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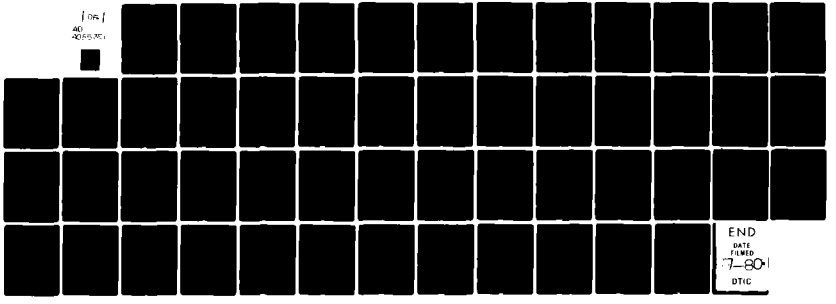

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DEVELOPMENT OF SPECIAL BIOLOGICAL PRODUCTS (U)

Annual Progress Report

by

Joseph L. DeMeio
Donald E. Craig
William J. Thomas
Chung K. Lee

January 1980

Supported by

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

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The Salk Institute
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Tissue Culture
Pasteurella Tularensis

20. ABSTRACT (Continue on reverse side if necessary and identify by block number) THIS REPORT COVERS THE FOLLOWING BIOLOGICAL RESEARCH AND DEVELOPMENTS:

(A). Rift Valley Fever (RVF) Vaccine Development -

RVF vaccine was prepared and tested. Experiments were done and lots tested for residual live virus. Shipments of vaccine were made to Israel and USAMRIID. Submission for Lots 4 through 10 was prepared. A vaccine prepared in BHK-21 cells for animal use was delivered. A second lot of diagnostic antiserum was prepared and shipped. Disrupted RVF was sent to Dr. Milstien. Vaccine prepared by Agouza Institute was tested; [redacted]

19. Continued
RVF HA Antigen
FRhL-2
MRC-5
BHK-21

20. Continued

(B) Rift Valley Fever (RVF) HA Antigen -
Five liters of antigen were prepared;

(C) Rift Valley Fever (RVF) H1849 Virus Strain -
The H1849 strain of RVF virus was tested;

(D) Rift Valley Fever (RVF) SA-51 Virus Strain -
Growth curves and potency tests were done;

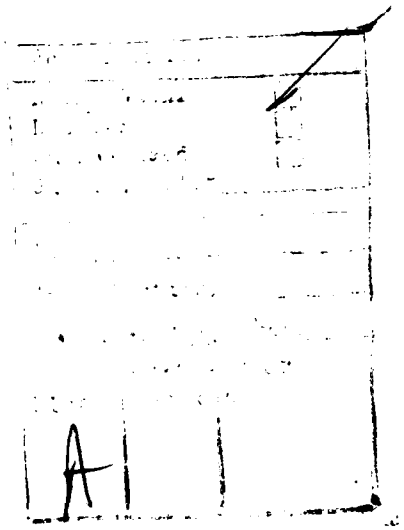
(E) VEE (C-84) Vaccine Development -
A pilot-run was conducted;

(F) Venezuelan Equine Encephalomyelitis (VEE) Vaccine Immunizations -
Plaque reduction neutralization tests were performed on sera from seven new employees;

(G) Tissue Culture -
Production lots of FRhL-2 and MRC-5 were stabilized and frozen. Two of the lots of FRhL-2 were certified;

(H) Pasteurella Tularensis, Live -
Seventeen vials of the SCHU-S5 strain were transferred to USAMRIID;

(I) Eastern Equine Encephalitis (EEE) Vaccine Development -
Two hundred vials were shipped.



Summary

A. Rift Valley Fever (RVF) Vaccine Development

The preparation and testing of Lots 7 through 19 and Lot 20 Run 1 of RVF vaccine have been completed. The potency test on Lot 20 Run 2 is in progress.

Experiments were performed in testing for residual live virus.

RVF vaccine Lots 1 through 14 were tested for residual live virus with negative results. Testing of Lot 15 by this procedure is in progress.

RVF vaccine was shipped to Israel and USAMRIID. Additional RVF vaccine was delivered to USAMRIID for clinical testing.

The vaccine submission for Lots 4 through 10 was prepared and submitted for approval.

An experimental vaccine prepared in BHK-21 cell cultures for animal use was delivered to USAMRIID.

A second lot of RVF diagnostic antiserum was prepared and sent to USAMRIID.

Chemically disrupted RVF virus was sent to Dr. Milstien for testing.

RVF vaccine prepared at the Agouza Institute was tested.

B. Rift Valley Fever (RVF) HA Antigen

The preparation of 5 liters of RVF antigen was completed this year. An additional 3½ liters with a titer of ≥1:1024 was requested. Work on this request is near completion.

Twenty-three batches of infected suckling mouse livers were harvested and 70 extractions made.

Antigens derived from suckling hamster livers had good titers but residual live virus was found during final safety testing.

C. Rift Valley Fever (RVF) H1849 Virus Strain

The H1849 strain of RVF virus was tested to determine its potential for use as a future vaccine.

D. Rift Valley Fever (RVF) SA-51 Virus Strain

Work was continued with the SA-51 strain of RVF virus which had been passaged during 1978.

Growth curves and potency tests were done to determine its potential as a candidate for future vaccine.

E. VEE (C-84) Vaccine Development

A pilot-run was conducted to garner information for preparation of 400 roller cultures from 2000 embryonated chicken eggs.

F. Venezuelan Equine Encephalomyelitis (VEE) Vaccine Immunizations

Plaque reduction neutralization tests were performed on sera from seven new employees given VEE Vaccine, Live, Attenuated.

G. Tissue Culture

Production lots of FRhL-2 and MRC-5 were stabilized and frozen. Two of the lots of FRhL-2 were certified. A total of 619 ampules of FRhL-2 were used to prepare 11 lots of RVF vaccine. Five lots of FRhL-2 and three of MRC-5 cells were depleted this year.

The karyology laboratory and dark room were established and put into operation.

H. Pasteurella Tularensis Vaccine, Live

Seventeen vials of the SCHU-S5 strain of P. tularensis were transferred to USAMRIID.

I. Eastern Equine Encephalitis (EEE) Vaccine Development

Two hundred vials of EEE Vaccine, Inactivated, Dried were shipped to USAMRIID.

Foreword

The authorization for the work contained herein was authorized under Contract No. DAMD17-78-C-8018, titled, "Development of Special Biological Products".

This annual report covers the period of January 1, 1979 to December 31, 1979. In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.

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Rift Valley Fever (RVF) Vaccine Development

I. Introduction

RVF vaccine production in FRhL-2 cell cultures continued through this period with the completion of Lots 7 through 19 and Lot 20 Run 1 as per the original vaccine submission. The potency test on Lot 20 Run 2 is in progress.

Experiments were performed in testing for residual live virus.

RVF vaccine Lots 1 through 14 have been tested for residual live virus by the Vero cell culture-weanling mouse test with negative results. Testing of Lot 15 by this procedure is in progress.

RVF vaccine Lots 1 through 3 were shipped to Israel and Lots 4 through 7 were sent to USAMRIID. Remaining quantities of these lots are stored at -20C to serve as file samples. Additional RVF vaccine was delivered to USAMRIID for clinical testing.

The vaccine submission for lots 4 through 10 was prepared and submitted for approval. The original submissions (Lots 1 through 3 and 4 through 10) were modified to include the residual live virus test and insert sheets forwarded to USAMRIID.

An experimental vaccine prepared in BHK-21 cell cultures for animal use was delivered to USAMRIID.

