AD-A191 626 ENZYME MINI-TEST FOR FIELD IDENTIFICATION OF LEISHMANIA ISOLATES FROM US MILITARY PERSONNEL(U) YOUNGSTOWN STATE UNIV OHIO R D KREUTZER 15 MAY 86 DAMD17-83-C-3119 1/1

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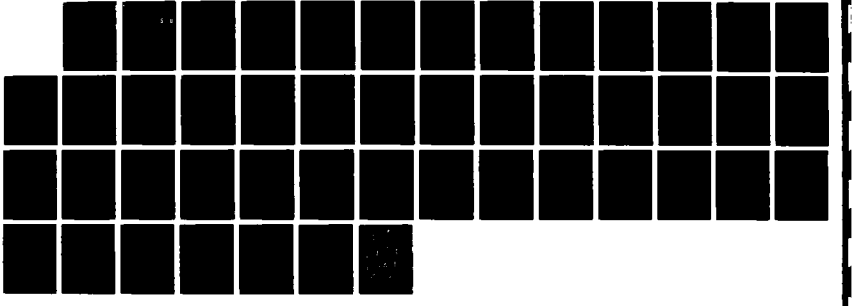

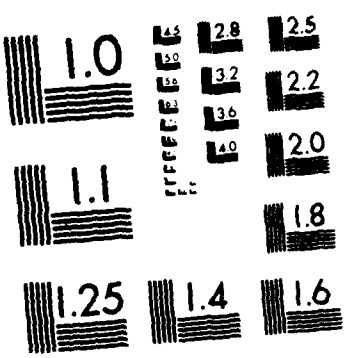

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# ENZYME MINI-TEST FOR FIELD IDENTIFICATION OF *LEISHMANIA* ISOLATES FROM U.S. MILITARY PERSONNEL

Annual and Final Report

RICHARD D. KREUTZER, PH.D.



MAY 15, 1986

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-83-C-3119

Youngstown State University
Youngstown, Ohio 44555

SECURITY CLASSIFICATION OF THIS PAGE

# REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

| | |
|---|---|
| 1a. REPORT SECURITY CLASSIFICATION: Unclassified | 1b. RESTRICTIVE MARKINGS |
| 2a. SECURITY CLASSIFICATION AUTHORITY | 3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited |
| 2b. DECLASSIFICATION/DOWNGRADING SCHEDULE | |
| 4. PERFORMING ORGANIZATION REPORT NUMBER(S) | 5. MONITORING ORGANIZATION REPORT NUMBER(S) |
| 6a. NAME OF PERFORMING ORGANIZATION: Youngstown State University; 6b. OFFICE SYMBOL (If applicable) | 7a. NAME OF MONITORING ORGANIZATION |
| 6c. ADDRESS (City, State, and ZIP Code): Youngstown, OH 44555 | 7b. ADDRESS (City, State, and ZIP Code) |
| 8a. NAME OF FUNDING/SPONSORING ORGANIZATION: U.S. Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable) | 9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: Contract No. DAMD17-83-C-3119 |
| 8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, Maryland 21701-5012 | 10. SOURCE OF FUNDING NUMBERS |

| PROGRAM ELEMENT NO. | PROJECT NO. | TASK NO. | WORK UNIT ACCESSION NO. |
|---|---|---|---|
| 62770A | 3M1-62770A870 | AI | 018 |

11. TITLE (Include Security Classification): Enzyme Mini-Test for Field Identification of Leishmania Isolates From U.S. Military Personnel

12. PERSONAL AUTHOR(S): Kreutzer, Richard D.

| 13a. TYPE OF REPORT | 13b. TIME COVERED | 14. DATE OF REPORT (Year, Month, Day) | 15. PAGE COUNT |
|---|---|---|---|
| Annual and Final Report | FROM 3/15/83 TO 3/31/86 | 1986 May 15 | 51 |

16. SUPPLEMENTARY NOTATION
Annual Report (for period August 15, 1985 - March 31, 1986)
Final Report (for period March 15, 1983 - March 31, 1986)

| 17. COSATI CODES | | |
|---|---|---|
| FIELD | GROUP | SUB-GROUP |
| 06 | 01 | |
| 06 | 13 | |

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number): Leishmania; electrophoresis; identification; isozymes

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

It is possible to identify Leishmania isolates by cellulose acetate electrophoresis (CAE) of up to 29 enzyme activities. Certain of these enzymes are polymorphic within a subspecies and therefore of limited value for identification; others are monomorphic and have taxonomic significance. Once large numbers of isolates from various geographical areas have been characterized and monomorphic enzymes identified, a simple, rapid, accurate field type identification test can be devised.

Data for up to 29 enzymes have been obtained from about 400 Leishmania isolates by CAE. Among the isolates were two groups designated as reference strains. Enzyme profiles have been established for many Leishmania subspecies based on CAE data from over 20 widely distributed isolates and reference strains. The Leishmania profiles thus established are as follows: L. braziliensis panamensis (L.b.p. 104 isolates), L. b. braziliensis (L.b.b. - 56), L. b. guyanensis (L.b.g. - 28), L. mexicana mexicana/-pifanoi (L.m.m./p. - 43/2),

| 20. DISTRIBUTION/AVAILABILITY OF ABSTRACT | 21. ABSTRACT SECURITY CLASSIFICATION |
|---|---|
| ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS | Unclassified |

| 22a. NAME OF RESPONSIBLE INDIVIDUAL | 22b. TELEPHONE (Include Area Code) | 22c. OFFICE SYMBOL |
|---|---|---|
| Mrs. Virginia Miller | 301/663-7325 | SGRD-RMI-S |

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

L. m. amazonensis/garnhami (L.m.a./g. - 47/2), L. m. aristedesi (L.ar. - 1), L. m. enriettii (L.m.e. - 2), L. donovani (L.d.d. - 28), L. chagasi (L.d.c. - 27), L. major (L.m.j. - 32), L. tropica (L.t. - 25), L. aethiopica (L.ae.- 7), L. hertigi hertigi (L.h.h. - 4), L. gerbilli (L.ge. - 1). No consistent CAE differences have been noted between L.m.m. and L.m.p. or between L.m.a. and L.m.g.; therefore, they have been noted as L.m.m./p. and L.m.a./g. respectively.

As the number of isolates studied from a particular geographical area increased so did the diversity of Leishmania subspecies; therefore, to obtain an accurate estimate of the Leishmania fauna in an area many isolates must be identified. Routine CAE identification was performed for certain WRAIR contractees and other sources. When appropriate, data from these isolates were included in subspecies enzymes profiles. Isolates obtained by the P.I. in Colombia and identified by CAE have been sent to and have been added to the WRAIR cryobank. It was suggested that preliminary and confirmatory CAE identification be made a part of all WRAIR Leishmania studies. The enzyme profile data from 400 isolates suggest that accurate CAE identification can be made from data of GPI, MPI and 6PGDH. The buffer and the stain components for these enzymes can be prepackaged, and data can be obtained from $10^6$ Leishmania cells.

The CAE data which have been generated in this and other studies have been compiled in a computer program. The data were recorded on a disk which was sent to the Leishmania Section at WRAIR and therefore are available to WRAIR personnel.

A detailed short test for Leishmania identification by CAE was included for the enzymes GPI, MPI, 6PGDH. The test included supplies and equipment with purchasing information needed for CAE, cell preparation for CAE procedures, complete set-up and running information, controls and confirming procedures, sequence of enzymes to study and diagrams of GPI, MPI and 6PGDH enzyme data. Most Leishmania could be identified from data obtained from this test.

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## Problem

*Leishmania* isolates from any source can be accurately and rapidly identified by cellulose acetate electrophoresis (CAE). Studies using 29 enzyme activities have shown that certain enzymes are polymorphic and of limited value in identification, while others are monomorphic and have taxonomic significance. It is, therefore, necessary to characterize isolates from a series of geographic areas by examining data from a number of enzymes to find a few monomorphic enzymes that can be used in a simple, rapid, accurate field identification test.

## Background

Electrophoretic study of gene-enzyme systems represents a powerful tool in systematics, genetics, ecology, ethology and applied biology. Studies on the systematic value of electrophoretic data reveal high levels of genetic similarity between conspecific populations, with up to 85% identity of their loci, while the percent of genetic similarities among closely related species are usually much lower.[1-4]

Recently enzyme electrophoresis has been used to identify parasites of the genus *Leishmania*[5-11] One study reported that 21% of the enzymes tested from particular subspecies were polymorphic.[11] This suggests that isolates which have profiles greater than 75% identical are the same subspecies, and isolates which are less than 75% identical are parasites from different subspecies. The enzyme profiles of some isolates in this study were identical, but in most cases, the profiles among isolates in a given subspecies were

different. Leishmania could be grouped into at least five major complexes according to enzyme profiles, braziliensis, mexicana, donovani, tropica and hertigi. These designations for the most part are consistent with the taxonomic categories used by others.[12] Within these complexes the isolates could be grouped into subspecies with varying levels of allozyme identity. It has been reported that the L. braziliensis complex is composed of two possibly three (L. b. guyanensis) subspecies, L. b. panamensis and L. b. braziliensis. The L. mexicana complex has at least three subspecies, L. m. mexicana, L. m. amazonensis and L. m. aristidesi. Minimal numbers of L. tropica and L. donovani had been examined and subspecies remained to be delineated. These data were consistent with those obtained by the principal investigator in an earlier less comprehensive study. Results of this study demonstrated that electrophoresis can be used for rapid and accurate subspecific identification of Leishmania.[11]

The data which have been reported on enzyme profiles of leishmanial groups have been obtained from small numbers of isolates. Among known species there is a certain amount of naturally occurring enzyme polymorphism. Isolates which have been identified as belonging to the same group by classical methods and their identification confirmed by CAE do have slight genetic differences as noted from their individual enzyme profiles. This type of population polymorphism is to be expected and has been noted in all groups of organisms which have been studied by electrophoresis.[1-4]

A taxonomic enzyme profile must include as much information on polymorphism as can be obtained. The problems which can result from establishing taxonomic enzyme profiles from small numbers of isolates are noted in the following example. If L. donovani WR130 from Khartoum and L. donovani WR352 from India are compared, 68% of 25 enzymes would be identical by CAE, but when the WR352 profile is compared to ten other L. donovani isolates, the levels of identity are greater. Furthermore, if the enzyme analysis were confined to MDH, ICD, GOT, ALAT, AK1, EST, ACP1, MPI (Table 1) there would have been 0% identity and the two isolates would have been considered as two different species complexes. Therefore, taxonomic profiles must be based on many systems and on data from a large number of isolates representing the entire geographical distribution of the group.

The identification of Leishmania based on 29 genetic loci produces more accurate results; however, it should be possible to make a rapid and accurate identification using only 2 or 3 enzymes once the geographic area of isolation is known. For example the WRAIR isolates WR209 (L. b. panamensis), WR359 (L. b. braziliensis), WR225 (L. m. amazonensis), WR381 (L. m. mexicana), and WR285 (L. chagasi) are all from Panama. These isolates can be separated as follows:

| | |
|---|---|
| GPI | 209 and 359 are identical. This pair differs from 225, 381 and 285 which differ from one another. |
| ALAT | 359 and 285 are identical. This pair differs from all others. |
| MPI | Each differs one from another. |

Isolates from the Ft. Sherman area in Panama would require the use of WR209, WR225 and WR285 as reference subspecies. Any of the three enzymes could be used to establish that an unknown is L. m. amazonensis and not the other subspecies, while MPI could be used to establish that the unknown as either L. b. panamensis or L. b. braziliensis. From this example it is evident that baseline studies on isozyme polymorphism are required to select the few taxonomically relevant enzymes to be used in a simplified identification procedure.

