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Report No. 44-95-1278-TSI001

DEVELOPMENT OF SPECIAL BIOLOGICAL PRODUCTS (U)

LEVEL II

Annual Progress Report

by

Armand N. DeSanctis
Joseph L. DeMeio
Donald E. Craig
Daniel S. Spicer
William J. Thomas

December 1978

Supported by

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

Contract No. DAMD17-78-C-8018

The Salk Institute
Government Services Division
P.O. Box 250
Swiftwater, PA 18370

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Rift Valley Fever
Q Fever
Venezuelan Equine Encephalomyelitis
Western Equine Encephalomyelitis
Chikungunya

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

A. Rift Valley Fever (RVF) Vaccine Development
RVF vaccine and antiserum were prepared.

B. Rift Valley Fever (RVF) HA Antigen
RVF HA antigen is being processed.

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19. Continued

Rocky Mountain Spotted Fever
Tissue Culture
FRhL-2
MRC-5
FCL-7
IMR-90
IMR-91

20. Continued

C. Rift Valley Fever (RVF) SA-51 Virus Strain
The SA-51 strain of RVF virus is being passaged.

D. VEE Vaccine Development
Testing of Lot C-84-1A VEE vaccine was accomplished and an addendum to IND 914 (MNLBR 109) was forwarded.

E. WEE Vaccine Development
Four WEE vaccines preparations were employed in potency testing with guinea pigs and rats as the test animals.

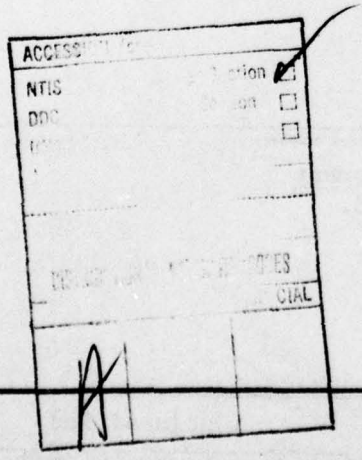
F. Chikungunya Vaccine Development
Chikungunya vaccine Lot 1 was completed and a submission followed by a procedures manual forwarded to USAMRIID.

G. Q Fever Program
A submission for Q Fever Skin Test Antigen Lot 1 was forwarded to USAMRIID.

H. Rocky Mountain Spotted Fever
A submission for RMSF vaccine Lots 1, 2 and 3 was forwarded to USAMRIID.

I. Tissue Culture
FHhL-2 and IMR-91 seed stocks were prepared. FRhL-2, MRC-5 and IMR-90 cells were certified. A total of 8235 cultures of FRhL-2 were processed for preparing 10 lots of RVF vaccine.

J. Inventory of Vaccines (1978)
An inventory of vaccines is given. This section is published separately as an For Official Use Only Addendum.



Contents:

Summary

A. Rift Valley Fever (RVF) Vaccine Development

Filtration and formalin-level inactivation experiments were completed.

A new isolate H1849 of RVF from a human serum sample was passed in FRhL-2 tissue culture cells.

One lot of antiserum to the Entebbe strain of RVF virus was prepared in rabbits and forwarded to USAMRIID.

The preparation and testing of six lots of RVF vaccine have been completed and an additional three lots of vaccine are in process.

An experimental vaccine without human serum albumin was made.

Plaque reduction neutralization tests were performed on 170 sera.

B. Rift Valley Fever (RVF) HA Antigen

The work on preparing 5 liters of RVF HA antigen has started, with 139 ml of Entebbe strain antigen having been processed.

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

The SA-51 strain of RVF virus is being passaged to determine its potential as a candidate for future vaccine.

D. VEE Vaccine Development

Testing of Lot C-84-1A VEE vaccine was accomplished and an addendum to IND 914 (MNLBR 109) was forwarded to USAMRIID.

E. WEE Vaccine Development

Four WEE vaccine preparations were employed in potency testing with guinea pigs and rats as the test animals.

F. Chikungunya Vaccine Development

Testing was completed on Lot 1 Chikungunya vaccine and a "Procedures Manual" was prepared.