A second lot of RVF diagnostic antiserum was prepared and sent to USAMRIID.

Chemically disrupted RVF virus was sent to Dr. Milstien for testing.

Lots 7 and 19, prepared by the Agouza Institute, Cairo, Egypt were tested.

II. Vaccine Production

During 1979 Lots 7 through 19 and Lot 20 Run 1 of RVF vaccine have been prepared and completely tested with satisfactory results as per the original vaccine submission. The outline of procedures and tests used is shown in Figure 2 of the 1978 Annual Progress Report (No. 44-08-1278-TS1001). Final container tests are summarized in Table 1. The potency test on Lot 20 Run 2 is in progress.

In performing the final container potency test, which was done using the two dose-antigen extinction test in mice, a vaccine prepared in African Green monkey kidney cell cultures, namely Lot 6 Run 2 (NDBR 127) was included in each test to serve as a standard. Values obtained for the standard vaccine are recorded below the vaccine under test in Table 1.

III. Residual Live Virus Testing

A. Determination of Most Sensitive Host

The various titrations of RVF virus described in the "Revised Protocol for Residual Live Virus Test for RVF Virus Vaccine" submitted to USAMRIID and dated 14 March 1979 were completed. The results of these titrations along with one additional titration, that employing 10-12 g weanling mice receiving 0.5 ml i.p. (as per our current live virus safety test on 24 and 72 hour post-formalin addition samples) are shown in Table 2.

Of all the hosts tested, Vero cell culture exhibited the highest sensitivity to infection by RVF virus in the presence of neutralized RVF vaccine. However, the increase in titer in Vero cell cultures which was indicated in the live virus confirmation test in hamsters was due to a considerable number of culture fluids which were negative by CPE actually contained live virus detectable by hamster inoculation. The data from the Vero cell-hamster confirmation is presented in Table 3 to illustrate this point.

This data indicates that a four day incubation period as outlined in the protocol is not sufficient when using Vero cell cultures to detect residual RVF virus.

The significant drop in virus titer observed in all but one of the animal groups when neutralized vaccine pool (0.003% formalin) was employed as a diluent, prompted a second experiment to determine the value of dialysis in removing virus inhibitors, i.e. residual formalin and/or sodium bisulfite.

Neutralized vaccine pool was dialyzed against two 50 ml volume changes of BME-2% HSA at 4C over a two day period. The dialyzed preparation was compared with the non-dialyzed pool and with BME-2% HSA as diluents for the virus at full log dilutions. Ten hamsters were used per dilution and results are as follows:

Host	Dose i.p.	Titer (log_{10} $IPLD_{50}$/ml) Diluent		
		BME-2% HSA	Neutr. Vaccine Pool	Dial. Neutr. Vaccine Pool
Hamster, Male (6 W.)	1.0	7.55	6.40	6.41

Data indicates that dialysis is of no value in increasing the test sensitivity in hamsters.

The Vero culture fluids titrated in hamsters in the initial investigation leading to the Residual Live Virus Test were titrated in weanling mice with the following confirmatory results:

Diluent	Vero ($TCID_{50}$/ml)	Hamster ($IPLD_{50}$/ml)	Weanling Mouse ($WMIPLD_{50}$/ml)
BME with 2% HSA	7.4	7.8	8.1
Neutralized Vaccine	7.4	7.91	8.32

Five weanling mice (10-12 g) were used per culture fluid with a 0.4 ml i.p. inoculum so as to test the same total volume of culture fluids tested in the hamster. Fluids were considered positive if one or more animals died after day one. Results shown represent the $\log_{10}$ of the titer.

As a result of the above testing the Vero cell culture-weanling mouse test, described in Dr. Cole's letter of 14 June 1979 (Ref. SGRD-UIV-I) was the procedure followed as the official test for residual live virus.

B. Testing of Production Lots

RVF vaccine Lots 1 through 14 have been tested for residual live virus by the Vero cell culture-weanling mouse test with negative results. Reconstituted vaccine (100 doses/run) was used for Lots 1 through 11 while 200 ml of a 72 hour post-inactivation liquid sample was tested for Lots 12 through 14.

IV. Vaccine Shipments

RVF vaccine Lots 1 through 3 were shipped to Israel and Lots 4 through 7 were sent to USAMRIID. Remaining quantities of these lots are stored at -20C to be retained as file samples.

Six 5.5 ml fill vials each of Lot 7 Run 1, Lot 8 Run 2, Lot 9 Run 1 and Lot 10 Run 2 RVF vaccine (TSI-GSD 200) were sent to USAMRIID for clinical testing and copies of potency tests conducted on both runs of Lots 1 through 10 were forwarded to USAMRIID to permit a comparison of mouse potency test results from our laboratory with responses obtained from clinical trials conducted at USAMRIID.

V. Submissions

A vaccine submission for Lots 4 through 10 was prepared and submitted for approval. The original submission for Lots 1 through 3 was modified to include the residual live virus test and, subsequently, the submission for Lots 4 through 10 was also modified to include this test and insert sheets were forwarded to USAMRIID.

VI. RVF Vaccine - For Use in Animals

RVF vaccine (TSI-GSD 204) prepared in BHK-21 cell cultures per instructions received from USAMRIID 22 May 1979 was transferred to Ft. Detrick. The shipment of this vaccine, designated NOT FOR HUMAN USE consisted of one hundred 21 ml fill vials. A separate report giving details of preparation and evaluation was sent to USAMRIID 6 November 1979. Table 4 contains results of the final container testing of this product.

VII. RVF Diagnostic Antiserum

A second lot of diagnostic antiserum (TSI-GSD 201) was prepared in rabbits using the same procedures as used for Lot 1. One hundred vials containing 0.5 ml each have been shipped to USAMRIID. Hemagglutination-inhibition titer of the serum when tested against a reference mouse liver antigen was 1:640.

VIII. RVF Virus - Disruption for Testing Purposes

Twenty milliliters of RVF virus fluid (vaccine, Lot 7, prior to formalin addition) was processed as outlined in Dr. Cole's letter of March 1979.

The high speed supernate was hand carried to Dr. Julie Milstien at the NIH for tests designed to show the presence or absence of mouse leukemia virus in the vaccine virus. No results have been received.

IX. Testing RVF Vaccine Prepared at the Agouza Institute

Lots 7 and 19, prepared by the Agouza Institute, Cairo, Egypt were tested in our laboratory and a report was submitted to USAMRIID November 26, 1979.

No immunogenicity was noted in either lot of vaccine by our potency test. Testing for formalin in the final product revealed at least four times the amount required to inactivate the virus. Finally, no residual live virus was detectable in either lot.