Approach

This study was designed to establish methods which the clinical laboratory technician can follow in the field to rapidly and accurately identify leishmanial isolates from U.S. military personnel. Emphasis was placed on identification of New World and Kenyan isolates in support of military operations in Central America and USAMRU-Kenya.

Isolates which are grown in any medium that can support sufficient growth of promastigotes are examined by CAE using up to 29 enzyme systems. Attempts were made to study at least 20 different isolates of each subspecies with adequate histories to obtain information on natural polymorphism. Standard enzyme profiles have been established in a previous study,[11] and unknown isolates were compared to these standard profiles. The enzyme profiles of each subspecies are compiled to determine which enzymes have taxonomic value.

Although the procedure for CAE identification of leishmanial isolates is simple, it should be possible to further reduce the time

and effort required to rapidly and accurately identify isolates. A simple kit designed for a particular geographic area will be assembled. This kit will contain instructions, buffers, stains, controls, and information on interpretation of results.

Technical Objectives

1. Determine the minimal biomass of cells needed to produce enzyme data for identification.
2. Characterize large numbers of isolates of each Leishmania to obtain information on population polymorphism which might interfere with identification.
3. Correlate data from 1 and 2 to prepare a short and accurate CAE test for identification.
4. Characterize and identify isolates from U.S. military personnel for at least 20 enzymes.
5. Concurrent with 1-4 search for possible parasite parameter/enzyme data relationships.
6. Continue to adapt additional enzyme systems to Leishmania cells searching for diagnostic enzymes.
7. Collaborate with WRAIR scientists and contractees.

Results

Isolates Identified

The isolates in this study have been characterized for the enzyme listed in Table 1. Certain isolates have been designated as WHO reference isolates, Table 2. The enzyme profiles of these enzymes have been used as standards, and the profiles of other

isolates have been compared to these reference profiles. A major objective of this study was to obtain CAE data from large numbers of isolates to obtain information on population polymorphism. The isolates which have been identified are listed in Table 3. Table 4 combines the information on geographical distribution of the isolates studies, and data on the number of isolates of each Leishmania which have been studied are included in Table 5.

In this study enzyme data from over 20 isolates of New World visceral, L. donovani chagasi and Old World visceral, L. d. donovani, have been obtained. For most of the enzymes studied (Appendix 1, Table 2) both groups are identical and monomorphic; however, for ASAT, GOT and MDH the allomorph frequency differences between the two groups of isolates are as follows:

| | ASAT* | | GOT* | | MDH | |
|---|---|---|---|---|---|---|
| | Fast | Slow | Fast | Slow | Fast | Slow |
| L.d.d. | 0.69 | 0.31 | 0.50 | 0.50 | 0.31 | 0.69 |
| L.d.c. | 0.00 | 1.00 | 0.00 | 1.00 | 0.00 | 1.00 |

*ASAT and GOT might be the same enzyme.

These data suggest that if a visceral isolate has the "slow" allomorph for ASAT and MDH, it is only 20% likely that it is LD. Similar data are not yet available for separating 1. infantum and L.d.c.

L. mexicana mexicana and L. m. amazonensis have distinct enzyme profiles. At present only two isolates of L. m. garnhami and two of L. m. pifanoi have been studied. No consistent differences have been noted between the profiles of L.m.a. and L.m.g. or the

profiles of L.m.m. and L.m.p.; therefore, until larger samples of LMG and LMP are studied, it is not possible to separate these pairs.

The profile of L. b. guyanensis is very similar to that of L. b. panamensis, but both profiles are quite different from the profile of L. b. braziliensis. The profiles of L.b.p. and L.b.g. differ as follows:

| ACP | | MDH | | ME | | 6PGDH | |
|---|---|---|---|---|---|---|---|
| L.b.p. | L.b.g. | L.b.p. | L.b.g. | L.b.p. | L.b.g. | L.b.p. | L.b.g. |
| A 1.00* | A 0.00 | A 0.68 | A 0.00 | A 0.05 | A 1.00 | A 0.00 | A 1.00 |
| B 0.00 | B 1.00 | B 0.32 | B 1.00 | A 0.84 | B 0.00 | B 0.33 | B 0.00 |
| C 1.00 | C 0.00 | | | C 0.06 | C 0.00 | C 0.67 | C 0.00 |
| D 0.00 | D 1.00 | | | | | | |

*Allomorph frequencies.

Therefore, data from ACP and 6PGDH can be used to identify isolates of these two subspecies.

In summary CAE enzyme profiles have been established for L.b.p., L.b.b., L.b.g., L.m.m./L.m.p., L.m.a./L.m.g., L. m. aristedesi, L. m. enriettii, L.d.d., L.d.c., L. major, L. tropica, L. aethiopica, L. hertigi hertigi and L. gerbilli.

Leishmania diversity

As noted in previous reports there appears to be a direct relationship between the number of isolates characterized from a particular geographical locale and the number of subspecies identified in that area. Among 83 isolates from Panama, 37 from Brazil, 50 from Colombia, and 14 from Belize there is a higher level of subspecies diversity than among isolates from other geographical

regions which have been less vigorously studied (Table 4). These supporting data on the relation between diversity and numbers of isolates examined emphasize the need to examine large numbers of isolates from all geographical areas from which leishmaniasis has been reported to obtain an accurage picture of the degree of Leishmania diversity.

Routine identification for Walter Reed Army Institute of Research (WRAIR) contractees

In addition to the WRAIR primary isolates, samples for CAE identification were received from and identified for individual WRAIR personnel and contractees. These are noted in Table 3. Certain of the identified Colombian isolates are not in the WRAIR cryobank. In each case the isolate identification was based on comparative data from previously identified WHO reference strains. In certain cases the CAE identification was made prior to initiation of other studies on the isolate and later follow up confirming identification was made during the course of studies by the contractee. It is suggested that groups involved in Leishmania research either establish CAE identification in their own laboratories or that identification confirmation be made at this laboratory prior to study. In addition CAE identification should be made a standard procedure in each project. Preliminary and confirmatory CAE identification is considered necessary, because certain isolates received at this laboratory from various sources and labeled as a particular species were identified by CAE as being other than the indicated species or as mixed cultures. Identification by CAE is too simple and accurate not to take advantage of the process.

Short test for isolate identification

General

Isolates of a particular subspecies have a high level of enzyme similarity (over 75%), but usually no two isolates are 100% identical. The allozyme differences among isolates are the result of natural polymorphism and can be of importance when studying biological parameters other than identification. Enzyme polymorphism can affect electrophoretic identification of Leishmania isolates. If, for example, 6PGDH were the only enzyme used for an identification, L.b.p., L.b.b., and L.b.g. could be separated even though two differently migrated bands have been observed among isolates of L.b.p. and L.b.b. (non-overlapping polymorphism). L.m.m. and L.m.a. which are also polymorphic for 6PGDH could not be separated because their polymorphism is overlapping. Then 6PGDH can identify L. braziliensis subspecies but not L. mexicana species. Enzymes chosen for a mini test identification should have either no or non-overlapping polymorphism, but ones which can separate Leishmania species and subspecies. Another consideration is the choice of enzymes which are very active (i.e. produce bands with small numbers of cells) and which are relatively simple to prepare. One monomorphic enzyme, simply prepared, with distinctly migrating bands for each Leishmania subspecies would be sufficient, unfortunately no single enzyme yet studied meets all of these requirements.

Population enzyme polymorphism is probably the most difficult parameter to determine. It requires study of many isolates (ideally 20) from the entire distributional range of the subspecies, but for

many Leishmania subspecies only a few isolates from a restricted geographical area have been studied. In this study attempts were made to reduce possible errors in biochemical identification by collecting data from many isolates with well documented (if possible already identified) histories from multiple geographical areas, Tables 3, 4 and 5. The data from the isolates already run indicate that enzyme polymorphism is either minor or not present for the enzymes GOT, GPI, $GSR_1$, ICD, MDH, MPI and 6PGDH; furthermore, these enzymes can be used to separate most New and Old World Leishmania. These enzymes produce good activity from small numbers of cells, and buffer/strain components have been changed so each system can be preweighed, sent through the mail and requires only the addition of distilled water prior to use. Although there are eight enzymes noted, the recommended short-test for Leishmania identification will include only GPI, MPI, and 6PGDH.

The preparation of this short test for identification of Leishmania is the major objective of this study. Coincidentally, it has been established that CAE identification plus confirmation data can be obtained for the enzymes GPI, MPI, 6PGDH from about $10^6$ cells. The idertification is accurate, and the procedures as enumerated in Appendix 1 should make it possible for any laboratory with the logistical capabilities necessary to culture the parasite with minor additional equipment and chemical purchase be able to identify their isolates by CAE.

In summary most Leishmania for which enzyme profiles are available can be separated by study of three enzymes, GPI, MPI and

6PGDH. These are active enzymes for which CAE procedures have been simplified and among which only minor enzyme polymorphism has been observed. Most of these data on the short test have already been made available to and are being used by personnel in the Leishmania section at WRAIR for isolate identification.

Computer analysis

The CAE data which have been generated by this and other studies have been compiled in a computer program so they are readily available to personnel at WRAIR. The program for the CAE data is menu operated and IBM compatible. Data from each Leishmania subspecies are combined into a file, and each file has columns for ID number, parasite species, date, enzyme and polymorphism. This system allows simultaneous determination of numbers of enzymes used for isolate identification, allomorphs of the isolate and a comparison of the isolate's enzyme polymorphism with the polymorphisms of all other isolates of the same subspecies. In addition it facilitates the polymorphism analysis of each subspecies, as well as providing a compiled visible picture of which enzymes remain to be tested for an isolate. Disk containing these data have been forwarded to the Leishmania section at WRAIR. It was suggested by Major Lovelace that it was necessary to include the tables from these disks in the final report; therefore, I have not included them but note their availability.

New enzyme

One new enzyme system has been developed, $GSR_2$. It is an isozyme with $GSR_1$. Data from $GSR_2$ can be used to separate the

Leishmania in the complexes L. braziliensis, L. mexicana and L. donovani, Fig. 1. Unfortunately, data from not one of the enzymes listed in Table 1 can separate all Leishmania, but as noted in Appendix 1 most can be separated by study of the enzymes GPI, MPI, and 6PGDH. The CAE procedures for $GSR_2$ has been included in Appendix 1, Table 1.

Publications and reports at meetings

Kreutzer, R. D., N. Souraty and P. B. McGreevy. 1983. New World diffuse cutaneous leishmaniasis:possibly one enzyme type. Annual meeting of Am. Soc. Trop. Med. Hyg.

McGreevy, P. B., R. D. Kreutzer, E. D. Franke, H. A. Stimson, C. N. Oster and L. D. Hendricks. 1983. Taxonomy, clinical pathology and prognosis of leishmaniasis in U. S. soldiers infected in Panama. Annual meeting Am. Soc. Trop. Med. Hyg.

