevalent clinical presentation of visceralizing Leishmania, kala-azar.

## MATERIALS AND METHODS

### PCR primers

20 different PCR primer pairs based on extensive literature [3-9] and DNA sequence database searches of Leishmania minicircle kDNA were synthesized (Synthetic Genetics, San Diego, CA). The primers finally chosen for systematic evaluation were designated JW-11-i and JW-12-i, which were 5' biotinylated to facilitate PCR product capture and analysis. JW-14 was the detection probe, 5' coupled to Horseradish Peroxidase (HRP). "I" = deoxyinosine.

5' primer JW-11-i: 5'-CCTATTTTACACCAACCCCIAGTTT-3'

3' primer JW-12-i: 5'-CGGGTAGGGGCGTTCTGCGAAAT-3'

probe JW-14: 5'-ATTGAACGGGGTTTCTGTATGCATTTTTCGAA-3'

### Sample collection and preparation

Peripheral blood was collected in LeucoPREP™ (Becton-Dickinson, Rutherford, NJ) vacutainer tubes that contained a gel matrix, and within 6 hrs spun at 1500-1800 x g for 20 minutes. The suspension of peripheral blood mononuclear cells (PBMC) above the matrix was removed, exposed to 0.1% saponin in 0.6% NaCl to lyse contaminating red cells, centrifuged (300 x g for 15 min), and then resuspended at a concentration of  $3 \times 10^7$  PBMCs/ml in lysis buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 2.5 mM MgCl<sub>2</sub>, 0.45% NP-40, 0.45% Tween 20, 240 ug/ml proteinase k) at 55°C for 1 hr and then at 95°C for 15 min to lyse the mononuclear cells.

### PCR amplification

For each sample assayed, 40 µl of lower PCR mix containing 10X PCR buffer (Promega, Madison, WI), MgCl<sub>2</sub>, primers, dNTP's, and 0.1 Unit UNG (uracil-N-glycosylase, Epicentre Technologies, Madison, WI) was added to each tube. A single AmpliWax™ bead (Perkin Elmer, Norwalk, CT) was placed in each tube, and the tubes were heated to approximately 70°C for five

minutes to melt the wax bead. After cooling to room temperature, 10  $\mu$ l of upper PCR mix containing 10X PCR buffer, 0.1 Unit UNG, and AmpliTaq™ DNA polymerase (Perkin Elmer, Norwalk, CT) was added. Fifty  $\mu$ l of lysed mononuclear cells, representing an original peripheral blood volume containing  $1.5 \times 10^6$  PBMCs, was then added. The final reaction volume of 100  $\mu$ l contained 1X PCR buffer (10mM Tris-HCl, pH 8.3, 50 mM KCl), 2.5 mM  $MgCl_2$ , 1.0  $\mu$ M each primer, 200  $\mu$ M of each dATP, dCTP, and dGTP (U.S. Biochemicals, Cleveland, OH), 300  $\mu$ M dUTP (Epicentre Technologies, Madison, WI), 2.5-5.0 Units AmpliTaq™ DNA polymerase, and 0.2 Unit UNG. Reactions were cycled in a Perkin Elmer model 9600 thermal cycler using the following conditions; 94°C for 5'0", 10 cycles of 97°C for 0'15", 55°C for 1'0", 72°C for 1'0", followed by 30 cycles of 92°C for 0'15", 55°C for 1'0", and 72°C for 1'0". PCR products were held at 72°C or frozen at -20°C until assayed.

#### PCR product analysis

Reaction products were detected by affinity-based hybridization analysis developed by SRA Technologies using the oligonucleotide probe (JW-14) to sequences bracketed by, but not overlapping, the primers [10,11]. Assay plates (Immulon 4, Dynatech Labs, Chantilly, VA) were coated with 100  $\mu$ l of 100  $\mu$ g/ml avidin D (Vector Labs, Burlingame, CA) in coating buffer (50 mM Sodium Bicarbonate pH 9.5, 150 mM NaCl, 0.01% Sodium Azide) per well. Ten  $\mu$ l from each PCR reaction containing biotinylated product DNA was heat-denatured for 5 minutes at 95°C, then transferred to an avidin-coated well containing hybridization solution (5X SSC, 5X Denhardt's, 0.5% SDS, 2% BSA, 50  $\mu$ g sheared salmon sperm DNA) and 1 pmol per well of HRP-coupled probe JW14 (Synthetic Genetics, San Diego CA). The reaction mixtures were incubated at 42°C for 20 minutes to allow simultaneous hybridization of the probe to the PCR product and binding of the biotinylated PCR product to the solid substrate. After the solution was discarded, each well was washed 4 times using an automatic microtiter plate washer. A substrate solution of 0.6 mg/ml o-phenylene-diamine dihydrochloride (OPD:

Sigma, St. Louis, MO) in citrate/phosphate buffer, pH 5.5, containing  $\text{H}_2\text{O}_2$  was then added. The reaction was incubated at room temperature for 10 minutes, stopped by the addition of 1N  $\text{H}_2\text{SO}_4$  and read at 490nm in a Molecular Devices UVmax microplate reader.