Table 1

Rift Valley Fever (RVF) Vaccine Development
RVF Vaccine (FRhL-2), Inactivated, Dried
(TSI-GSD 200)

Final Container Tests

Test	Vaccine Lot Number 7		8		9		10	
	Fill Size (ml)							
	Run 1 5.5	Run 2 21.0	Run 1 21.0	Run 2 5.5	Run 1 5.5	Run 2 21.0	Run 1 21.0	Run 2 5.5
Sterility	S*	S	S	S	S	S	S	S
General Safety	S	S	S	S	S	S	S	S
Formalin Content (%)	0.002	0.003	0.008	0.008	0.007	0.004	0.008	0.009
Moisture Content (%)	0.23	0.22	0.26	0.24	0.28	0.21	0.40	0.22
Potency (ED_{50},ml)	0.006	0.018	0.015	0.01	0.02	0.017	0.017	0.016
Potency, Ref. Vaccine[1]	0.003	0.004	0.001	0.003	0.004	0.003	0.003	0.004

*S = Satisfactory

[1] GMK Vaccine

Table 1 -- continued

Rift Valley Fever (RVF) Vaccine Development
RVF Vaccine (FRhL-2), Inactivated, Dried
(TSI-GSD 200)

Final Container Tests

Test	Vaccine Lot Number							
	11		12		13		14	
	Fill Size (ml)							
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
	5.5	21.0	21.0	5.5	5.5	21.0	21.0	5.5
Sterility	S*	S	S	S	S	S	S	S
General Safety	S	S	S	S	S	S	S	S
Formalin Content (%)	0.008	0.007	0.006	0.005	0.005	0.003	0.008	0.008
Moisture Content (%)	0.30	0.24	0.27	0.24	0.27	0.22	0.38	0.23
Potency (ED_{50},ml)	0.009	0.006	0.006	0.011	0.004	0.007	0.009	0.007
Potency, Ref. Vaccine[1]	0.003	0.003	0.003	0.003	0.002	0.003	0.001	0.003

*S = Satisfactory

[1] GMK Vaccine

Table 1 -- continued

Rift Valley Fever (RVF) Vaccine Development
RVF Vaccine (FRhL-2), Inactivated, Dried
(TSI-GSD 200)

Final Container Tests

Test	Vaccine Lot Number 15		16		17		18	
	Fill Size (ml) Run 1 5.5	Run 2 21.0	Run 1 21.0	Run 2 5.5	Run 1 5.5	Run 2 21.0	Run 1 21.0	Run 2 5.5
Sterility	S*	S	S	S	S	S	S	S
General Safety	S	S	S	S	S	S	S	S
Formalin Content (%)	0.003	0.002	0.003	0.004	0.008	0.003	0.006	0.003
Moisture Content (%)	0.51	0.24	0.58	0.27	0.28	0.27	0.20	0.27
Potency (ED_{50},ml)	0.005	0.009	0.013	0.014	0.006	0.005	0.018	0.009
Potency, Ref. Vaccine[1]	0.001	0.002	0.002	0.002	0.001	0.002	0.004	0.002

*S = Satisfactory

[1] GMK Vaccine

Table 1 -- continued

Rift Valley Fever (RVF) Vaccine Development
RVF Vaccine (FRhL-2), Inactivated, Dried
(TSI-GSD 200)

Final Container Tests

Test	Vaccine Lot Number		
	19		20
	Fill Size (ml)		
	Run 1 5.5	Run 2 21.0	Run 1 21.0
Sterility	S*	S	S
General Safety	S	S	S
Formalin Content (%)	0.004	0.003	0.004
Moisture Content (%)	0.31	0.22	0.22
Potency (ED_{50},ml)	0.005	0.011	0.008
Potency, Ref. Vaccine[1]	0.002	0.006	0.004

*S = Satisfactory

[1] GMK Vaccine

Table 2

Rift Valley Fever (RVF) Vaccine Development

Live Virus Detection Study

Host	Dose-IP	Titer ($log_{10}IPLD_{50}$/ml) Diluent (½ log dilutions) BME-2% HSA	Neutralized Vaccine Pool
Hamster, Male (6 w)	1.0 ml	7.95	5.61
Mouse, Male (28-35 d)	1.0 ml	6.10	5.32
Mouse, Male (10-12 g)	0.5 ml	6.47	5.62
Mouse, Male (10-12 g)	0.1 ml	6.50	6.42

All groups except the last were observed for 14 days. This was observed for 10 days as per protocol.

Vero Cell (75 cm^2)*	1.0 ml	7.4 (CPE)	7.4 (CPE)
Vero confirmation in hamsters using 2 animals/culture, 1.0 ml IP (10^{-7}, $10^{-7.5}$, $10^{-8.0}$ fluids)		7.8	7.91

*Seed not removed as per protocol. Experimental data obtained after the protocol was written indicated no significant toxicity to cell sheet.

Table 3

Rift Valley Fever (RVF) Vaccine Development

Live Virus Detection Test in Vero Cell Culture

Confirmation in Hamsters

Virus Dilution	Negative[1] as Negative[2]	Negative[1] as Positive[2]	Positive[1] as Positive[2]	Positive[1] as Negative[2]	Total
BME-2% HSA					
$10^{-7.0}$	0	2	8	0	10
$10^{-7.5}$	2	4	3	0	9
$10^{-8.0}$	4	3	1	2	10
Neutralized Vaccine Pool					
$10^{-7.0}$	0	1	9	0	10
$10^{-7.5}$	1	5	3	1	10
$10^{-8.0}$	5	4	1	0	10

Test period for Vero cell cultures - 4 days, hamsters - 14 days.

[1] CPE

[2] Death in one or more hamsters.

Table 4

Rift Valley Fever Vaccine, Inactivated, Dried
(TSI-GSD 204) (NOT FOR HUMAN USE)

Lot 1
Prepared from BHK-21 Cell Cultures

Final Container Testing

1.	Bulk Sterility Testing	
	a. Fluid Thioglycollate Medium	NEOC[1]
2.	Final Container Sterility Testing	
	a. Fluid Thioglycollate Medium	NEOC[1]
	b. Fluid Soybean-Casein Digest Medium	NEOC[1]
3.	General Safety Test	
	a. Mouse and Guinea Pig	Passed[2]
4.	Residual Moisture Content	0.3%[3]
5.	Potency (ED_{50}/dose)	0.0038 ml[4]
6.	Safety Test for RVF Residual Live Virus	Passed[5]

---

[1] No evidence of contamination as per Section 610.12, CFR 21, April 1, 1977 and TSI SOP 1-2, July 1, 1978.

[2] As per Section 610.11, CFR 21, April 1, 1977 and TSI SOP 2-0, April 18, 1978.

[3] As per Section 610.13, CFR 21, April 1, 1977 and TSI SOP 4-0, March 31, 1978.

[4] As per TSI SOP 3-0, March 31, 1978.

[5] As per TSI SOP 2-1, September 4, 1979.

Rift Valley Fever (RVF) HA Antigen

I. Introduction

The preparation of 5 liters of RVF antigen, BPL inactivated (Ref. SGRD-UIZ-A 9/12/78) was completed this year with the shipment of Lots 1-78, 1-79 and 3-79. An additional 3½ liters of antigen with a titer of ≥1:1024 was requested (Ref. SGRD-UIV-I 3/1/79 and 7/17/79) this year. Work on this request is nearing completion.

A total of 23 batches of infected suckling mouse livers were harvested and 70 extractions were made. Of the 70 extractions, 12 were used for Lot 1-79, 15 for Lot 3-79, 19 are reserved for Lot 1-80 (in process), 8 were lost through a poor filtration, 8 are being re-tested and 8 were unusable due to poor titer.

Antigens derived from suckling hamster livers had good titers but residual live virus was found in Lot 2-79 during the final safety test and several subsequent batches showed the presence of residual live virus upon testing in Vero cells. Although it was found possible to re-inactivate these antigens with 0.1% BPL, work with hamster tissues was suspended to concentrate on the more-dependable mouse tissues.

II. Processing

A. Infected Livers

It was found that passing the mouse serum virus 184-Be once in mice and using a 10% liver suspension diluted to 10^{-3} as an inoculum was best. Mice were inoculated with 0.2 ml i.p. and harvested at 40-42 hours when 30-50% mortality had occurred. Litters were harvested in a random fashion to prepare more uniform tissue harvests. The harvested livers were rinsed once in HBSS, dabbed on sterile gauze and deposited in small jars before weighing and storing at -65°C.