Kreutzer, R. D. and N. Souraty. 1984. Accurate identification of Leishmania isolates by study of three enzymes. Annual meeting Am. Soc. Trop. Med. Hyg.

Kreutzer, R. D., N. Souraty, P. B. McGreevy and E. D. Franke. In review. A New World Leishmania which can cause either cutaneous or diffuse cutaneous leishmaniasis in human hosts. Am. J. Trop. Med. Hyg.

Chulay, J. D., C. N. Oster, P. B. McGreevy, R. D. Kreutzer and L. D. Hendricks. In review. American cutaneous leishmaniasis: clinical presentation and problems of patient management. Annals Intern. Med.

Kreutzer, R. D., N. Souraty and M. E. Semko. In review. Biochemical identities and differences among Leishmania species and subspecies. Am. J. Trop. Med. Hyg.

Table 1. Enzymes tested in this study.

| Enzyme | Enzyme Abbreviation |
|---|---|
| **Oxidoreductases** | |
| Lactate dehydrogenases (1.1.1.27) | LDH |
| Malate dehydrogenase (1.1.1.37) | MDH |
| Malic enzyme (1.1.1.40) | ME |
| Isocitrate dehydrogenase (1.1.1.42) | ICD |
| Phosphogluconate dehydrogenase (1.1.1.44) | 6PGDH |
| Glucose-6-phosphate dehydrogenase (1.2.1.49) | G6PDH |
| Gluthione reductase (1.6.4.2) | $GSR_1$, $GSR_2$ |
| **Transferases** | |
| Glutamate-oxaloacetate transaminase (2.6.1.1) | GOT & ASAT |
| Glutamate-pyruvate transaminase (2.6.1.2) | ALAT |
| Hexokinase (2.7.1.1) | HK |
| 6-Phosphofructokinase (2.7.1.11) | FK |
| Phosphoglucomutase (2.7.5.1) | $PGM_1$, $PGM_2$ |
| **Hydrolases** | |
| Acid phosphatase (3.1.3.2) | ACP |
| Peptidase D (3.4.13.9) | PEPD |
| **Lyases** | |
| Fumerate hydratase (4.2.1.2) | FUM |
| **Isomerases** | |
| Mannose phosphate isomerase (5.3.1.8) | MPI |
| Glucose phosphate isomerase (5.3.1.9) | GPI |

Table 2. WHO Reference isolates.

| Species | Reference Number |
|---|---|
| L. braziliensis panamensis (L.b.p.) | MHOM/PA/71/LS94 WR576 |
| L. b. guyanensis (L.b.g.) | MHOM/BR/75/M4147 WR577 |
| L. b. braziliensis (L.b.b.) | MHOM/BR/75/M2903 WR675 |
| L. mexicana amazonensis (L.m.a.) | IFLA/BR/67/PH8 WR670 |
| | MHOM/BR/73/M2269 WR669 |
| L. m. garnhami (L.m.g.) | MHOM/VE/76/JAP78 WR673 |
| L. m. mexicana (L.m.m.) | MNYC/BZ/62/M379 WR668 |
| | MHOM/BZ/82/BZ21 WR667 |
| L. m. pifanoi (L.m.p.) | MHOM/VE/57/LL167 WR671 |
| L. m. aristedesi (L.m.ar.) | MORY/PA/68/GML-3 WR681 |
| L. m. enriettii (l.m.e.) | MCAV/BR/45/L88 WR678 |
| L. donovani chagasi (L.d.c.) | MHOM/BR/74/M2682 WR685 |
| L. d. donovani (L.d.d.) | MHOM/ET/67/L82 WR684 |
| | MHOM/IN/80/DD8 WR657 |
| L. d. infantum (L.d.i.) | MHOM/FR/80/LEM235 WR658 |
| L. major (L.mj.) | MHOM/IL/67/Jericho II WR662 |
| | MHOM/SU/73/5-ASKH WR661 |
| | MRHO/SU/59/NGAL P WR663 |
| L. tropica (L.t.) | MHOM/IQ/65/LRC-L32 |
| | MHOM/SU/60/LRC-L39 WR683 |
| | MHOM/SU/74/K27 WR664 |
| L. aethiopica (L.ae.) | MHOM/ET/72/L100 WR666 |
| L. hertigi hertigi (L.h.h.) | MCOE/PA/65/C8* WR679 |
| L. gerbilli (L.ge) | MRHO/CN/60/LUMP1575 WR683 |

Table 2. Continued

| | |
|---|---|
| L. tarentolae (L.ta.)* | ATAR/DZ/34/TARII |
| L. adleri (L.ad.)* | RLAT/KE/54/LRC-L123 |
| L. agamae (L.ag.)* | RAGA/IL/29/LRC-27 |

*Not WHO reference isolates.

TABLE 3. CAE isolate identification.

| WRAIR No. | Category | Parasite Species | Host Species | Source | Locality | Other Designation |
|---|---|---|---|---|---|---|
| 271B[1] | OWV | LD[10] | Man | USAMRU-K | Kenya | LV650 |
| 307 | OWV | LD | Man | USAMRU-K | Kenya | 209762/400-78 |
| 310 | OWV | LD | Man | USAMRU-K | Kenya | 298807 |
| 312 | OWV | LD/LMM-P | Man | USAMRU-K | Kenya | 307024 |
| 326 | OWV | LD | Man | USAMRU-K | Kenya | 321827 |
| 417 | NWC | LMA-G | Hamster | LAN-SHAW | Brazil | LV79, MI841, LRCL309 |
| PJ001[2] | NWV | LC | Man | P.Jackson/ Reed | Brazil | BA3 |
| PJ002 | NWV | LC | Man | P.Jackson/ Reed | Brazil | BA4 |
| PJ003 | NWV | LC | Man | P.Jackson/ Reed | Brazil | BA7 |
| PJ004 | NWV | LC | Man | P.Jackson/ Reed | Brazil | BA9 |
| PJ005 | NWV | LC | Man | P.Jackson/ Reed | Brazil | BA10 |
| PJ006 | NWV | LMJ | - | P.Jackson/ Reed | Brazil | BA11 |
| PJ007 | NWV | LC | Man | P.Jackson/ Reed | Brazil | BA12 |
| PJ008 | NWV | LC | Man | P.Jackson/ Reed | Brazil | BA13 |
| CL002B[3] | NWC | LMM-P | Man | Corredor | Colombia | MHOM/CO/84/CL002B |
| CL005B | NWC | LMM-P | Man | Corredor | Colombia | MHOM/CO/84/CL005B |
| CL018A | NWC | LMM-P | Man | Corredor | Colombia | MHOM/CO/84/CL018A |
| CL020A | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/84/CL020A |
| CL021 | NWC | LMA-G | Man | Corredor | Colombia | MHOM/CO/84/CL021 |
| CL022 | NWC | LBB | Man | Corredor | Colombia | MHOM/CO/84/CL022 |
| CL023 | NWC | LBB | Man | Corredor | Colombia | MHOM/CO/84/CL023 |
| CL024 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/84/CL024 |
| CL026 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/84/CL026 |
| CL027 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/84/CL027 |
| NO32[4] | NWC | LBB | Man | Sacks | - | Torrez |
| NO33 | NWC | LB/LMA-G | Man | Sacks | - | Broos |
| NO34 | NWC | LBB | - | Sacks | - | WR608 |
| NO35 | NWC | LBB | - | Sacks | - | WR605 |
| NO36 | NWC | LBB | - | Sacks | - | WR604 |
| NO37 | NWC | LBB | - | Sacks | - | WR603 |
| NO39 | OWV | LD | Man | Neva | India | Mongi |
| NO40 | OWC | LAE | Man | Neva | Ethiopia | Hulum |
| NO41 | OWC | LAE | Man | Neva | Ethiopia | Kassahun |
| NO42 | OWC | LAE | Man | Neva | Ethiopia | Gede |
| NO43 | OWC | LAE | Man | Neva | Ethiopia | Degu |
| NO44 | OWC | LAE | Man | Neva | Ethiopia | Kassaye |
| NO45 | OWV | LD | Man | Neva | India | Sebalek |
| NO46 | OWV | LD | Man | Neva | India | Child |
| NO47 | NWC | LMM-P | Man | Neva | - | Morton |

TABLE 3. CAE isolate identification - continued.

| WRAIR No. | Category | Parasite Species | Host Species | Source | Locality | Other Designation |
|---|---|---|---|---|---|---|
| 047 | OWC | LT | Man | Bray | Bengal | L117RG, LRCL160, WR221B |
| 221 | OWC | LT | Man | Bray | Bengal | L117RG, LRCL160, WR047 |
| 339 | OWV | LD | Man | USAMRU-K | Kenya | 1336 |
| 350 | OWC | LT | Man | - | Iraq | LRCL32, LV142, WR296 |
| 353 | OWC | LMJ | Tatera sp. | Baringo | Kenya | LRCL119, LV181, LUMP1972 |
| 372 | OWV | LD | Man | USAMRU-K | Kenya | L89 |
| 375 | OWV | LD | Man | USAMRU-K | Kenya | IDH1908, 380544 |
| 576 | OWC | LT | - | - | - | - |
| 625 | NWC | LBP | Man | WRAIR | Panama | - |
| 626 | NWC | LBP | Man | WRAIR | Panama | - |
| 626B | NWC | LBP | Man | WRAIR | Panama | - |
| 627 | NWC | LBP | Man | WRAIR | Panama | - |
| 637 | NWC | LBP | Man | WRAIR | Panama | - |
| 638 | NWC | LBP | Man | WRAIR | Panama | - |
| 639 | NWC | LBP | Man | WRAIR | Panama | - |
| 640 | NWC | LBP | Man | WRAIR | Panama | - |
| 641 | NWC | LBP | Man | WRAIR | Panama | - |
| 642 | NWC | LBP | Man | WRAIR | Panama | - |
| 642B | NWC | LBP | Man | WRAIR | Panama | - |
| 643 | NWC | LBP | Man | WRAIR | Panama | - |
| 644 | NWC | LBP | Man | WRAIR | Panama | - |
| 645 | NWC | LBP | Man | WRAIR | Panama | - |
| 646 | NWC | LBP | Man | WRAIR | Panama | - |
| 654 | NWC | LBP | Man | WRAIR | Panama | - |
| CL001B | NWC | LBP | Sandfly | Corredor | Colombia | ITRA/CO/81/CL001B |
| CL002C | NWC | LMM-P | Sandfly | Corredor | Colombia | MHOM/CO/84/CL002C |
| CL005C | NWC | LBP | Sandfly | Corredor | Colombia | MHOM/CO/84/CL005C |
| CL006B | NWC | LBP | Sandfly | Corredor | Colombia | ITRA/CO/81/CL006B |
| CL015B | NWC | LBP | Sandfly | Corredor | Colombia | ITRA/CO/84/CL015B |
| CL018B | NWC | LMM-P | Man | Corredor | Colombia | MHOM/CO/84/CL018B |
| CL031 | NWC | LBB | Man | Corredor | Colombia | MHOM?CO/84/CL031 |
| CL033 | NWC | LBB | Man | Corredor | Colombia | MHOM/CO/84/CL033 |
| CL035 | NWC | LBB | Man | Corredor | Colombia | MHOM/CO/84/CL035 |
| CL038 | NWC | LBB | Man | Corredor | Colombia | MHOM?CO/84/CL03? |
| CL039 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO?84/CL039 |
| CL041 | NWC | LBB | Man | Corredor | Colombia | MHOM/CO/84/CL041 |
| CL042 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/84/CL042 |
| CL043 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/84/CL043 |
| CL044 | NWV | LC | Man | Corredor | Colombia | MHOM/CO/84/CL044 |
| KO30 | NWC | LBG | - | Keithly | - | - |
| KO31 | NWC | LBG | - | Keithly | - | - |
| KO32 | NWC | LBG | - | Keithly | - | - |
| KO33 | NWC | LBG | - | Keithly | - | - |
| KO34 | NWC | LBG | - | Keithly | - | - |
| KO35 | NWC | LMA-G | - | Keithly | - | - |
| KO36 | NWC | LMA-G | - | Keithly | - | - |