Background in the assay is typically less than 0.06  $\text{OD}_{490}$ , with a positive signal generally 0.700  $\text{OD}_{490}$  or greater. All samples were tested in duplicate reactions with a negative control (all PCR reagents but no DNA). Since 10-20 samples (with 2 experimental and one negative control reaction per sample) were analyzed on each 96-well plate, the mean and standard deviation (SD) for negative control values could be calculated from the 10-20 negative control reactions for each 96-well plate. A sample was defined as positive if the  $\text{OD}_{490}$  of both experimental reactions was greater than or equal to the mean  $\text{OD}_{490}$  of the negative controls for that plate plus three SD.

## Determination of sensitivity and specificity using laboratory samples

In preliminary work, we found that the DNA equivalence of one promastigote of L. donovani and L. chagasi, as well as of L. tropica (the species for which the primers were originally designed), was detected by this PCR method. When clinical specimens were simulated by infecting the PBMCs of normal volunteers with Leishmania amastigotes, 10 and 5 L. tropica-infected macrophages in 8 ml blood were detected each of 4 times, 1 infected macrophage was detected 3 of 4 times, and uninfected macrophages gave no PCR signal on each of

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simulated by infecting the PBMCs of normal volunteers with Leishmania amastigotes, 10 and 5 L. tropica-infected macrophages in 8 ml blood were detected each of 4 times, 1 infected macrophage was detected 3 of 4 times, and uninfected macrophages gave no PCR signal on each of 8 occasions. The absence of PCR product when DNA from >10,000 organisms of other genera (Trypanosoma, Toxoplasma, Plasmodia, Pneumocystis, Histoplasma, Mycobacterium, or Salmonella) were used indicates that this PCR technique is 100% biologically specific.

## Clinical data

To determine if this PCR technique could be used to diagnose kala-azar, blood was drawn from 75 Indians, 11 Kenyans, and 10 Brazilians with disease proven by visualization of Leishmania amastigotes in splenic aspirates (Kenyan and Indian patients) or in bone marrow aspirates (Brazilian patients). Blood was also drawn from 5 Indians, 7 Kenyans, 1 Brazilian, and 27 Americans who were not suspected to be infected with Leishmania.

A majority of specimens were drawn pre-therapy (Table 1). Approximately 90% of these were positive for Leishmania kDNA. The percent of PCR-positive samples for the different endemic areas was 80% for Brazil, 90% from India, and 100% from Kenya. There were 11 patients for whom blood was drawn on each of two consecutive days. There was complete

concordance between the duplicate specimens. 3 patients had blood that was PCR positive in both

instances, and 2 patients had blood that was PCR negative in both instances.

There was no correlation between PCR results and the important clinical parameter of spleen size. For the two false-negative Brazilian samples, the patients' spleen sizes were 6 and

7 cm below the right costal margin. For the 5 PCR-positive samples in that group, the patients' spleen sizes ranged from 4 to 13 cm (mean = 8.6 cm). The second batch of Indian kala-azar samples contained 3 samples of blood that were negative by PCR. The spleen sizes for these patients ranged from 5 to 11 cm (mean = 8.7 cm). For the 12 PCR-positive samples in that batch, the mean patient spleen size was 6.4 cm.

There were 33 Indian patients for whom blood was drawn during or after therapy (Table 1). These patients were treated either with the classic agent sodium stibogluconate @20 mg/kg/day for 40 days [12] or amphotericin B @1 mg/kg every-other-day for 20 injections [13]. For patients in the first half of therapy, blood was PCR positive in approximately 90% of cases, nearly the same percent as for patients whose blood was taken before therapy. For patients in the second half of therapy, blood was positive in only 37%. In addition, there were 13 patients in whom drug treatment had apparently been successful by clinical criteria (loss of fever, weight gain, decrease in spleen size). By 1-6 months after the end of therapy, blood was positive in 14% to 0% of cases, respectively.

The blood of all 40 healthy volunteers was Leishmania kDNA negative by PCR.

## DISCUSSION

We were able to diagnose 90% of cases of parasitologically proven kala-azar by PCR on the patients' peripheral blood. The cases came from the 3 major endemic areas of the world--India, Africa, and Brazil. Since PCR was positive for laboratory samples in which merely one infected macrophage was spiked into 8 mls of blood, the 10% PCR negative samples are thought to be due to biologic false-negativity (the organisms were present in infected organs but were not in the peripheral circulation) rather than to technical false negativity (the organisms were in the blood but were undetected by PCR). Clinical parameters did not differentiate the 90% of patients whose blood was PCR positive from the 10% of patients whose blood was PCR negative, and no patient who was PCR negative in one sample was PCR positive on subsequent samples taken a few days later. We conclude that when blood samples are drawn over a period of a few days, infected macrophages enter the peripheral circulation from diseased organs in 90% but not 100% of patients.