B. Extractions

A revised outline of the extraction procedure is given in Table 5 . A Sorvall Omni-Mixer set at 10 was used for tissue homogenization in sucrose after allowing the tissue to thaw slowly in cold water (10-15 minutes). The rate of dropping the sucrose homogenate into cold acetone was adjusted to be no more than 2 ml/min. Efforts were made to maintain the pH between 7.4-7.6 (Steps 11, 14) although this was difficult.

C. Antigen Pools

Lots 1-78, 1-79 and 3-79 were completed, tested for safety and shipped to USAMRIID. Table 6 shows the essential data. Lot 2-79, prepared from infected suckling hamster liver was withdrawn when residual live RVF virus was shown in the final safety test in suckling mice. This occurrence prompted us to check subsequent extractions in Vero cells (10 days plus 10 day sub-pass) as part of our control.

Pooling and testing of antigens to prepare 3½ liters with a titer of ≥1:1024 is in progress.

One pool of normal suckling mouse liver antigen (445 ml) was prepared from 5 extractions, filtered and is frozen in bulk. This is intended for use as a tissue control in the CF test.

D. Residual Live Virus - Reinactivation

Aside from Lot 2-79 (hamster liver) antigen showing residual live virus, the Vero test picked up four additional hamster batches with live virus present (Batches 18-79-1, 17-79-1, 17-79-2 and 17-79-3). Reinactivation with 0.3% BPL caused a one tube drop in HA titer, 0.2% BPL showed no loss and 0.1% BPL re-treated antigen showed a one-tube increase in HA titer. All were inactivated after secondary inactivation. Subsequently, the initial concentration of BPL was raised to 0.4% BPL for initial hamster antigen inactivation. However, in 6 trials (Lot 18) the titers were 1 to 2 tubes lower than the re-inactivated antigen, possibly caused by more acid production during BPL hydrolysis.

E. Testing

The micro-HA and micro-CF tests were incorporated into our system in the latter half of the year.

III. Conclusion

Regulation of pH during the preparation of RVF HA antigens appears to be one of the more important points to watch. Perhaps the use of a mini-osmotic pump during inactivation would be one approach to solve this problem.

The Vero safety test, as a control measure on individual batches prior to pooling, works well and is an effective screen for live virus.

Table 5
RVF HA Antigen Preparation
(12/31/79)

RVF Strain_____ Lot #_____
Tissue source_____ Date_____
Mortality_____

1. Tissue weight (liver)_____g.
   Vol. sucrose_____ml (4 volumes/g tissue) Lot #_____
   Vol. homogenate_____ml Vol. acetone_____ml (20 x homogenate)

2. Homogenation (2 10 second bursts - 2 pauses - 10 min, 1 min)
   Start_____ Finish_____

3. Homogenate into acetone Start_____ Finish_____ Rate_____ml/min

4. Centrifugation - 5 min/1800 RPM/4°C

5. Decant - save sediment - add acetone _____ml
   Hold 4°C/1 hr Start_____ Finish_____

6. Centrifugation - 5 min/1800 RPM/4°C

7. Decant - save sediment. Add glass beads. Dry 1 hr 4°C
   Drying Start_____ Finish_____

8. Shake well to disperse sediment.

9. Rehydrate - (1 volume borate saline - F/Mc pH 9.0) - hold overnight on ice.
   (Vol. sucrose homogenate _____ x 0.4 = _____ ml; per bottle_____ml)

10. Add equal volume borate saline (#9) + 0.6% BPL (mouse) or 0.8% BPL (hamster).
    Mix well - covering all internal surfaces - keep on ice.

11. Hold for 3 hours - checking pH after 1 and 2 hours. Time start_____.

Time		pH	Adjustment* (Na_2HPO_4-ml/NaOH-ml)	Post Adj. pH
_____	1 hr	_____	_____	_____
_____	2 hr	_____	_____	_____

12. Centrifuge - 1 hr/10,000 rpm/0°C. Finish_____

13. Decant supernate (antigen) and pool. Volume_____ pH_____

14. Check pH for 3-4 hrs and after 18 hours at 4°C (keep above 7.2).

Date	Time	Hour	pH	Adjustment* (Na_2HPO_4-ml/NaOH-ml)	Post Adj. pH
_____	_____	0	_____	_____	_____
_____	_____	1	_____	_____	_____
_____	_____	2	_____	_____	_____
_____	_____	3	_____	_____	_____

Table 5 -- continued
RVF HA Antigen Preparation

14. (continued)

Date	Time	Hour	pH	Adjustment* (Na_2HPO_4-ml/NaOH-ml)	Post Adj. pH
_____	_____	4	_____	_____	_____
_____	_____	18	_____	_____	_____

15. Hold antigen at 4°C - 4 days. Date finished_____

16. Dilute antigen 1:1 with Borate Saline + 0.8% (W/V) HSA, pH 9.0.
Amount added_____ml Final volume _____ml

17. Freeze-thaw antigen 3X.

18. Test for HA, live virus (Vero-10 days + 10 day subpass).

19. Maintain antigen at -65°C until ready for pooling or use.

*Na_2HPO_4(1N) adjustment: Vol sucrose homogenate x 0.05 = ml per dose.

1N NaOH: added to finish pH adjustment.

Table 6

RVF (Entebbe Strain) HA Antigen Preparation
(Sucrose-Acetone Extracted Suckling Mouse Liver - BPL Inactivated)

Lot no.	Date freeze-dried	No. batches in pool	Pool volume (liters)	HA titer	Moisture content	No. bottles (1 ml) shipped
1-78	1/22/79	7*	1.5	1:128	0.32%	1500
1-79	8/2/79	12	1.2	1:512	0.36%	1152
3-79	10/11/79	15	3.1	1:512	0.31%	2736

*1978 extractions

Rift Valley Fever (RVF) H1849 Virus Strain

I. Introduction

At the request of USAMRIID (9 March 1979) experiments were started to test the RVF virus strain H1849 for possible use as a vaccine candidate seed virus.

The virus had been isolated from infected human serum H1849 obtained on 13 May 1975 from a male veterinarian in South Africa during the second day of illness. This serum was used to infect FRhL-2 diploid cells and subsequently a second virus passage was made. A trial vaccine was prepared from virus fluid of the third passage in diploid cells. A standard potency assay performed in mice employing this vaccine showed that it was equivalent to the Entebbe (MK) reference vaccine.

II. Experimental

A. Virus Passage and Vaccine Preparation

1. An aliquot of the original serum sample was diluted 10^{-3} and used to infect cultures of certified FRhL-2 cells (Pass 17, Lot 18). The maintenance medium was Eagle Basal Medium (BME) containing human serum albumin (0.5%, W/V). Virus fluid was collected on the seventh day when the cytopathic effect (CPE) had involved 80-90% of the cells. Fifty ampules, containing 1 ml/amp, were stored at -65C with control fluid from uninfected cultures.

A second passage was prepared in the same lot of certified cells using the first pass virus fluid diluted 10^{-3} and the same type of medium. Virus containing fluids collected on day four when CPE was 100% were stored at -65C (50 x 1 ml).

The titers of virus passages in mice and tissue culture are shown in Table 7 .

B. Trial Vaccine

1. A trial vaccine was prepared in FRhL-2 cells infected with second passage virus diluted 1:10,000 (MOI = 0.001). The fluid was harvested on day three when CPE was 90-100%. The virus pool was filtered (0.04μ filter) and inactivated with formalin (0.05%) at 37C for 72 hours.

Potency of the trial vaccine was determined by the standard two dose antigen-extinction test in mice. The challenge virus for this test

was the Entebbe strain of RVF virus (7.9 $\log_{10}$/0.1 ml ip). The ED_{50} of the trial vaccine was 0.004 and the reference vaccine (Entebbe, MK) ED_{50} was 0.003 in the same group of mice. In view of these results no further vaccine work is contemplated with the H1849 virus strain of RVF.