TABLE 3. CAE isolate identification - continued.

| WRAIR No. | Category | Parasite Species | Host Species | Source | Locality | Other Designation |
|---|---|---|---|---|---|---|
| 126 | NWC | LMM-P | Man | WRAIR | Texas | WR127, ATCC30883, LV468 |
| 363 | NWC | LMA-G | Man | WRAIR | Brazil | LBO16C, M1287 |
| 367N | NWC | LBB | Man | WRAIR | Brazil | LTBO014, WR386 |
| 411B | OWC | LT | Man | WRAIR | Texas | |
| 503N | VIS | LC/D | Dog #5 | WRAIR | Oklahoma | WR373R |
| 603 | NWC | LBB | Man | WRAIR | Brazil | LTB564A |
| 606 | NWC | LBB | Man | WRAIR | Brazil | LTB560 |
| 609N | NWC | LBB | Man | WRAIR | Brazil | LTB559 |
| 618 | NWC | LBB/BP | Man | WRAIR | Honduras | H-12 |
| 618N | NWC | LBB/BP | Man | WRAIR | Honduras | H-12 |
| 621 | NWC | LBB/BP | Man | WRAIR | Honduras | See WR620 |
| 621N | NWC | LBB/BP | Man | WRAIR | Honduras | See WR620 |
| 626N | NWC | LBP | Man | WRAIR | Panama | |
| 626BN | NWC | LBP | Man | WRAIR | Panama | |
| 628N | NWC | LBP | Man | WRAIR | Panama | |
| 655 | NWV | LC | Man | WRAIR | - | |
| 656 | NWC | LBP | Man | WRAIR | - | |
| 657* | OWV | LD | Man | WRAIR | India | MHOM/W/80/DD8 |
| 658* | OWV | LI | Man | WRAIR | - | LEM235 |
| 661* | OWC | LMJ | Man | WRAIR | - | SASKH |
| 662* | OWC | LMJ | Man | WRAIR | Israel | MHOM/IL/67/Jer[illegible] |
| 664* | OWC | LT | Man | WRAIR | ? | K27 |
| 666* | OWC | LAE | Man | WRAIR | Ethiopia | MOHM/ET/72/L10C |
| 667* | NWC | LMM | - | WRAIR | Belize | BEL 21 |
| 668* | NWC | LMM | *Nyctomys* | WRAIR | Belize | MNYC/BZ/74/M379 |
| 669* | NWC | LMA | - | WRAIR | - | M2269 |
| 670* | NWC | LMA | Sandfly | WRAIR | Brazil | IFLA/BR/67/Ph8 |
| 671* | NWC | LMP | Man | WRAIR | Venezuela | MHOM/VE/57/LL1 |
| 673* | NWC | LMG | - | WRAIR | - | JAP78 |
| 675* | NWC | LBB | Man | WRAIR | Brazil | MHOM/BR/75/M29C[illegible] |
| 676* | NWC | LBP | Man | WRAIR | Panama | MHOM/PA/71/L594 |
| 677* | NWC | LBG | Man | WRAIR | Brazil | MHOM/BR/75/M41[illegible] |
| 678* | | LME | - | WRAIR | - | L88 |
| 679* | | LHH | Coendou | WRAIR | Panama | MCOE/PA/65/C8 |
| 681* | | LMAR | - | WRAIR | - | GML3 |
| 682* | | LGE | Gerbili | WRAIR | - | GERBILLI |
| 683* | OWC | LT | Man | WRAIR | USSR | MHOM?SO/60/LRC-[illegible] |
| 684* | OWV | LD | Man | WRAIR | Ethiopia | MHOM/ET/67/L83 |
| 685* | NWC | LC | Man | WRAIR | Brazil | MHOM?BR/74/M268[illegible] |
| 394B | NWC | LBB? | - | WRAIR | - | - |
| 674 | NWC | LMM-P | CAT | WRAIR | - | |
| 675N | NWC | LBB | Man | WRAIR | Brazil | MHOM?BR/75/M29C[illegible] |
| 710 | VIS | Lc/LI | - | WRAIR | - | |
| 663* | OWC | LMJ | Man | WRAIR | - | |
| R125 | | ? | - | WRAIR | - | |
| R170 | OWC | LT? | - | WRAIR | - | |
| R300A | | LT/LMA | - | WRAIR | - | |
| R300B | | LD/LMA | - | WRAIR | - | |
| R335 | OWC | LT? | - | WRAIR | - | |
| TX HAMS | NWC | LMA-G | - | WRAIR | - | |

TABLE 3. CAE isolate identification - continued.

| WRAIR No. | Category | Parasite Species | Host Species | Source | Locality | Other Designation |
|---|---|---|---|---|---|---|
| CLO17B | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/85/CLO17B |
| CLO19A | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/84/CLO19A |
| CLO20B | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/84/CLO20B |
| CLO25 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/84/CLO25 |
| CLO29 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/85/CLO29 |
| CLO46 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/85/CLO46 |
| CLO47 | NWC | LBG | Man | Corredor | Colombia | MHOM/CO/85/CLO47 |
| CLO48 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/85/CLO48 |
| CLO49 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/85/CLO49 |
| CLO52 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/85/CLO52 |
| CLO58 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/85/CLO58 |
| CLO60 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/85/CLO60 |
| CLO63 | NWC | LBB | Man | Corredor | Colombia | MHOM/CO/85/CLO63 |
| CLO64 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/85/CLO64 |
| CLO66 | NWC | LBP | Man | Corredor | colombia | MHOM/CO/85/CLO66 |
| CLO69 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/85/CLO69 |
| CLO70 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/85/CLO70 |
| CLO72 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/85/CLO72 |
| CLO73 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/85/CLO73 |
| CLO79 | NWC | LBP | Man | Corredor | Colombia | MHOM/CO/85/CLO79 |
| CLO80 | NWC | LBB | Man | Corredor | Colombia | MHOM/CO/85/CLO80 |
| CLO82 | NWC | LBG | Man | Corredor | Colombia | MHOM/CO/85/CLO82 |
| CLO83 | NWC | LBB | Man | Corredor | colombia | MHOM/CO/85/CLO83 |
| CLO85 | NWC | LC | Man | Corredor | Colombia | MHOM/CO/85/CLO85 |
| KO38 | OWC | LMJ | - | Keithly | - | |
| KO39 | NWC | LBG | - | Keithly | - | |
| KO41 | NWC | LBB | - | Keithly | - | |
| KO42 | NWC | LBP | - | Keithly | - | |
| KO43 | NWC | LBP | - | Keithly | - | |
| 003 | NWC | LBP | Man | - | Panama | Husbands |
| 004 | NWC | LBP | Man | - | Panama | Marlow |
| 111C | NWC | LBP | Man | - | Belize | Salgado |
| 111LN | NWC | LBP | Man | - | Belize | Salgado |
| 132 | NWC | LBP | Man | - | Panama | Tush |
| 154 | NWC | LBP | Man | - | Panama | Hernandez |
| 176 | NWC | LBP | Man | - | Panama | Watterson |
| 179 | NWC | LBP | Man | - | Panama | Legoas |
| 211 | NWC | LBP | Man | - | Panama | Fizer |
| 232 | NWC | LBP | Man | - | Panama | Barstow |
| 241 | NWC | LBP | Man | - | Panama | Martin |
| 246 | NWC | LBP | Man | - | Panama | Boynton |
| 282 | NWC | LBP | Man | - | Panama | Morrison |
| 322 | NWC | LBP | Man | - | Panama | Kingsley |
| 360 | NWC | LBP | Man | - | Panama | Clonts |
| 390 | NWC | LBG | Man | - | Brazil | Ward |
| 442 | NWC | LBP | Man | - | Panama | Bennett |
| 446 | NWC | LBP | Man | - | Panama | Burgos |
| 470 | NWC | LBP | Man | - | Panama | Belisle |
| 475 | NWC | LBP | Man | - | Panama | Varsel |
| 486 | NWC | LBP | Man | - | Panama | Reinertsen |
| 487 | NWC | LBP | Man | - | Panama | Tyus |
| 491 | NWC | LBP | Man | - | Panama | Reinertsen |

TABLE 3. CAE isolate identification - continued.

| WRAIR No. | Category | Parasite Species | Host Species | Source | Locality | Other Designation |
|---|---|---|---|---|---|---|
| 492 | NWC | LBP | Man | - | Panama | Reinertsen |
| 493 | NWC | LBP | Man | - | Panama | Reinertsen |
| 505 | NWC | LBP | Man | - | Panama | Reyes |
| 525 | NWC | LBP | Man | - | Panama | Dominques L1 |
| 526 | NWC | LBP | Man | - | Panama | Dominques L2 |
| 539 | NWC | LBP | Man | - | Panama | Carillo |
| 530 | NWC | LBP | Man | - | Panama | Schoonmaker |
| 063 | NWC | LBG | Man | - | Peru | Terborgh/Muco |
| 294 | NWC | LBB | Man | - | Brazil | Jurandi |
| 359 | NWC | LBB | Man | - | Panama | Courtwright |
| 410 | NWC | LBB | Man | - | Panama | Askew |
| 508 | NWC | LBB | Man | - | Brazil | Barbosa |
| 524 | NWC | LMA-G | Man | - | Panama | Alderman |
| 531 | NWC | LMA-G | Man | - | Panama | Castro |
| 140 | NWC | LMM-P | Man | - | Peru | UTA |
| 381 | NWC | LMM-P | Man | - | Panama | Peters |
| 453 | NWC | LMM-P | Man | - | Dominican Republic | - |
| 457 | NWC | LMM-P | Man | - | Venezuela | - |
| 527 | NWC | LMM-P | Man | - | Venezuela | Gladys, DCL |
| 311 | OWV | LD | Man | - | Kenya | David Wambua |
| 285 | NWV | LC | Man | - | Panama | Wade |
| 317 | NWV | LC | Man | - | Panama | Natuel |
| 341 | NWV | LC | Man | - | Panama | Furr |
| 481 | - | LMAR | Man | - | Panama | Oryzomys |
| 528 | NWC | LMP | Man | - | Venezuela | NR |
| 316 | NWC | LSP | Man | - | Panama | Wilcoxen |
| 077 | NWC | LBP | Man | USAMRU-P | Canal Zone | - |
| 526 | NWC | LBP | Man | WRAIR | Panama | Dominquez |
| 535 | NWC | LBP | Man | WRAIR | Panama | Tyus |
| 539 | NWC | LBP | Man | WRAIR | Panama | Carrillo |
| 556 | NWC | LBP | Man | WRAIR | Panama | Evans |
| 566 | NWC | LBP | Man | EVans | Panama | LS94, LV44 |
| 568 | NWC | LBG | Man | Evans | Brazil | M4147, LV476 |
| 049 | NWC | LBB | Man | USAMRU-P | Canal Zone | Sablan, WR242 |
| 540 | NWC | LBB | Man | Evans | Belize | BZ10 |
| 541 | NWC | LBB | Man | Evans | Belize | BZ14 |
| 542 | NWC | LBB | Man | Evans | Belize | BZ15 |
| 543 | NWC | LBB | Man | Evans | Belize | BZ17 |
| 545 | NWC | LBB | Man | Evans | Belize | BZ25 |
| 557 | NWC | LBB | Man | Evans | Belize | BZ16 |
| 562 | NWC | LBB | Man | Evans | Brazil | LV436, M2903 |
| 348 | NWC | LMM-P | Man | Neal | Costa Rica | LV191 |
| 548 | NWC | LMM-P | Man | Evans | Belize | BZ18 |
| 549 | NWC | LMM-P | Man | Evans | Belize | BZ26 |
| 347 | NWC | LMM | Nyctomys | Neal | Belize | WR450, WR458, M379, LUMP1641-1965, LV4, L11, GML92 |