A serum neutralizing antibody titer of 1:10 against Chikungunya virus appears to protect mice.

over

G. Q Fever Program

A submission for Q fever Skin Test antigen Lot 1 was forwarded to USAMRIID.

H. Rocky Mountain Spotted Fever

A submission for RMSF vaccine Lots 1, 2 and 3 was forwarded to USAMRIID.

I. Tissue Culture

FRhL-2 and IMR-91 seed stocks were prepared. FRhL-2, MRC-5 and IMR-90 cells were certified. A total of 8235 cultures of FRhL-2 were processed for preparing 10 lots of RVF vaccine.

J. Inventory of Vaccines (1978)

An inventory is supplied giving amounts of vaccines on hand at the end of 1978 and quantities withdrawn during the year. This section is published separately as an For Official Use Only Addendum.

FOREWARD

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, 1978 to December 31, 1978. 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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Distribution:

Rift Valley Fever (RVF) Vaccine Development

I. Introduction

During this period filtration and formalin-level inactivation experiments were completed to confirm the feasibility of vaccine production. At USAMRIID's direction, a vaccine production seed was prepared and vaccine preparation initiated.

In addition, a new isolate of RVF was obtained from a human serum sample supplied by USAMRIID.

One lot of antiserum to the Entebbe strain of RVF virus was prepared in rabbits and forwarded to USAMRIID.

Previous experiments in these laboratories indicated the feasibility of preparing RVF vaccine suitable for human use from the Entebbe strain of the virus as propagated in stationary FRhL-2 cell cultures.

To date, the preparation and testing of six lots of RVF vaccine have been completed and an additional three lots of vaccine are in various stages of production.

An experimental vaccine was made without human serum albumin.

Plaque reduction neutralization tests were performed on 170 sera.

II. Experimental

A. Virus Filtration

To determine the possible loss that might be encountered when RVF virus fluids are filtered through a sterilizing membrane filter as commonly used in vaccine production the following experiment was performed.

Fluids for filtration were prepared from the fifth passage of the Entebbe strain of RVF virus in FRhL-2 cell culture. FRhL-2 cultures (Lot 13, passages 23, 24) were washed twice with HBSS and seeded with 2 ml of a 10^{-4} dilution of the fourth tissue culture passage of the virus. After a one hour seeding period at 25C with occasional rocking, 40 ml of BME-0.5% (W/V) HSA was added to each 150 cm^2 cell culture and the cultures placed at 36C. Fluids were harvested and pooled when the cell sheets were 50% destroyed.

After removing a sample for plaque titer, the fluid pool was filtered through a 142 mm Millipore filter equipped with an AP25 pad followed by a 0.45 micron membrane filter. Filtration was completed easily with less than 5 lbs/m^2 of pressure applied to the filter.

As the filtrate emerged from the filter, sequential fractions of 200 ml each were collected. A sample for plaque titer from each of these was removed and the fractions pooled and a sample for plaque titer removed.

The plaque titers obtained for the samples taken pre and post filtration are shown in Table 1. Little or no loss of infectivity is indicated.

B. Formalin Ianctivation

Earlier GMK grown RVF vaccine as well as the experimental FRhL-2 grown vaccines prepared in these laboratories employed 0.1% formalin to inactivate the Entebbe strain of the virion. At USAMRIID's request, the feasibility of using 0.05% formalin levels for this purpose was explored in the following manner.

The filtered virus pool described earlier under Filtration was divided into two equal portions and warmed to 36-37C. Ten percent formalin in water was added to one portion of the virus pool to obtain a final concentration of 0.05% formalin and to the other to obtain a 0.1% level. Both samples were constantly stirred and maintained at 36-37C. At appropriate intervals, samples were removed and neutralized with sodium bisulfite for testing in weanling mice. Sampling was discontinued at 24 hours post formalin addition, but the bulk solutions were maintained at 36-37C for 72 hours at which time they were designated as vaccines and stored at 4C until tested. Neutralized samples along with the zero time sample were tested for live virus in weanling mice and results shown in Table 2 . Live virus was undetectable in solutions inactivated at either formalin level 18 hours post addition.