Table 7

Virus Titer of RVF Strain H1849 in Tissue Culture and Mice

Virus Passage[1]	Mice[2] IC	Mice[2] IP	Vero Cells[3] (log_{10}/ml)	FRhL-2 Cells[4] (log_{10}/ml)
1	6.8	5.9	6.8	5.5
2	7.3	6.2	7.5	6.4
3	-	-	7.5	6.9

[1] Passages made in certified FRhL-2 cells. Pass 1 is the isolation passage. Pass 3 virus fluid was used to prepare the trial vaccine.

[2] Weanling mice (10-12 g) inoculated 0.03 ml IC, 0.1 ml IP.

[3] Plaque sizes ranged from 0.5 mm to 3.0 mm, average = 1.0 mm.

[4] Titration in FRhL-2 diploid cells done by observation of CPE.

Rift Valley Fever (RVF) SA-51 Virus Strain

I. Introduction

The annual progress report for 1978 (No. 44-95-1278-TSI001) contained the passage history of the SA-51 RVF virus to be evaluated as a candidate seed virus for vaccine purposes. The above report also gave titers by CPE and plaque count of the second passage of SA-51 virus in certified FRhL-2 cell cultures.

The current report compares the growth curves and vaccine potency of the SA-51 RVF strain propagated in pass 17 and pass 25 FRhL-2 cell cultures. Growth curves of the virus were similar in both cell passes. The vaccine prepared in pass 17 cells was equivalent to that of the Entebbe reference vaccine, however, the vaccine from the higher cell pass 25 failed to meet the minimum potency standard.

Vaccines prepared with SA-51 virus in FRhL-2 cells from fluids harvested at 48, 60 and 72 hours after infection show that those fluids harvested at 60 hours, or later, had potency equivalent to the Entebbe vaccine, but vaccines prepared from 48-hour fluid failed potency.

Mouse sera were obtained for a Mouse Antibody Production (MAP) Test and stored at -65C.

Work on this project was terminated in March.

II. Experimental

A. Preparation of Vaccines with SA-51 RVF Virus in FRhL-2 Cells of Pass 17 and 25.

1. Growth Curves

Strain SA-51, passaged twice in FRhL-2 cells, was used to initiate infection of FRhL-2 cells prepared at passes 17 and 25. Virus samples obtained at 12-hour intervals post infection were titrated in Vero cells. As shown in Table 8 the virus grows quite well in both cell pass levels, although CPE develops more slowly at pass 25.

2. Vaccine Potency

FRhL-2 cells prepared from pass 17 and 25 were infected with second passage SA-51 virus and vaccine fluids were harvested 72 hours later when the CPE of pass 17 cells was 95 percent and pass 25 was 75 percent.

After inactivating and safety testing, the vaccines were subjected to a standard RVF antigen extinction assay in weanling mice. The results of these tests reveal that the vaccine prepared in pass 17 cells was equivalent to Lot 6 Run 2 (NDBR 103) RVF vaccine prepared in GMK with a potency of 0.004 ml/dose ED_{50} for both vaccines. On the other hand pass 25 vaccine failed to meet our potency standard (Table 9).

It may be that RVF vaccines prepared from virus propagated in pass 25 cells and allowed to incubate for 96 hours when CPE has proceeded further might result in a vaccine with potency equal to that of Lot 6 (NDBR 103).

B. Potency of Vaccines Prepared From Virus Fluids Obtained at 48, 60 and 72 Hours After Infection

FRhL-2 cells (pass 17) were infected with the SA-51 virus and fluids obtained from groups of cultures at 48, 60 and 72 hours post-infection were titrated in Vero cells. The vaccines prepared from inactivated viruses then were tested in the standard mouse potency assay.

Test data shown in Table 10 reveal that the early harvest fluid, when CPE was 20 percent, did not meet the potency standard. Fluids harvested later, when more extensive CPE had occurred, had potency values equivalent to the reference vaccine (NDBR 103). The virus titers from the two latter infected fluids were equivalent.

C. MAP Test Serum

In order to prepare mouse serum for this adventitious agent safety test a group of "virus-free" mice (Chas. River, Portage, MI) first received two doses of an undilute experimental vaccine (one week apart, 0.2 ml ip/dose prepared from the H1849 strain of RVF virus). One week after the second vaccination the undilute test virus (SA-51) was inoculated into 16 mice 0.5 ml ip and 0.05 ml intranasally (standard procedure for the MAP test).

During the following 28-day period six of the test mice were found dead (38%) on days eight to thirteen. The cause of death though not determined, may have been due to the SA-51 virus.

The surviving animals and appropriate control mice were bled and the sera stored at -65C. The MAP test was never completed since this project was terminated.

Table 8

SA-51 RVF Virus Propagated in FRhL-2 Cell Pass 17 or Pass 25 and Tested at Various Time Intervals

Hrs.-Post Infection	Percent CPE[1]		Titer (TCD_{50}/ml log_{10})[2]	
	Pass 17	Pass 25	Pass 17	Pass 25
12	0	0	2.3	2.6
24	0	0	4.4	4.2
36	5	5	6.4	6.1
48	30	30	7.3	7.4
60	70	40	7.4	7.3
72	90	70	7.1	7.0
84	100	80	7.5	7.1
96	100	90	7.5	7.3

[1] Cytopathic effect estimated.

[2] Virus samples titrated in Vero cells. Average of duplicate tests.

Table 9

Potency of SA-51 RVF Virus Vaccines Prepared from Pass 17 or Pass 25 FRhL-2 Cells 72 Hours Post Infection

Vaccine	Percent CPE Live Virus [1]	Live Virus [2] Titer (TCD_{50}/ml log_{10})	Vaccine Potency [3] (ED_{50},ml)
Reference [4]	--	--	0.004
Pass 17	95	7.50	0.004
Pass 25	75	7.25	0.020

[1] Cytopathic effect estimated.

[2] Virus titrations done in Vero cells.

[3] Standard RVF weanling mouse antigen extinction test.

[4] RVF vaccine, Lot 6, Run 2 (NDBR 103).

Table 10

Potency of SA-51 RVF Virus Vaccines Prepared with Virus Fluids Harvested 48, 60 or 72 Hours After Infection of FRhL-2 Pass 17 Cells

Hrs.-Post Infection	Percent CPE Live Virus [1]	Live Virus [2] Titer (TCD_{50}/ml log_{10})	Vaccine Potency [3] (ED_{50}, ml)
Reference [4]	--	--	0.005
48	20	7.8	0.070
60	50	7.6	0.005
72	100	7.6	0.005

[1] Cytopathic effect estimated.

[2] Virus titrations done in Vero cells.

[3] Standard RVF weanling mouse antigen extinction test.

[4] RVF vaccine Lot 6, Run 2 (NDBR 103).

VEE (C-84) Vaccine Development

I. Introduction

A pilot-run, scaled at one-quarter production level, was conducted to assimilate information concerning equipment, time and personnel. Function sheets for preparation of approximately 400 roller cultures from 2000 embryonated chicken eggs and subsequent viral infection, harvest and inactivation were drawn up.

II. Process Studies

A. Tissue Culture

1. Embryo Harvest

Five hundred fertile eggs were received from SPAFAS and incubated. Thirty eggs were candled-out after 7 days and 2 on day 8 (6.4% mortality). During decapping, 2 eggs were crushed and one was dropped, leaving 465 embryos in the harvest. Heads were removed and tissue was rinsed three times in cold Pucks A. The total tissue weight was 195 g (0.42 g/embryo). The tissue was washed three additional times in HBSS and placed in the refrigerator in HBSS for overnight holding.