TABLE 3. CAE isolate identification - continued.

| WRAIR No. | Category | Parasite Species | Host Species | Source | Locality | Other Designation |
|---|---|---|---|---|---|---|
| 364 | NWC | LMA-G | Man | Keithly | Brazil | UISS150492, WR384, WR421 |
| 563 | NWC | LMA-G | Lutzomyia | Evans | Brazil | PH8, LV10 |
| 561 | NW | LHH | Coendou | Evans | Panama | LV42 |
| 206 | NWC | Lsp | Man | WRAIR | Brazil | Collier |
| 544 | NWC | Lsp | Man | EVans | Belize | Bz21 |
| 553 | NWC | Lsp | Man | Keystone | Ecuador | Chest |
| 554 | NWC | Lsp | Man | Keystone | Ecuador | Face |
| 560 | OWV | LD | Man | Evans | Ethiopia | WR354, HV3, L82, LRC-L133, LV9 |
| 564 | OWC | LT | Man | Evans | USSR | LRC-L39, LV357 |
| 565 | OWC | LAE | Man | Evans | Ethiopia | WR298, L100, LRC-L147, LV24 |
| 547 | OWC | LMJ | Man | R. Beach | Kenya | LRC-L137 |
| 551 | OWC | LMJ | Rodent | R. Beach | Kenya | NLB095 |
| 552 | OWC | LMJ | Sandfly | R. Beach | Kenya | NLB144 |
| 558 | OWC | LMJ | Man | Hendricks | Kenya | Beach, NLB173 |
| 559 | OWC | LMJ | Man | Hendricks | Kenya | Beach, NLB175 |
| 567 | OWC | LMJ | Man | Evans | Israel | LRC-L137, JERICHOII, LV561 |
| 550 | OWC | UNK | Sandfly | R. Beach | Israel | NLB136A |
| 007 | NWC | LBB | Man | USAMRU-P | Panama | Flores |
| 134 | NWC | LBP | Man | USAMRU-P | Panama | Acosta |
| 336 | DCL | LMM-P | Man | NIH | Dominican Republic | Ceclilo |
| 354 | OWV | LD | Man | Evans | Ethiopia | |
| 355 | OWC | LMJ | Man | ? | Jericho | |
| 357 | NWC | LMA | Man | Schnur | Brazil | |
| 409 | NWC | LBG | Man | Keithly | Surinam | Davis |
| 463 | NWC | LBG | Man | Evans | Brazil | Desouza |
| 467 | NWC | LBP | Man | Tulane | Colombia | Calcedo |
| 578 | NWC | LBP | Man | WRAIR | Panama | Placenta |
| 579 | OWC | LT | ? | Sacks/NIH | ? | Clone 121 |
| 580 | NWC | LBB | Man | Neva | ? | MUCO |
| 581 | OWC | LT | Man | NIH | ? | Ackerman |
| 582 | OWC | LT | ? | Sacks/NIH | ? | Clone 12 |
| 584 | OWC | LT | ? | Sacks/NIH | Afghan | Niazy |
| 366 | NWC | LBP | Man | Zeledon | Costa Rica | LB012, HSJD1 |
| 367 | NWC | LBB | Man | Keithly | Brazil | LTB014, WR386 |
| 369 | NWC | LMA-G | Man | Zeledon | Costa Rica | LB011, HSJD11 |
| 467 | NWC | LBP | Man | Tulane | Colombia | LTB 111 |
| 472 | NWC | LBB | Man | Marsden | Brazil | Aurelino |
| 484 | NWV | LC | Man | Reed | UNK | Hevania, BA7 |
| 485 | NWV | LC | Man | Reed | UNK | Wilton, BA1 |
| 513 | NWV | LC | Man | Reed | Brazil | Risia, BA3 |
| 514 | NWV | LC | Man | Reed | Brazil | Marcos, BA11 |
| 515 | NWV | LC | Man | Reed | Brazil | Elson, BA12 |

Table 3. CAE isolate identification - continued.

| WRAIR No. | Category | Parasite Species | Host Species | Source | Locality | Other Designation |
|---|---|---|---|---|---|---|
| 516 | OL | LME | Guinea Pig | Weinstein | UNK | |
| 517 | NWV | LC | Man | Reed | Brazil | Ranilson, BA4 |
| 518 | NWV | LC | Man | Reed | Brazil | Jose, BA7 |
| 519 | NWV | LC | Man | Reed | Brazil | Dominqos, BA8 |
| 520 | NWV | LC | Man | Reed | Brazil | Francisco, BA9 |
| 529 | OL | LME | Guinea Pig | Weinstein | UNK | |
| 585 | NWC | LMA | | WRAIR | Panama | WR227 |
| 586 | NWC | LMJ | | WRAIR | ? | WR367, WR386 |
| 587 | NWC | LMJ | ? | WRAIR | ? | WR508 |
| BE004[6] | ? | LMA-G | Hamster | WRAIR | Berman | Foot |
| BE006 | ? | LMA-G | Hamster | WRAIR | Berman | Spleen |
| BE008 | ? | LMJ | ? | WRAIR | Berman | 173 |
| BE009 | ? | LMJ | ? | WRAIR | Berman | 220 |
| BE005 | ? | LMJ | ? | WRAIR | Berman | LTB |
| BE010 | ? | LMJ | ? | WRAIR | Berman | 420 |
| BE007 | ? | LMJ | ? | WRAIR | Berman | 15A |
| G001[7] | ? | LD/LC | ? | Hanson | | G128A |
| G002 | ? | LD/LC | ? | Hanson | | G128B |
| G003 | ? | LD/LC | ? | Hanson | | G128C |
| G004 | ? | LD/LC | ? | Hanson | | G128D |
| G005 | OWV | LD | Man | Hanson | Khartoum | Parent strain, G378 |
| G006 | OWV | LD | Man | Hanson | Khartoum | Resistant, G555 |
| G007 | NWC | LBP | Man | WRAIR | Panama | Carrillo, G539A |
| G008 | NWC | LBP | Man | WRAIR | Panama | G539B |
| K028 | NWC | LMA-G | ? | Keithly | ? | CD5/84 |
| K026 | NWC | LMM-P | ? | Keithly | ? | CB5/85 |
| K029 | NWC | LMA-G | ? | Keithly | ? | $CD_2$5/84 |
| K009 | ? | LBG | ? | Keithly | ? | M1142 |
| K010 | ? | LBG | ? | Keithly | ? | M2904 |
| K025 | ? | LBB | ? | Keithly | ? | CA |
| K026 | ? | LMM-P | ? | Keithly | ? | CB |
| | ? | LBB | ? | Keithly | ? | M1287 |
| K014 | ? | LBG | ? | Keithly | ? | Wild. CP |
| K015 | ? | LBG | ? | Keithly | ? | Clone 8-2. CX |
| K016 | ? | LBG | ? | Keithly | ? | Clone 8-3. CY |
| K017 | ? | LBG | ? | Keithly | ? | Clone 7-D. CZ |
| K018 | ? | LBG | ? | Keithly | ? | CAPCP |
| K019 | ? | LMM-P | ? | Keithly | ? | $CBS_1P$ |
| K020 | ? | LMA-G | ? | Keithly | ? | $CCS_6P$ |
| K021 | ? | LD?LC | ? | Keithly | ? | $CDPCP^8$ |
| K022 | ? | LBB | ? | Keithly | ? | $CES_5P$ |
| K023 | ? | LMA-G | ? | Keithly | ? | $CFS_6P$ |
| K024[8] | ? | LBB | ? | Keithly | ? | $CGS_1P$ |
| B001 | OWV | LD | Man | Marr | Ethiopia | $B_1$ |
| B002 | OWV | LD | Man | Farrell | Ethiopia | $B_1^2$ |
| B003 | OWV | LD | Man | Blackwell | Ethiopia | $B_3$ |
| 233 | NWC | LMA-G | Proechimys | Lainson | Brazil | WR302, LUMP1718-1899, LV78, M1845, LB016C, M1287 |

TABLE 3. CAE isolate identification - continued.

| WRAIR No. | Category | Parasite Species | Host Species | Source | Locality | Other Designation |
|---|---|---|---|---|---|---|
| 321 | NWC | L? | Man | UNK | Brazil | 211.497, GOTAS |
| 363 | NWC | LMA-G | Man | Lair | Brazil | |
| 374 | NWC | Lsp | Man | Beach | Surinam | Pepper Trail |
| 405 | NWC | LBB | Man | Keithly | Brazil | Ferreira. LTB082 |
| 473 | NWC | LBB | Man | Marsden | Brazil | Julio |
| 489 | NWC | LMG | Man | Peters | Venezuela | JAP78, LUMP1568 |
| 508 | NWC | LBB | Man | Marsden | Brazil | LTB0012 |
| 509 | NWC | LMA | Man | Marsden | Brazil | Marino, B05-2 |
| 601 | UNK | LD | UNK | UNK | UNK | |
| 604 | NWC | LBB | Man | UNK | BRazil | LTB558 |
| 607 | NWC | LBB | Man | UNK | Barbosa | LTB12, Mucosa |
| 608 | NWC | LBB | Man | UNK | Brazil | LTB300 |
| 609 | NWC | LBB | Man | UNK | Brazil | LTB559, Corte de Pedro |
| 610 | NWC | LBB | Man | UNK | Manause | |
| 611 | NWC | ?? | Lutzomyia | UNK | Brazil | |
| CL002A | NWC | LMM-P | Man | Tesh | Colombia | Sanchez, Y-JS |
| CL013A | NWC | LBP | Man | Tesh | Colombia | Posado, Y-BP |
| CL005A | NWC | LBP | Man | Tesh | Colombia | Maturana, Y-LOM |
| CL016 | NWC | LBP | Man | Tesh | Colombia | Artamide, Y-LA |
| A0019 | UNK | L? | UNK | Anthony | UNK | |
| A002 | UNK | L? | UNK | Anthony | UNK | |
| A003 | UNK | LMJ | UNK | Anthony | UNK | |
| A004 | UNK | lMJ | UNK | Anthony | UNK | |
| A005 | UNK | L? | UNK | Anthony | UNK | |
| A006 | UNK | LMJ | UNK | Anthony | UNK | |
| A007 | UNK | L? | UNK | Anthony | UNK | |
| A008 | UNK | LMM | UNK | Anthony | UNK | |
| A009 | UNK | L? | UNK | Anthony | UNK | |
| BE001 | UNK | LMJ | UNK | Berman | UNK | Ullman, from amastigotes |
| BE002 | UNK | LMJ | UNK | Berman | UNK | 508B |
| BE003 | UNK | lMJ | UNK | Berman | UNK | Old pros. 508A |
| K001 | UNK | LBG | UNK | Keithly | New World | CUMC1 7/84 |
| K002 | UNK | LBB/LD | UNK | Keithly | New World | CUMC2, 7/84 |
| K003 | UNK | LBB/LD | UNK | Keithly | Old World | CUMC3, 7/84 |
| K004 | UNK | LBG | UNK | Keithly | New World | X 9/84 |
| K005 | UNK | LBG | UNK | Keithly | New World | Y 7/84 |
| 346 | NWC | LMA | Orizomys capito | Neal | Brazil | LV81, M1824 |