The vaccines resulting from this study were tested in weanling mice for potency along with a GMK produced vaccine using the standard two dose-antigen extinction method using 20 animals per dilution. The test was concluded at 14 days post challenge with the following results.

Vaccine	Cell Line	Percent Formalin	ED_{50} (ml)
314-36A	FRhL-2	0.05	0.004
36B	FRhL-2	0.1	0.006
Lot 6, Run 2	GMK	0.1	0.004

Challenge - 10^3 $WMIPID_{50}$ as 185th mouse passage of the Entebbe strain of RVF virus.

III. New Isolate

Two samples labelled H1849 and H1853 were received from USAMRIID. These consisted of viremic sera derived from a patient ill with Rift Valley Fever.

At USAMRIID's direction, two passages of the agent(s) present in these sera were made in certified FRhL-2 cell culture for potential use as a vaccine seed source.

The passage history of the agent(s) is recorded in Table 3 . Suitable aliquots of each harvest and their control fluids were stored at -65C.

A sample of the second passage of the agent from serum H1849 and the corresponding control fluid were sent to USAMRIID. The identity of this agent was indirectly confirmed by the following experiment.

Another second passage of the agent present in serum H1849 was completed in non-certified FRhL-2 cell culture for use in preparing the vaccine necessary to perform the Mouse Antibody Production (MAP) test on the vaccine Production seed described later in this report. The preparation of the virus fluid was essentially the same as that described earlier under Filtration in this report. Four stationary cultures (150 cm^2) were employed and these were seeded with a 10^{-3} dilution of the first virus passage of agent H1849. After harvest, the fluids were lightly clarified by centrifugation and filtered through a 0.45 micron disposable filter (Falcon, 150 cm^3 unit). The filtrate was inactivated with 0.1% formalin at 37C for 72 hours.

The pertinent data associated with this vaccine are as follows:

Pre-filtration	3.5×10^7 PFU/ml (Vero cell)
Post-filtration	3.7×10^7 PFU/ml (Vero cell)
Potency (ED_{50}/ml)	0.003 (vs. Entebbe strain challenge)

IV. RVF Antiserum Production

The production of antiserum to RVF virus was completed during this period as follows.

Rabbits weighing from 2.5 - 3.0 kg were pre-bled and each animal inoculated with RVF (Entebbe strain) mouse serum seed 185A. Inoculations were 0.1 ml intradermally at each of two sites and 0.2 ml subcutaneously at one site. Animals were bled out by cardiac puncture on day 28 and their sera collected and pooled. After sterile filtration the pool was dispensed into 3 ml vials at 0.5 ml/vial. Sterility tests were satisfactory and the hemagglutination-inhibition titer of this antiserum was found to be 1:1280 when tested against the standard in-house RVF hemagglutinating antigen of mouse liver origin. Vials were labelled and stored at -20C. One hundred and ten vials have been shipped to USAMRIID.

V. Vaccine Production Seed (317-16B)

A. Preparation

The primary mouse serum production seed 184 Ba, Entebbe strain of RVF virus, was passed twice in certified FRhL-2 cell culture to obtain

a seed suitable for vaccine production. An outline of the preparation of the Production Seed (317-16B) is presented in Fig. 1 . The bulk of the seed was dispensed as 1.2 ml amounts, labelled and stored at -65C. Production Seed is used at a 10^{-4} dilution.

B. Testing

Sterility and Mycoplasma tests were satisfactory. The infectivity of the Production Seed was found to be 1 x 10^7 PFU/ml as determined in Vero cell culture (25 cm^2 plastic flasks).