2. Trypsinization

Embryos were removed from the refrigerator and rinsed three times with HBSS. Tissue was passed through a 50 cc disposable syringe, equipped with a short 15 g needle, into a 2 liter bottle containing HBSS. After 6 rinses with HBSS and one with trypsin, the tissue was placed into an 8 liter flask on a magnetic stirrer at 37°C (6½ ml trypsin/embryo) for 30 minutes. Cells were collected through sterile gauze into a 4 liter bottle containing fetal calf serum (60 ml/3000 ml trypsin). Tissue was re-trypsinized and cells collected after another 30 minutes in a like manner. Cells were collected by sedimentation, pooled and washed once in growth medium. A total of 21 ml cell pack was achieved containing 3.12×10^{10} cells, 99% viable.

3. Planting

Cells were resuspended in growth medium and distributed to 100 roller vessels containing 150 ml growth medium. They were rolled at ¼ rpm over the weekend.

4. Washing

Growth medium was removed and each bottle was rinsed with HBSS prior to placing 150 ml M199 + 2% HSA (maintenance) on each culture.

Cultures were incubated at 37°C overnight. One additional rinse of 50 ml HBSS was done the next day.

B. Virus

1. Infection

The rinse was removed and 20 ml of 10^{-4} C83 VEE (in M199 + 2% HSA) was placed on each of 80 cultures for one hour at 35°C/½ rpm. The viral inoculum was removed and cultures were rinsed with 50 ml HBSS.

2. Viral Proliferation

The rinse was removed and 150 ml maintenance placed on each culture. Incubation was at 35°C/¼ rpm.

3. Harvest

After 20 hours, controls were harvested and after 22 hours, infected cultures were completely harvested into a 20 L Millipore pressure tank. A 200 ml bulk sample was removed and the virus was filtered through an AP20 and HA pad into a 20 L bottle. Another 200 ml sample was removed.

4. Inactivation

A total of 11,500 ml of virus was warmed at 37°C and 115 ml of 10% Formalin (in Hanks) was added slowly with magnetic stirring. A zero time sample was removed, neutralized with sodium bisulfite and frozen. The bulk virus was refiltered into a fresh container (24 hrs) and after 72 hours the bulk virus was placed at 4°C with magnetic stirring for 4 additional days. Appropriate samples were taken during this period.

C. Testing

As of the time of this report, little testing has been achieved. The Formalin content of the bulk vaccine was 0.093%, by assay. Preliminary tissue culture assay in CEC indicated virus to be approximately $10^{-9.8}$/ml.

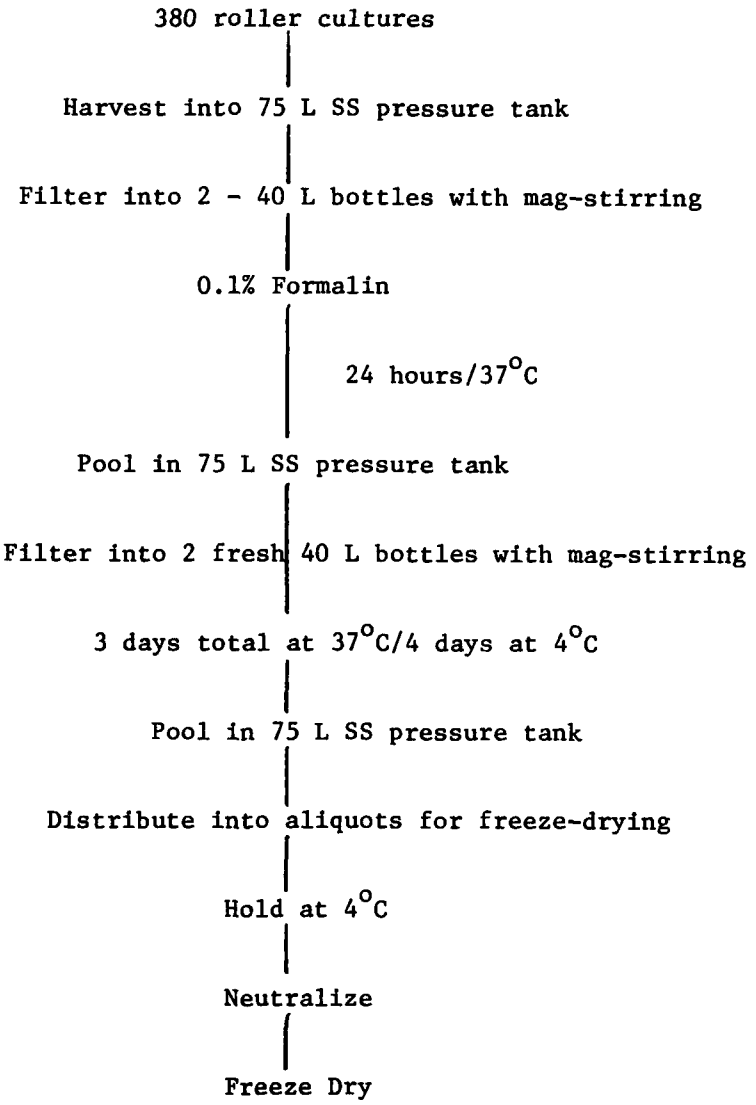
III. Conclusion

One major problem is obtaining a vessel large enough to contain the projected 50-60 liters during inactivation. The schematic solution is shown in Figure 1. Some difficulty was incurred in holding temperature in the individual Bellco incubators at desired levels. This will be eliminated during production by using the large walk-in incubator for cell incubation and infection. Other equipment is being put together to aid in the production of this vaccine. Egg mortality should be reduced by receiving pre-incubated 8 day-old eggs from SPAFAS.

Figure 1

C-84 VEE Vaccine (Inactivated)

Schematic Production



Venezuelan Equine Encephalomyelitis (VEE) Vaccine Immunizations

I. Introduction

Plaque reduction neutralization tests were performed on pre- and post-vaccination sera from seven new employees inoculated with VEE Vaccine, Live, Attenuated (TC-83, NDBR 102). None of the pre-sera had pre-existing antibody to VEE virus. After immunization five of the seven subjects developed titers greater than the required 1:80.

II. Experimental

The plaque reduction neutralization tests were performed in accordance with protocols in current use at USAMRIID.

The challenge virus was TC-83 attenuated vaccine virus. Neutral red in agarose was added 48 hours after inoculation of Vero cell monolayers with the serum-virus test fluids. The 80% plaque reduction serum titer was calculated by use of a standard formula.

The data in Table 11 show that serum obtained from each vaccinee prior to inoculation with TC-83 vaccine had no measurable antibody to VEE virus. The post-vaccination serum of one person (VG) contained no antibody, and the serum of a second person (RC) had less than the required 1:80 titer. These two people will be inoculated with an inactivated VEE vaccine and their sera will be tested following this procedure.

Table 11

Neutralizing Antibody Titers in Sera Following Administration of VEE Vaccine, Live, Attenuated (TC-83) (NDBR 102)

Vaccinee	Antibody Titer*	
	Reciprocal of Serum Dilution	
	Pre-immunization Serum	Post-immunization Serum
RC	<1:10	1:56
VG	<1:10	<1:10
DM	<1:10	>1:640
JS	<1:10	1:122
CL	<1:10	1:117
AH	<1:10	>1:640
DS	<1:10	>1:640

*Plaque reduction neutralization test, 80% endpoint.