[1]Walter Reed isolates
[2]P. Jackson isolates
[3]Colombia isolates
[4]NIH isolates
[5]Keithly isolates
[6]Berman isolates
[7]Hanson isolates
[8]Bonventre isolates
[9]Anthony isolates
[10]CAE identification

LMM-P - Leishmania mexicana mexicana - pifanoi
LMA-G - L. m. amazonensis - garnhami
LMG - L. garnhami
LMP - L. pifanoi
LBB - L. braziliensis braziliensis
LBP - L. b. panamensis
LBG - L. b. guyanensis

TABLE 3. CAE isolate identification - continued.

LT - L. tropica
LMJ - L. major
LMAR - L. m. aristidesi
LAE - L. aethiopica
LD - L. donovani
LC - L. chagasi
LI - L. infantum
LME - L. enrietti
LHH - L. hertigi hertigi
LGE - L. gerbilli

Table 4. Geographical frequency distribution of isolates.

| Old World | New World |
|---|---|
| Afghanistan 1-L.t. | Belize 7-L.b.b., 5-L.m.m. |
| Algeria 1-L.ta. | |
| China 1-L.ge. | Brazil 2-L.b.g., 13-L.b.b., 10-L.m.a. 4-L.m.m, 1-L.m.e., 10-L.d.c., 1-L.h.h. |
| Ethiopia 1-L.d.d., 6-L.ae. | Colombia 33-L.b.p., 3-L.b.g., 19-L.b.b., 2-L.m.a., 3-L.m.m., 5-L.d.c. |
| France 2-L.d.i. | Costa Rica 2-L.b.p., 1-L.m.a. |
| India 5-L.d.d., 1-L.t. | Dominican Republic 6-L.m.m. |
| Iran 1-L.d.d. | Guatemala 3-L.m.m. |
| Iraq 1-L.t. | Honduras 1-L.d.c. |
| Israel 3-L.mj., 1-L. ag. | Nicaragua 1-L.b.p. |
| Kenya 12-L.d.d., 6-L.mj., 1-L.t., 1-L.ad. | Panama 48-L.b.p., 4-L.b.b., 12-L.m.a., 1-L.m.m., 1-L.m.ar., 4-L.d.c., 2-L.h.h. |
| Malta 1-L.t. | Peru 1-L.b.g., 1-L.m.m. |
| Senegal 1-L.mj. | Suriname 1-L.b.g. |
| Soviet Union 2-L.mj., 1-L.t. | United States 1-L.m.a., 1-L.m.m. |
| Sudan 6-L.d.d. | Venezuela 2-L.m.a., 1-L.m.g., 5-L.m.m., 2-L.m.p. |

Table 5. Numbers of isolates in each subspecies or species of *Leishmania* tested. Note: 20 of each type should be required to produce a valid profile of the group. Isolates are from various laboratories.

| Species or Subspecies | Number of Isolates |
|---|---|
| *L.g.p.* | 104 |
| *L.b.b.* | 56 |
| *L.m.m.* | 43 |
| *L.m.a.* | 47 |
| *L.d.c.* | 27 |
| *L.d.i.* | 2 |
| *L.d.d.* | 28 |
| *L.mj.* | 32 |
| *L.t.* | 25 |
| *L.b.g.* | 28 |
| *L.m.e.* | 2 |
| *L.ae.* | 7 |
| *L.ar.* | 1 |
| *L.m.p.* | 2 |
| *L.m.g.* | 2 |
| *L.h.h.* | 4 |
| *L.ge.* | 1 |
| *L.ta.* | 1 |
| *L.ad.* | 1 |
| *L.aq.* | 1 |

*At present it is not possible to separate *L.m.m.* from *L.m.p.*, *L.m.a.* from *L.m.g.*, *L.d.c.* from *L.d.i.*

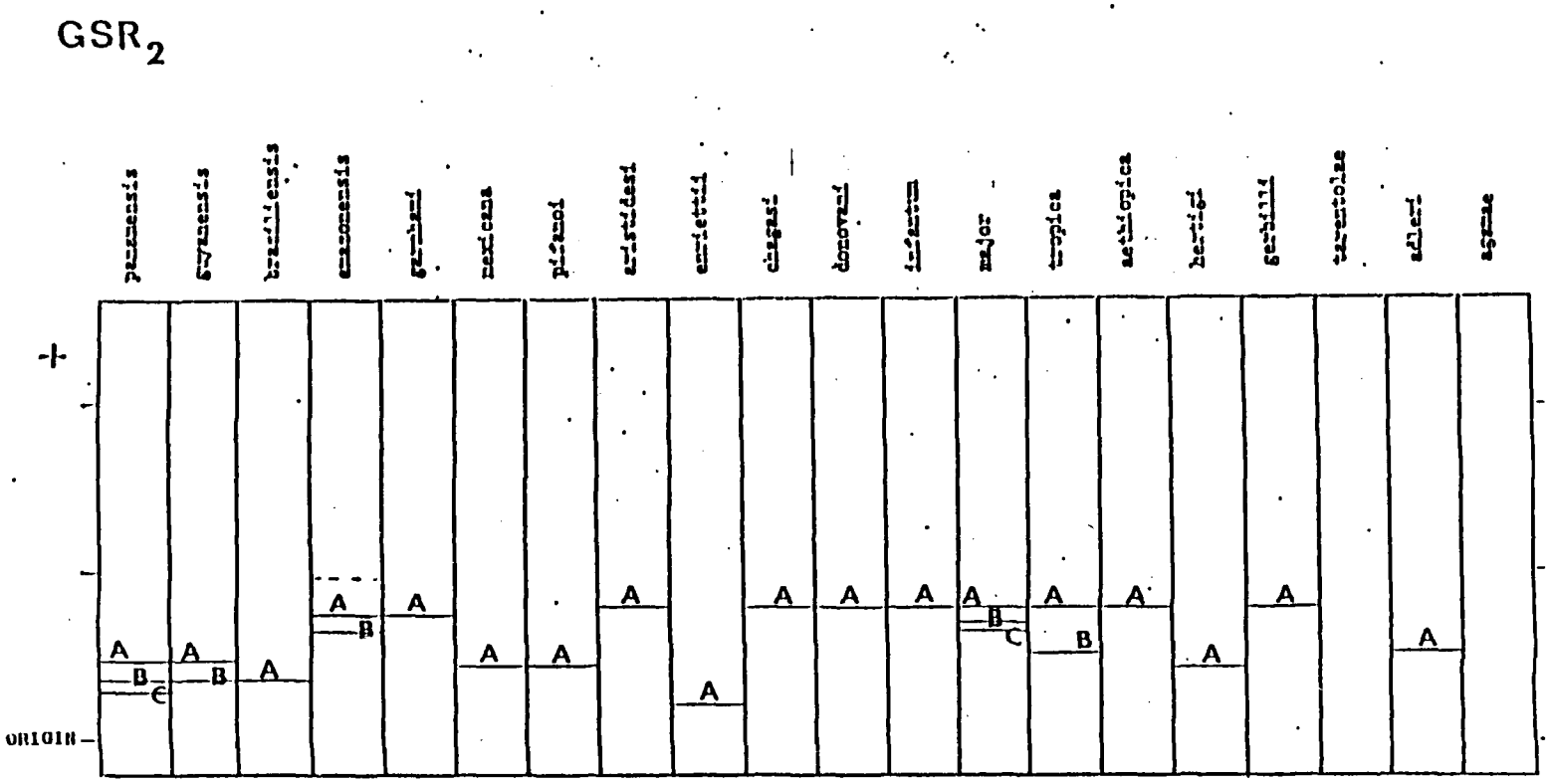



Figure 1. Diagrammatic representations of the CAE patterns of $GSR_2$ for various Leishmania. Note: More than one band for a Leishmania indicates different allomorphs or natural polymorphism.

Appendix 1. Short test procedure.

Test for identification of a $10^6$ cell biomass of Leishmania.

Data from over 400 Leishmania isolates indicate that an accurate identification of most Leishmania can be made from cellulose acetate electrophoresis (CAE) data from the enzymes glucose phosphate isomerase (GPI) EC #5.3.1.9, mannose phosphate isomerase (MPI) EC #5.3.1.8, phosphogluconate dehydrogenase (6PGDH) EC #1.1.1.44.

Materials needed:

- Nobel agar
- Distilled water
- Chemicals - see Appendix 1, Table 1
- Normal saline

Equipment needed:

- Centrifuge - 1,000 g potential (cooled preferred)
- Mettler type balance - 4-5 place
- Nunc tubes - 2 ml
- Vortex
- pH meter
- Stirring hotplate plus sitrring bars
- Stirrer
- Microscope slides
- Cryomarker
- Microdispenser - 1-10 µl
- Pipettes - 1-100 µl and 100-1,000 µl
- Petri dishes (100 x 45 mm)
- CAE equipment (Helena Labs, Beaumont, Texas)

Titan Power Supply (Cat. No. 1500, 110V)

Zip Zone Chamber (Cat. No. 1283)

Disposable Wicks (Cat. No. 5081)

Super Z Applicator Kit (Cat. No. 4088)

a. Applicator

b. Sample Well Plate

c. Aligning Base

Zip Zone Plates (Cat. No. 3023)

Procedure

1. Cell/medium separation and cell preparation for CAE.

   A. About $10^6$ cells (a visible biomass) are needed; however, visible cell masses grown on and picked off blood agar medium in petri dishes without a liquid overlay are sufficient.

   B. Pour cells cultured in any liquid medium into centrifuging tubes and centrifuge at about 1,000 g for 5-10 min.

   C. Pour off medium.

   D. Add 0.5 ml normal saline to the pellet.

   E. Transfer saline and cells to a 2 ml nunc tube and centrifuge at about 1,000 g for 5-10 min.

   F. Remove all saline using a pipetter if necessary.

   G. Add 6 µl/$10^6$ cells of buffer (14 parts distilled water: 1 part cell buffer 1 or 2 - Appendix 1, Table 1) to the cells. For larger cell pellets add buffer about 1/3 the size of the pellet.