The presence of murine agents in the Production Seed was screened by the use of the murine antibody production (MAP) test. Weanling mice weighing 10-12 g. were immunized with an RVF vaccine prepared from the South African human isolate described earlier in this report, see part III. Mice were immunized in the usual fashion and challenged with the Production Seed one week following the second immunization. Complete protection was afforded the immunized mice and their sera were collected on day 14 post challenge. Appropriate controls were included and all sera were submitted to Microbiological Associates, Bethesda, Md. for murine antibody testing. None of the sera submitted contained detectable murine agent antibody when screened against eleven murine agents. Test results are shown in Table 4.

VI. Vaccine Production

To date, the preparation and testing of six lots of FRhL-2 cell culture grown RVF vaccine have been completed and an additional three lots are in various stages of production.

The six completed lots averaged 24.35 liters in volume and an outline of the procedures and tests used to produce them is shown in Fig. 2 with final container tests summarized in Table 5 .

In performing the final container potency test, which was done using the standard two dose-antigen-extinction test in mice, a green monkey kidney cell culture prepared vaccine, namely Lot 6, run ?, 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 5 .

A vaccine submission, including data from the first three lots of vaccine completed, was prepared as an addendum to the green monkey kidney cell culture vaccine NDBR103 and forwarded to USAMRIID for approval. The quantities of the completed vaccines which are stored at 4C are recorded elsewhere in the inventory section of this report.

An additional three lots of vaccine are in progress and a summary of their status is shown in Table 6.

VII. Experimental Vaccine

At USAMRIID's request efforts were made to produce a small volume of RVF vaccine without the use of human serum albumin in either the wash fluid

or in the maintenance medium. Results to date have indicated that low virus titers are obtained with additional losses after filtration when human serum albumin is not employed in vaccine preparation.

VIII. Plaque Reduction Neutralization Test (PRNT)

One hundred and seventy serum samples received from USAMRIID were tested for RVF antibody using the PRNT. Results have been forwarded to USAMRIID.

Table 1

Rift Valley Fever (RVF) Vaccine Development

RVF Virus [1] Filtration

Preparation		Volume (ml)	Infectivity [2] $\log_{10}$ PFU/ml
Bulk Harvest Pool			7.3
Filtrate [3] Fraction	1	200	6.3
	2	200	6.9
	3	200	7.2
	4	200	7.0
	5	200	7.3
	6	200	6.9
	7	200	7.2
	8	200	7.0
	9	200	7.2
Filtrate Pool			7.2

1] Passage 5 in FRhL-2 cell culture, Entebbe strain

2] Plaque titer in Vero cell culture, 25 cm^2 plastic flasks

3] Filtered through 142 mm AP25 pad followed by 0.45 μ membrane. No prior clarification.

Table 2

Rift Valley Fever (RVF) Vaccine Development
Formalin Inactivation of Filtered Virus Fluid

	Titer in Weanling Mice,[1] $\log_{10}$ $WMIPID_{50}$/ml	
	Formalin Level (%)	
Sampling Time	0.05	0.1
0	7.1	7.1
15 min.	5.5	3.5
30 min.	4.5	<4.0
1 hr.	2.91	<3.0
4 hrs.	<3.0	<3.0
8 hrs.	<2.0	<2.0
18 hrs.	<1.0 [2]	<1.0 [2]
24 hrs.	<1.0	<1.0

[1] Buckburg mice, 10-14 g.

[2] Undetectable at undilute, 0.1 ml IP.

Table 3

Rift Valley Fever (RVF) Vaccine Development
South African Isolate [1] History

	H1853 Serum			H1849 Serum	
Passage [2]	Dilution	Harvest Titer $\log_{10}$ PFU/ml (Vero)	Passage [2]	Dilution	Harvest Titer $\log_{10}$ PFU/ml (Vero)
1	10^{-1}	7.16	1	10^{-1}	7.04
	10^{-2}	7.04		10^{-2}	7.3
	↓			↓	
2	10^{-3}	7.15	2	10^{-3}	7.38
	10^{-4}	6.8		10^{-4}	7.31
	10^{-5}	7.03		10^{-5}	6.92
	10^{-5}	6.88		10^{-6}	6.78
	10^{-7}	6.65		10^{-7}	6.80
	10^{-8}	≥4.0		10^{-8}	5.7