Tissue Culture

I. Introduction

During 1979, three Production Lots of FRhL-2 and two of MRC-5 were stabilized and frozen. Two of the three FRhL-2 lots were certified while the two lots of MRC-5 were not certified since they were prepared specifically for experimental use. A total of 619 ampules of frozen FRhL-2 were processed into 8707 cultures for preparing 11 lots of RVF vaccine. Five lots of FRhL-2 and three of MRC-5 cells were depleted this year.

The karyology laboratory and dark room were established and put into operation and the roller culture incubators were installed this year.

II. Process Studies

A. Production Cells

1. Primary Duck Cells

No work was done.

2. WI-38 Diploid Cell Line

No work was done.

3. FRhL-2 Diploid Cell Line

Three production lots of FRhL-2 were prepared as shown in Table 12. Lots 19 and 20 were completely tested while a few tests remain to be completed on Lot 21. The chromosome analyses on Lots 19 and 20 (Metpath) are shown in Table 13. The high polyploidy shown for Lot 20 (12%) will be re-examined in our laboratories.

Lots 15, 16, 17, 18 and 19 were depleted this year for RVF vaccine production or through shipment to USAMRIID. A total of 619 ampules were used to prepare 8707 cultures for RVF production. Assorted cultures for experimental RVF virus seed preparation utilized another 27 ampules.

Shipments included 200 ampules Lot 18, 50 ampules Lot 15, 363 ampules Lot 19 (all certified) and 54 ampules of Lot 21 (not certified).

4. Primary Chick Embryo Cells

Several small batches of CEC were prepared for training purposes. One pilot run of 100 rollers was made for VEE (C-84) Vaccine Development.

5. MRC-5 Diploid Cell Line

Two lots (#6 and 7) of MRC-5 cells were prepared for experimental use (uncertified). Data on these lots are shown in Table 14. More cells were obtained in Lot 7 by adjusting the time between last passage and harvest by 2 days. All of Lot 6 (350 amps) and part of Lot 7 (204 amps) were shipped to USAMRIID along with Lot 3 (300 amps) and Lot 4 (342 amps) certified cells.

One lot of cells was prepared and used to test the automatic filler-sealer. The machine had to be slowed when good sealing was not effected, due to the chilled ampules and suspension used. Slowing the machine caused glass to stick to the lifting arms creating a non-operative condition. We used most of the "run" trying to correct these defects. Ampules and cell suspension maintained at room temperature help overcome these difficulties.

6. IMR-90 Diploid Cell Line

This cell line was used in safety tests.

7. IMR-91 Diploid Cell Line

No work was done.

8. FCL-7 Diploid Cell Line

No work was done.

9. Certified Canine Kidney Cells (Dow Chemical Co.)

Approximately half (41 dog lots) of the canine kidney cell lots in storage were tested for sterility after four previously tested lots (141, 148, 149, 150) came down with mold. Testing consisted of thawing one amp from lots 151 through 209, planting, changing media after one day and 3-4 days and maintaining all cultures and media-pouroffs for at least 30 days. Three failed, #188-mold; #197 and #200-bacterial contamination. The remaining 38 lots showed no evidences of mold or bacterial contamination.

A total of 88 ampules from dogs #141, 148, 149, 150 and 151 were shipped to USAMRIID.

III. Experimental

A. Production in Roller Cultures and "Cell Factories" (Nunc)

Cell yield data are summarized in Table 15 comparing cell yield data obtained from rolling bottles (Falcon 3027- 850 cm^2 plastic) and "Cell Factories" (Nunc-6000 cm^2 plastic). Comparative data from Lot 21 FRhL-2 are included since the same fetal calf serum (Reheis) was used. Schedules

were kept fairly uniform, allowing one media change prior to harvest. A higher concentration of trypsin (0.5% vs. 0.25%) was needed to detach FRhL-2 cells in the "Cell Factories". It was also found that 4 out of 27 Cell Factories tended to leak, probably due to the complex construction of the 10 trays glued together. Control cultures had to be used to evaluate sheeting in the Cell Factories since microscopic observation was not possible directly.

As indicated, cell yields in rolling cultures were 25% that of Lot 21 while Cell Factories yielded only 6% as compared by cells/cm^2. Cell yields in both systems could be improved by additional media changes and incubation time, but would not be practical for cell production or be economical.

B. Microcarrier Cell System

Cytodex I and Biosilon were compared in one experiment using MRC-5 cells. Cell growth of 5-fold increase was observed in the Biosilon culture between days 3 and 9. The Cytodex I culture was discontinued due to contamination. Additional work is planned as time becomes available.

C. BHK-21

A total of 92 rollers from 20 ampules was prepared for RVF work (Experimental Lot 1 - Not for Human Use).

D. Serum

One lot of serum from Sterile Systems (Hyclone 100210) was tested. It was sterile, free of PPLO and was superior to our present serum (Reheis S45006) in sheeting cells from small inoculums and in the total numbers of cells produced/culture. It was obtained and placed in -20°C storage.

E. Vero Cells

One lot of Vero cells was frozen at P137 for use in safety tests. The preparation is summarized in Table 16.

F. BSC-1

Two ampules of P57 were shipped.

G. L-929

The cell line was acquired from the ATCC at P556.

IV. Cell Inventory

A summary of the inventory and use of ampules for the year is in

Table 17. As shown, 1343 ampules of certified and 608 ampules of uncertified normal cells were shipped to USAMRIID. A total of 886 ampules of normal cells were used here for testing and vaccine work. Of the various cells used for testing, two ampules were shipped to USAMRIID and 79 ampules were used here.

V. Conclusion

The certified cell system has continued to work well this year. The addition of the karyology laboratory is a valuable adjunct to this work. Little, sustained experimental work was accomplished this past year due to the pressure of other work. It is hoped that the microcarrier cell work may be investigated more thoroughly in the coming year.

Table 12

Testing Status of Three Lots of Male Fetal Rhesus Lung Diploid Cells - FRhL-2

Items		Lot 19 Pass 16	Lot 20 Pass 16	Lot 21 Pass 16
1.	Surface area harvested (cm^2)	106,200 cm^2	107,100 cm^2	106,500 cm^2
2.	Total cells (x 10^9)	15.7	15.1	11.6
3.	Cells/cm^2 (x 10^5)	1.5	1.4	1.08
4.	No. ampules frozen	395	376	388
5.	Cells/ampule (x 10^6)	37.6	41.2	29.8
6.	Percent viability (aqueous trypan blue)	95	94	93
7.	Bulk sterility - CFR 610.12	S*	S	S
8.	2-week hold of cell samples after harvest	S	S	S
	hemadsorption (g. pig RBC)	Neg.	Neg.	Neg.
9.	30-day hold of harvest fluids	S	S	S
	PPLO - CFR 610.30	Neg.	Neg.	Neg.
10.	PPLO: frozen-thawed cells(3X)-CFR 610.30	Neg.	Neg.	Neg.
11.	Sheetability: 1 amp-1 roller-850 cm^2 (no.)	3 days	3 days	2 days
	1 amp-2 rollers	4 days	4 days	ND
	1 amp-10 x 75 cm^2 plastics	2 days	3 days	2 days
	1 amp-20 x 75 cm^2 plastics	5 days	5 days	3 days
12.	Hemadsorption-sheetability test (g.pig RBC)	Neg.	Neg.	Neg.
13.	PPLO: sheetability test - CFR 610.30	Neg.	Neg.	Neg.
14.	M. tuberculosis (Lowenstein-Jensen)	Neg.	Neg.	Neg.
15.	Tissue culture safety-CFR 630.13: CEC	S	S	ND
	CV-1 & subpass	S	S	ND
	IMR-90	S	S	ND
	MRC-5	S	S	ND
	RK-13	S	S	ND
16.	Embryonated egg safety (allantoic)-CFR 610.13(4)	S	S	ND
17.	Oncogenicity (new-born hamsters/ALS)	Neg.	Neg.	Neg.
18.	Karyology (Metpath, Inc.)	Passes	Passes	ND

*S = test satisfactory; ND= not done.