   H. Resuspend the cells in the buffer with a vortex.

I. Rapidly freeze and thaw three times.

a. Store $10^6$ cells/buffer at -70°C until needed.

b. For larger cell masses separate lysate from the cell debris at about 1,000 g for 5-10 min., remove the lysate from the cell debris, place lysate in a 2 ml nunc tube and store at -70° C until needed.

2. Buffer/stain preparation and conditions for CAE. See Appendix 1, Table 1. If a pH meter is available, it is recommended that cell buffer 1 be used for GPI.

A. Buffer preparation.

1. Cell buffers 2-5 and reaction buffers B-G can be prepackaged, sealed and stored at room temperature indefinitely.

2. Combine the buffer components with the appropriate amounts of distilled water and stir until dissolved.

3. Buffers can be prepared for any final volume, the amounts noted in the table are for preparation of one liter of each buffer.

B. Stain preparation.

1. Stain components can be prepackaged, sealed and stored at -30° C indefinitely.

2. Combine 25 ml of the appropriate reaction buffer with 0.5 g nobel agar and bring to boiling on a stirring hotplate.

3. Cool to 50° C.

4. While the agar is cooling, add 25 ml of the reaction buffer to the substrate/stain components and stir until dissolved. It might be necessary to use a glass rod to break up large particles which do not readily dissolve.
5. Pour the cool (50° C) agar into the substrate stain solution and continue stirring for 5 seconds.
6. Pour the mixture into 100 x 15 mm petri dishes (5-6 dishes) and store at 4° C.
7. It is recommended that the stain plates be used as soon as possible.

3. Electrophoresis

A. Preparation of the Zip Zone chamber.

1. Add 100 ml of cell buffer to each outer well of the Zip Zone Chamber and apply a paper wick to each center rib. Remove all air bubbles.
2. If cooling is necessary, add ice cubes to the center wells. Ice should not touch the wicks.

B. Zip Zone Plates should be carefully placed in the membrane buffer (prevent trapping air bubbles in the plate) and soaked for 5-10 minutes prior to use.

C. Place 3 µl portions in the slots of the Sample Well Plate and dilute as noted in Appendix 1, Table 1.

D. Remove Zip Zone Plates from the buffer, blot dry and place on the Aligning Base parallel to the second line from the top of the Base.

E. Use the Applicator to transfer the lysate from the Sample Well Plates to the Zip Zone Plate.

F. Transfer the Zip Zone Plate to the Zip Zone Chamber. The place is applied lysate side down and application side to the negative side of the chamber. The plate should be perpendicular to the paper wicks. Place microscope slides on the plate to insure wick/plate contact.

G. Set appropriate time and voltage.

1. After electrophoresis remove the Zip Zone Plate, blot the ends dry, cut the plate to fit the petri dish and apply it enzyme side down to the substrate/stain/agar plate. Carefully press out any air bubbles.

H. Monitor the plates and mark the bands as they appear. Mark the plates on the plastic side with a cryomarker.

I. After all samples have been marked, remove the plate, place it in 5% Acetic acid for 30 seconds, wash in tap water and allow to air dry.

4. Controls. To identify an isolate a comparison of the unknown to a known must be made. Control Leishmania isolates are any which have already been identified by electrophoresis and are prepared as noted above. It is recommended that only isolates designated as reference isolates by WHO be used as controls. The object of the control is to produce a band of migration so unknown isolate bands can be compared with it. Two bands

which migrate equally indicate identity. If a known isolate and an unknown produce identically migrating bands for GPI, MPI and 6PGDH, the unknown and the known are the same Leishmania.

A. Preliminary run controls in Sample Well Plate for a New World isolate.

1. L.b.b. well 3.
2. L.b.p. well 4.
3. L.m.a. well 5.
4. L.d.c. well 6.
5. Wells 1, 2, 7, 8 for unknowns or if necessary other controls.

B. Confirming run controls. After a preliminary identification has been made, a second run should be made. Each unknown should be placed next to the suspected control. This will confirm both bands migrate identically.

C. Old World isolates will require different controls.

5. Sequence of enzymes leading to identification.

A. Run GPI, Appendix 1, Figure 1.

1. Separate - L.b. complex subspecies - L.b.p., L.b.b., L.b.g.; L.d. complex subspecies - L.d.d., L.d.c., L.d.i.; L.m.m./p; L.m.a./g.; L.ar.; L.t.; L.h.h.; L.ae.-L.g.e.
2. Identical band: L.b. complex - L.mj.-L.m.e.; L.d. complex; L.h.h.; L.ae.-L.ge.

B. After GPI, run MPI Appendix 1, Figure 2.

1. Separate (in addition to data in 5A1 above - L.b.b., L.ae., L.ge., L.mj., L.m.e., L.h.h.

2. Identical band: L.d. complex, L.b.p.-L.b.b.

C. After GPI and MPI, run 6PGDH Appendix 1, Figure 3.

1. Separate (in addition to data in 5A1 and 5B1 above) - L.b.p.-L.b.b.-L.b.g.

2. Identical band: L.d. complex.

D. L.d. complex subspecies identification.

1. Geographical area of isolation.

2. Run GOT (ASAT) and MDH with L.d.d. and L.d.c. controls - If both allomorphs are "slow", it is 35% likely that the isolate is L.d.d. or 65% likely it is L.d.c. Appendix 1, Table 2.

3. At present no reliable CAE procedures are available to separate LI from LD or LC, L.m.a. from L.m.g., L.m.m. from L.m.p.

Cell buffers:

1. 0.1 M. Tris (12.11 g/l)/0.1 M Maleic acid (M-0379) (11.62 g/l)/0.01 M EDTA (ED-2SS) ($Na_2$)(2.92 g/l)/0.01 M $MgCl_2$ (M-0250) (2.03 g/l); adjust to pH 7.4 with 40% NaOH.
2. 0.1 M Tris (12.11 g/l)/0.05 M Maleic Acid (5.81 g/l)/0.01 M EDTA (2.92 g/l)/0.11 M Sodium phosphate dibasic (S-0876) (16 g/l), pH 7.4.
3. 0.2 M Phosphate buffer: 7.1 g $Na_2HPO_4$/3.24 g $NaH_2PO_4$ (S-0791) in 385 mL distilled water, pH 7.0.
4. 0.29 M Tris (28.12 g/l)/0.09 M Citric acid monohydrate (C-7129) (1.89 g/l), pH 7.0.
5. 0.05 M Tris (6.06 g/l)/0.05 M $NaH_2PO_4$ (6.0 g/l), pH 7.5.

Reaction buffers:

A. 0.1 M Tris (12.11 g/l), adjust to pH 8.0 with 50% HCl.
B. 0.06 M Tris (7.28 g/l)/0.04 M Sodium phosphate monobasic (4.72 g/l), pH 8.0.
C. 0.25 M Tris (30.24 g/l)/0.103 M Sodium phosphate monobasic (12.34 g/l), pH 8.4.
D. 0.1 M Tris (12.11 g/l)/0.069 M Sodium phosphate monobasic (8.21 g/l), pH 8.0.
E. 0.018 M Sodium phosphate monobasic/0.082 M Sodium phosphate dibasic, pH 7.4.
F. 0.1 M Tris (12.11 g/l)/0.101 M Sodium phosphate monobasic 12.12 g/l), pH 7.5.

Number of applications of the aliquot to the cellulose acetate plate:

Dilutions are made using 1 part cell buffer either buffer 1 or 2 and 14 parts distilled water.
1:1 dilution then 1X: GPI, MPI.
1X from lysate: GOT, $GSR_2$, MDH, 6-PGDH.

*To make 50 mL of stain (about 6 petri dishes). All chemicals from Sigma.

**The membrane buffers are dilutions of 1 part cell buffer: distilled water.

***The CA plates after electrophoresis are placed on substrate petri dishes for + 10 min. at 37°C; then blotted dry and placed on the stain petri dishes in which the bands are monitored.

[a]These systems require cooling during electrophoresis.

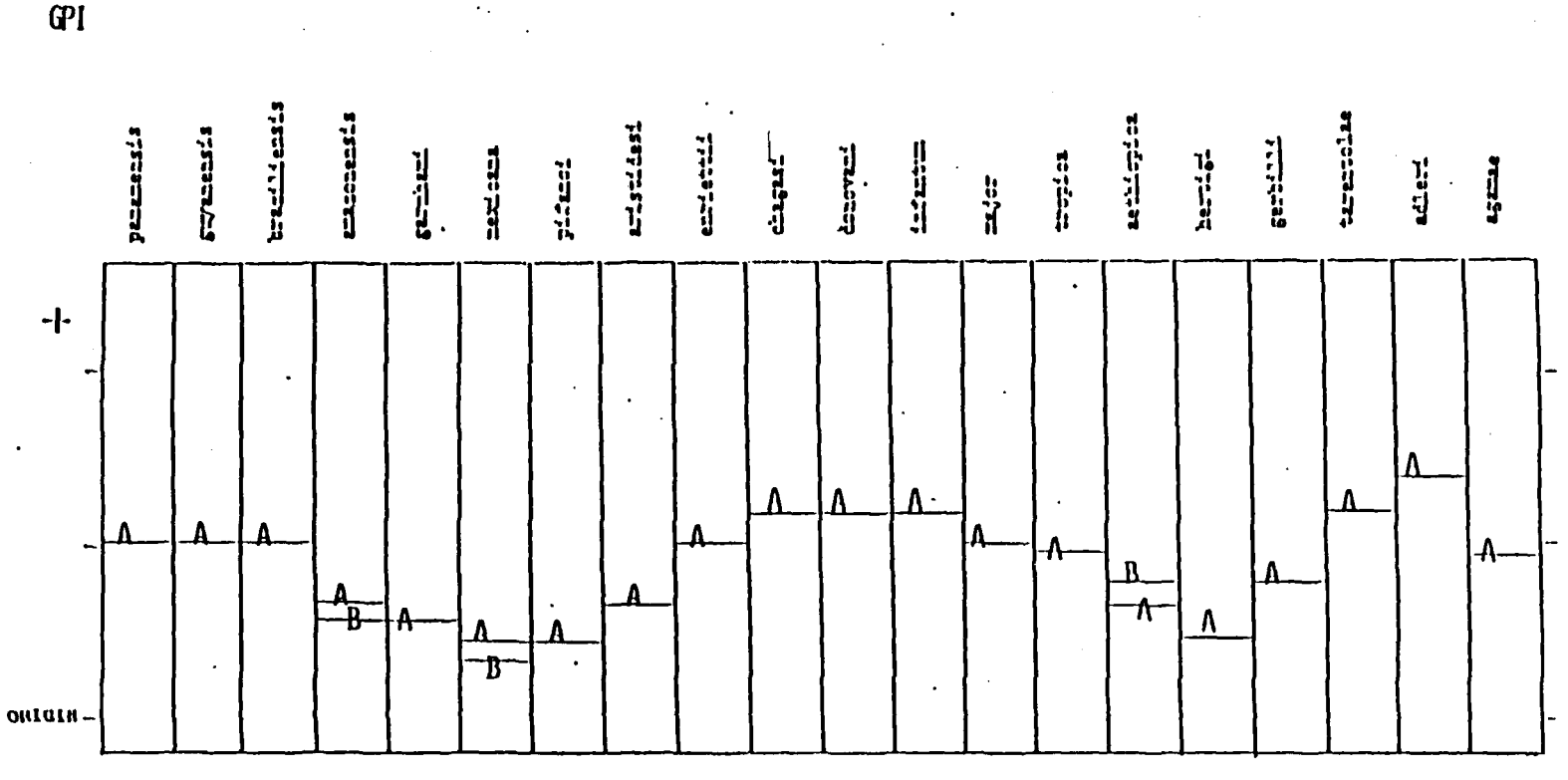
[b]This system requires viewing with Ultra-Violet light for the bands to be visible.