These results represent a substantial clinical advance compared to previous reports on diagnosis of visceral leishmaniasis via PCR. For example, for 7 Indian kala-azar patients prior to treatment for whom the blood was analyzed by another PCR technique, 3 PCR reactions were negative, 2 were positive, one was faintly positive, and one was positive via an alternative detection band [8].

The PCR procedure described herein has potential applicability for the clinical management of kala-azar cases. The 100% specificity indicates that if the blood of a patient suspected of having kala-azar is PCR positive, that patient would not have to undergo invasive organ biopsy for a parasitological diagnosis. The 90% sensitivity indicates that the vast majority of patients with kala-azar will be diagnosed via this technique. For example, it is reasonable to assume clinical judgement is sufficiently accurate so that half of clinically suspected cases are eventually proven to have kala-azar. For every 100 suspected cases, of whom 50 would actually have kala-azar, 45 cases (90%) should be diagnosed on the basis of

blood PCR. Invasive diagnostic tests would only have to be performed on the remaining 55 PCR negative persons to distinguish the 50 who are uninfected from the 5 who would have biological false negative PCR results.

Post therapy, 1 of 13 patients clinically cured of kala-azar was PCR positive in our study, and this patient had blood drawn only 1 month after the end of therapy. The low percent of PCR positivity post therapy suggests that further study of successfully treated kala-azar patients may reveal a time post-therapy when virtually all cases are PCR negative. This PCR technique therefore has the potential to obviate invasive parasitological tests both prior to and post therapy.

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**TABLE 1: POLYMERASE CHAIN REACTION RESULTS FOR KALA AZAR PATIENTS**

Table depicts countries from which batches of patient samples were obtained, time in relation to treatment that blood for PCR was obtained [Pretreatment (pre-RX); in the first half of treatment (< 0.5 RX); in the second half of treatment (0.5-1.0 RX); or 1, 2, or 6 months after treatment] and PCR results (+ or -). The total number of PCR+ and PCR- results for each country and for all countries are also shown.

**POLYMERASE CHAIN REACTION RESULTS (+ OR -) FOR EACH PATIENT  
AT THE FOLLOWING TIMES WITH RESPECT TO THERAPY**

| <b>COUNTRY/<br/>BATCH #</b>     | <b>PFE<br/>RX</b> | <b>&lt; 0.5<br/>RX</b> | <b>0.5--1.0<br/>RX</b> | <b>1 MONTH<br/>POST RX</b> | <b>2 MONTH<br/>POST RX</b> | <b>6 MONTH<br/>POST RX</b> |
|---------------------------------|-------------------|------------------------|------------------------|----------------------------|----------------------------|----------------------------|
| <b>INDIA #1</b>                 |                   |                        |                        |                            |                            |                            |
| positive                        | 3                 | 5                      | 1                      | [none]                     | [none]                     | [none]                     |
| negative                        | 0                 | 0                      | 2                      | [none]                     | [none]                     | [none]                     |
| <b>INDIA #2</b>                 |                   |                        |                        |                            |                            |                            |
| positive                        | 12                | 4                      | 1                      | [none]                     | 0                          | [none]                     |
| negative                        | 3                 | 0                      | 0                      | [none]                     | 1                          | [none]                     |
| <b>INDIA #3</b>                 |                   |                        |                        |                            |                            |                            |
| positive                        | 23                | 2                      | 1                      | 1                          | 0                          | 0                          |
| negative                        | 1                 | 1                      | 5                      | 6                          | 3                          | 2                          |
| <b>INDIA TOTAL</b>              |                   |                        |                        |                            |                            |                            |
| positive                        | 38                | 11                     | 3                      | 1                          | 0                          | 0                          |
| negative                        | 4                 | 1                      | 5                      | 6                          | 4                          | 2                          |
| <b>KENYA TOTAL</b>              |                   |                        |                        |                            |                            |                            |
| positive                        | 11                |                        |                        |                            |                            |                            |
| negative                        | 0                 |                        |                        |                            |                            |                            |
| <b>BRAZIL #1</b>                |                   |                        |                        |                            |                            |                            |
| positive                        | 5                 |                        |                        |                            |                            |                            |
| negative                        | 0                 |                        |                        |                            |                            |                            |
| <b>BRAZIL #2</b>                |                   |                        |                        |                            |                            |                            |
| positive                        | 3                 |                        |                        |                            |                            |                            |
| negative                        | 2                 |                        |                        |                            |                            |                            |
| <b>BRAZIL TOTAL</b>             |                   |                        |                        |                            |                            |                            |
| positive                        | 8                 |                        |                        |                            |                            |                            |
| negative                        | 2                 |                        |                        |                            |                            |                            |
| <b>TOTALS FOR ALL COUNTRIES</b> |                   |                        |                        |                            |                            |                            |
| ✓ positive                      | 57                | 11                     | 3                      | 1                          | 0                          | 0                          |
| negative                        | 6                 | 1                      | 5                      | 6                          | 4                          | 2                          |
| % positive                      | 90                | 92                     | 37                     | 14                         | 0                          | 0                          |