Table 13

Chromosome Analysis on Two Lots of
FRhL-2 (Pass 17)
(Metpath, Inc.)

	Lot 19	Lot 20
	(no. of cells)	
No. of chromsomes		
<42	0	0
42	98	99
43	2	1
>43	0	0
Polyploidy	4%	12%
Breaks and/or Gaps	1	1
Total no. cells counted	100	100
Modal No.	42	42

Table 14

Preparation of Two Lots of MRC-5 P23 (Uncertified) for Experimental Use

Item	Lot No. 6	Lot No. 7
1. Surface area harvested	107,850 cm^2	107,850 cm^2
2. Total cells (x 10^9)	13.2	27.5
3. Cells/cm^2 (x 10^5)	1.2	2.6
4. No. amps frozen	394	428
5. Cell count/amp (x 10^6)	33.3	64.5
6. Cell viability (aqueous trypan blue)	93%	96%
7. Bulk sterility	Passed	Passed
8. 2-week hold harvest cells	Passed	Passed
hemadsorption	Passed	Passed
9. 30-day hold harvest fluids	Passed	Passed
10. PPLO-frozen-thawed cells	Passed	Passed
harvest pool	Passed	Passed
11. Sheetability: 1 amp/10 x 75 cm^2	3 days	1 day
1 amp/20 x 75 cm^2	4 days	3 days

Table 15

Comparison of FRhL-2 Cell Yields in Three Production Procedures

	Rolling Bottle	Cell Factories	Lot 21 150 cm^2 Flasks
Area harvested	127,500 cm^2	162,000 cm^2	106,500 cm^2
No. containers	150	27	729
Total cells (x 10^9)	3.46	1.04	11.6
Cells/cm^2 x 10^5	0.27	0.064	1.08

Table 16

Preparation of One Lot of Vero Cells P137* for Use in Testing

	Item	Result
1.	Surface area harvested	6825 cm^2
2.	Total cells (x 10^9)	3.34
3.	Cells/cm^2 (x 10^5)	4.9
4.	No. amps frozen	103
5.	Cell count/amp (x 10^6)	32.4
6.	Cell viability (aqueous trypan blue)	96%
7.	Bulk sterility	Passed
8.	30-day hold harvest fluids	Passed
9.	PPLO-frozen-thawed cells harvest fluids	Negative Negative
10.	Sheetability: 1 amp/ 2 x 75 cm^2 1 amp/ 5 x 75 cm^2 1 amp/10 x 75 cm^2	1 day 3 days 5 days

*Passages began from Passage 124 cells frozen in thin-film freezing (in situ) and stored at -65C/11 months.

Table 17

Cell Inventory and Use
1979

Item #	Cell	Lot # *	Pass	Date Frozen	Ampule Cell Count (x 10^6)	Viability (%)	No. Amps Jan. 79	Amps Shipped	Amps Used	Current Inventory	Use
1	FRhL-2	PS	10	2/14/78	6.4	94-98	90	-	6	84	Certified Cells for Vaccine Prep.
		8-OPS	17	11/23/76	8.0	75	284	-	-	284	
		14	17	2/22/78	26.0	96-98	307	-	-	307	
		15	16	3/22/78	38.0	95-100	50	50	-	0	
		16	16	7/12/78	34.8	95-100	241	-	241	0	
		17	16	9/13/78	31.1	91-100	311	-	311	0	
		18	16	9/20/78	31.2	95-100	350	200	150	0	
		19	16	1/2/79	37.6	95	-	363	32	0	
		20	16	2/1/79	41.2	94	-	-	31	345	
		21**	16	3/20/79	29.8	93	-	54	34	300	
2	FCL-7	Seed	7	12/22/77 (rec'd.)	-	-	1	-	-	1	
		OPS	16	5/17/77	5.4	90-94	193	-	-	193	
3	IMR-90	MS	10	5/16/77	4.9	94	180	-	-	180	
		PS	14	6/1/77	3.4	99	46	-	-	46	
		1	21	11/14/77	37.7	94-97	315	-	6	309	
4	IMR-91	MS	10	8/31/78	5.2	100	98	-	-	98	

Table 17

Cell Inventory and Use
1979
(continued)

Item #	Cell	Lot # *	Pass	Date Frozen	Ampule Cell Count (x 10^6)	Viability (%)	No. Amps Jan. 79	Amps Shipped	Amps Used	Current Inventory	Use
5	MRC-5	PS	17	6/7/77	7.0	100	32	-	3	29	← Certified Cells for Vaccine Prep. →
		3	23	12/9/77	27.0	94-98	300	300	-	0	
		4	23	12/21/77	32.0	94-98	343	342	1	0	
		5	23	1/11/78	42.0	92-96	352	-	3	349	
		6**	23	8/6/79	33.3	93	-	350	5	44	
		7**	23	9/14/79	64.5	96	-	204	27	196	
6	DEC (Duck)	1	Primary	2/26/75	152.0	93	6	-	-	6	
7	(Dog Kidney)	Dow Chem.	Primary	4/5/77 (rec'd.)	-	-	1508	88	45	1375	
8	BSC-1	-	76	2/14/75	14.0	84-87	21	2	-	19	← Test Cells →
9	CV-1	-	29	12/21/76	1.3	85	11	-	1	10	
			36	10/20/78	-	-	81	-	-	81	
10	KB	-		3/18/75	14.0	89-98	52	-	1	51	
11	LLC-MK2	-	264	2/11/75	4.0	78	32	-	-	32	
12	RK13	-	73	6/16/75	9.0	83	40	-	1	39	

Table 17

Cell Inventory and Use
1979
(continued)

Item #	Cell	Lot # *	Pass	Date Frozen	Ampule Cell Count ($\times 10^6$)	Viability (%)	No. Amps Jan. 79	Amps Shipped	Amps Used	Current Inventory	Use
13	Vero	-	122	4/24/75	2.0	82	56	-	9	47	Test Cells
		-	137	8/30/79	32.4	96	-	-	12	91	
14	BHK-21	-	57	5/14/79 (rec'd.)	-	-	-	-	3	5	
			58	6/12/79	30.0	98	-	-	6	21	
15	L-929	-	556	12/18/79 (rec'd.)	-	-	-	-	1	1	

*PS = production seed; OPS = old production seed; MS = master seed

**Shipped as uncertified cells.

Pasteurella Tularensis Vaccine, Live

I. Introduction

Thirteen vials of Lot 5 and 4 vials of Lot 6 SCHU-S5 strain of Pasteurella tularensis were transferred to USAMRIID.

Eastern Equine Encephalitis (EEE) Vaccine Development

I. Introduction

Two hundred 10 dose vials of EEE Vaccine, Inactivated, Dried (NDBR 104) were shipped to USAMRIID.

Distribution

5 copies	Commander US Army Medical Research Institute of Infectious Diseases Fort Detrick Frederick, Maryland 21701
4 copies	HQDA (SGRD-SI) Fort Detrick Frederick, MD 21701
12 copies	Defense Technical Information Center (DTIC) ATTN: DTIC-DDA Cameron Station Alexandria, Virginia 22314
1 copy	Dean School of Medicine Uniformed Services University of Health Sciences 4301 Jones Bridge Road Bethesda, Maryland 20014
1 copy	Superintendent Academy of Health Sciences, US Army ATTN: AHS-COM Fort Sam Houston, Texas 78234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