Appendix 1, Table 1. Conditions for electrophoresis and components for developing zymograms.

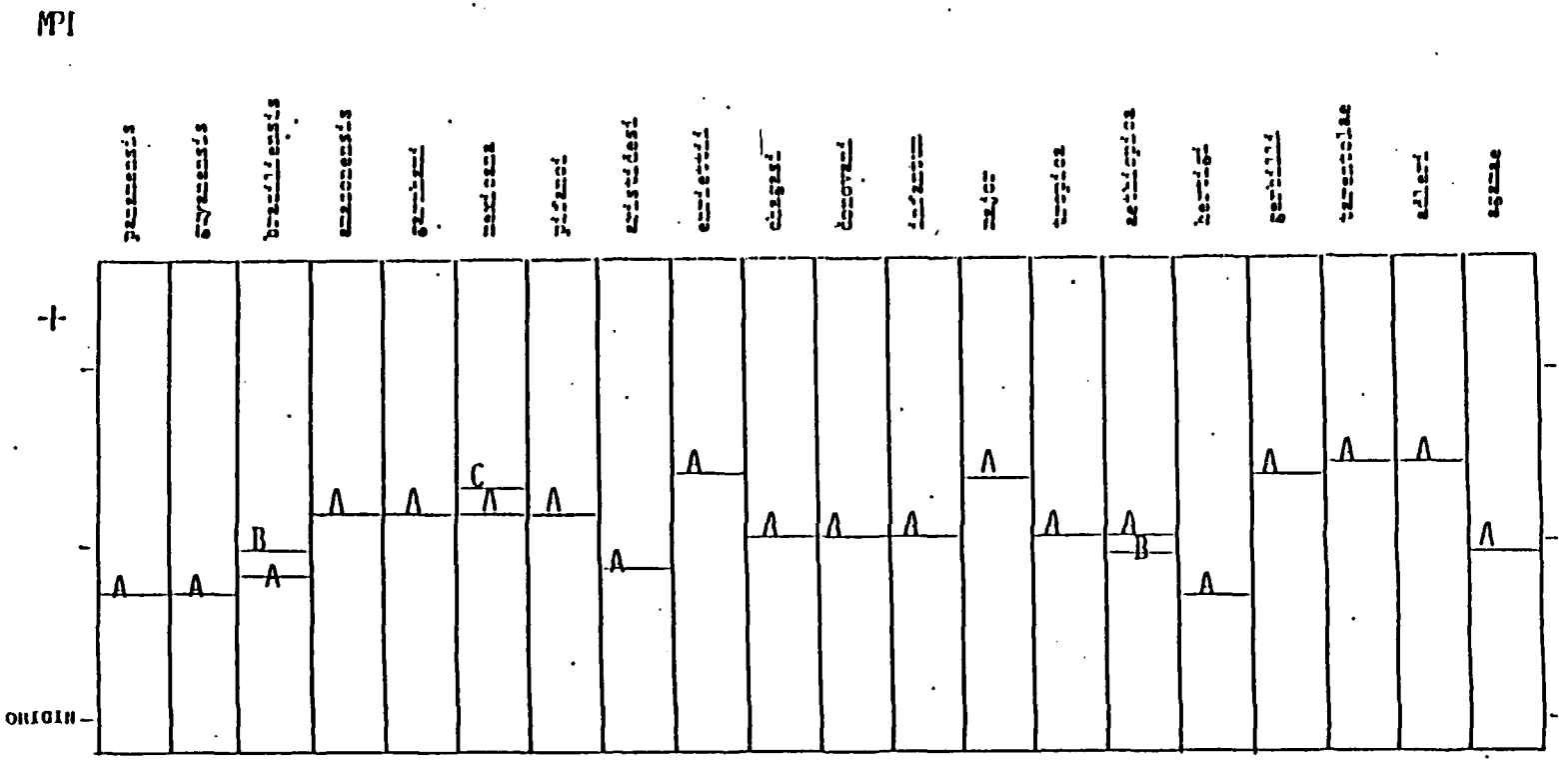
| Enzyme | Cell Buffer | Membrane** Buffer | Voltage | Run Time (Minutes) | Reaction Buffer | Substrate/Stain Components* |
|---|---|---|---|---|---|---|
| GOT | 1 or 2 | 1:14 | 180 | 15 | A or D | Substrate: 100 mg L-Aspartic acid (A-9256); 75 mg $\alpha$-Keto-glutaric acid (K-1875), readjust to pH 8.0; add 10 mg Pyridoxal-5-phosphate (P-9255); pour this mixture with agar*** by itself in petri dishes. Stain: (second petri dish) 75 mg Fast blue BB (F-3378). |
| GPI | 1 or 2 | 1:14 | 180 | 15 | B | 20 mg Fructose-6-phosphate (F-1502); 15 mg MTT Tetrazolium (M-2128); 15 mg $\beta$-TPN (Na salt) (N-0505); 10 mg Phenazine methosulfate (P-9625) (PMS); 60 mg EDTA; 25 units (1 flake) Glucose-6-phosphate dehydrogenase (G-6378) (Bakers yeast); 120 mg $MgCl_2$. |
| $GSR_2$[a] | 3 | 1:9 | 200 | 15 | C | 30 mg Oxidized Glutathione (G-4376); 5 mg $\beta$-NADH (N-8129); < 1 mg 2, 6-Dichlorophenol-indophenol (D-1878); 15 mg MTT. |
| MDH[b] | 4 | 1:9 | 200 | 15 | E | 15 mg oxalacetic acetic (340-48); 15 mg $\beta$-NADH ($Na_2$ salt). |
| MPI | 5 | 1:5 | 160 | 15 | F | 15 mg Mannose-6-phosphate (M-8754); 10 mg $\beta$-NADP ($Na_2$); 10 mg MTT; 5 mg PMS; 1 mg Glucose phosphate isomerase (P-8391); 15 units (1 flake) Glucose-6-phosphate dehydrogenase; 40 mg $MgCl_2$. |
| 6-PGDH[a] | 3 | 1:14 | 180 | 15 | B | 15 mg 6-Phosphogluconic acid (P-6888) ($Na_3$ salt); 15 mg MTT; 15 mg $\beta$-TPN; 10 mg PMS; 60 mg EDTA ($Na_2$ salt); 120 mg $MgCl_2$. |

Appendix 1, Table 2. Allomorph frequencies for ASAT and MDH in L.d.c. and L.d.d.

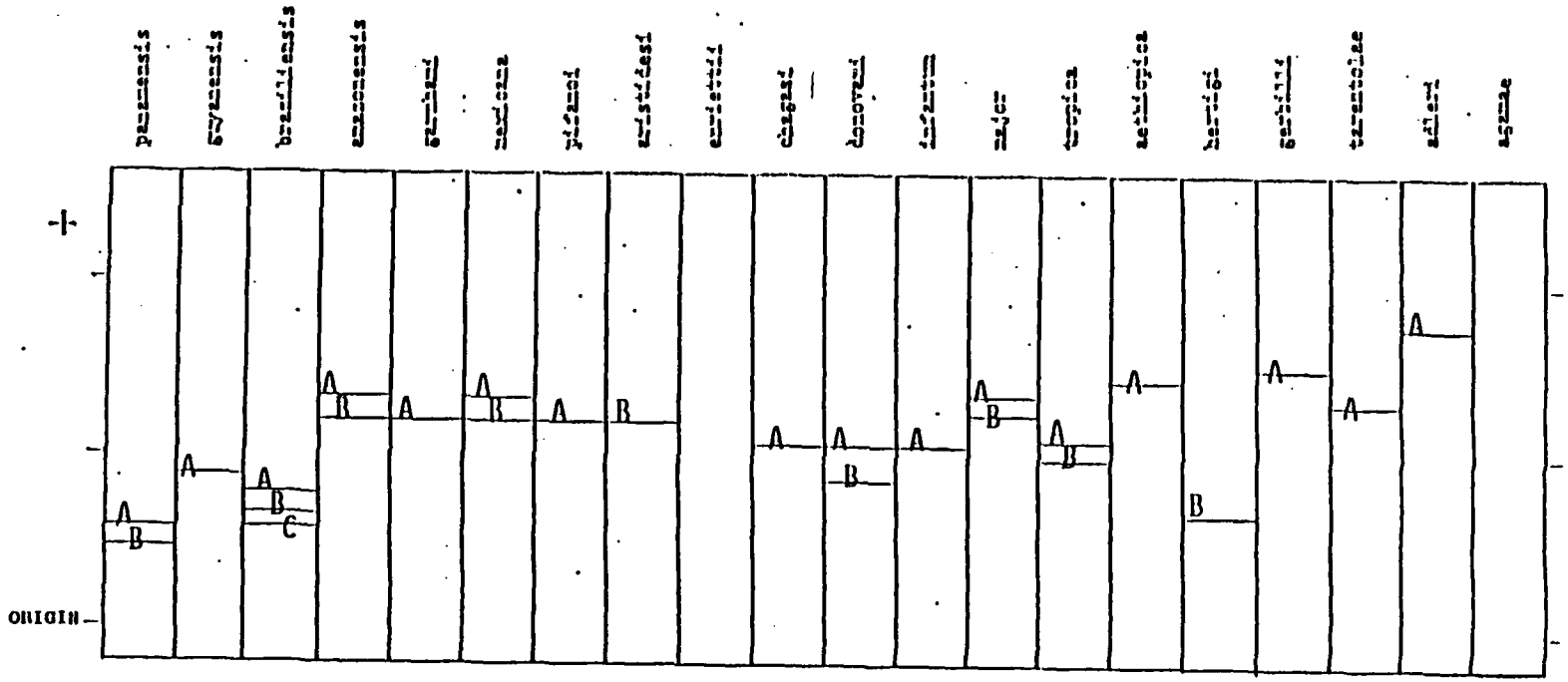
| | ASAT | | | MDH | |
|---|---|---|---|---|---|
| | L.d.c. | L.d.d. | | L.d.c. | L.d.d. |
| Fast | 0.0 | 0.7 | Fast | 0.0 | 0.32 |
| Slow | 1.0 | 0.3 | Slow | 1.0 | 0.68 |




Appendix 1, Figure 1. Diagrammatic representations of the CAE patterns of GPI for various *Leishmania*. Note: More than one band for a Leishmania indicates different allomorphs or natural polymorphism.




Appendix 1, Figure 2. Diagrammatic representations of the CAE patterns of MPI for various Leishmania. Note: More than one band for a Leishmania indicates different allomorphs or natural polymorphism.




Appendix 1, Figure 3. Diagrammatic representations of the CAE patterns of 6PGDH for various Leishmania. Note: More than one band for a Leishmania indicates different allomorphs or natural polymorphism.

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