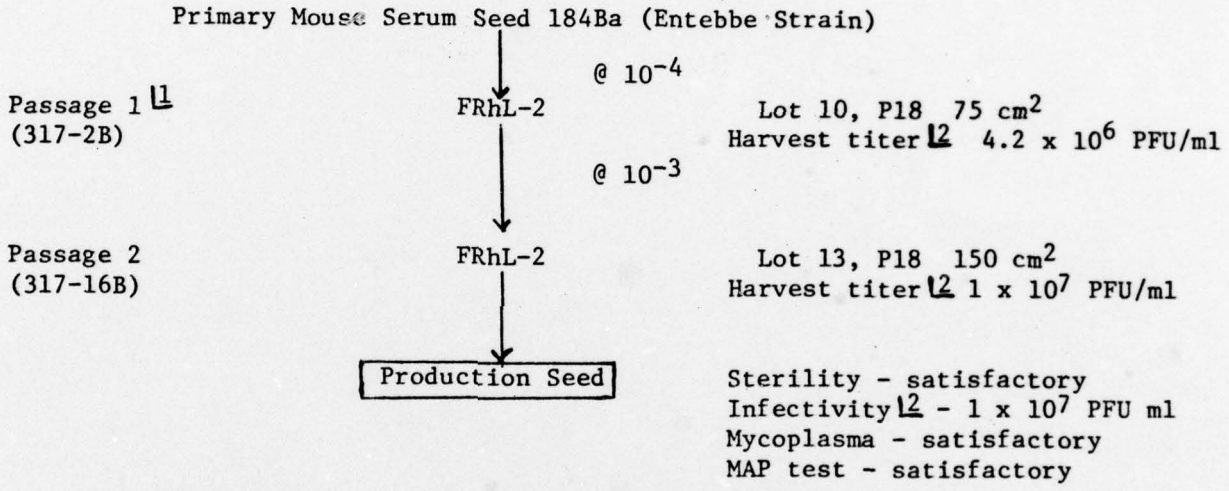
Control tissue passed at 10^{-1} dilution

[1] Both sera from same individual; collected one day apart.

[2] Passage 1 FRhL-2 cell cultures, 25 cm^2, L8, P18
Passage 2 FRhL-2 cell cultures, 25 cm^2, L&, P18

Figure 1

Rift Valley Fever (RVF) Vaccine Development
Vaccine Production Seed
Preparation



[1] Control tissue fluids passed at 10^{-3} dilution

[2] Plaque titer in Vero cell culture. Two 25 cm^2 cultures per dilution were employed.

Microbiological Associates

5221 RIVER ROAD • BETHESDA, MARYLAND 20016
TELEPHONE: (301) 654-3400

DIAGNOSTIC SERVICES TEST REPORT

Table 4

Our Code MVS 6220

MAP Test

TO: Dr. Daniel S. Spicer
The Salk Institute
P.O. Box 250
Swiftwater, Pa. 18370

FROM: Michael J. Collins, Jr. Ph.D. (MJC)
DATE: August 4, 1978
TEST: Murine virus antibody determination
SPECIMEN: 18 mouse sera
RECEIVED: July 20, 1978

	Hemagglutination Inhibition							Complement Fixation				
SAMPLE #	PVM	Reo3	GDVII	K	Poly	Ectro	MVM	Sendai	MAD	MHV	LCM	
328-47-1	-	-	-	-	-	-	-	-	-	-	-	Environ. Contr.
2	-	-	-	-	-	-	-	-	-	-	-	1 ↓
3	-	-	-	-	-	-	-	-	-	-	-	3
13	-	-	-	-	-	-	-	-	-	-	-	Human Isolate,
14	-	-	-	-	-	-	-	-	-	-	-	S.A., Vaccine 13
15	-	-	-	-	-	-	-	-	-	-	-	↓ 15
25	-	-	-	-	-	-	-	-	-	-	-	Human Isolate,
26	-	-	-	-	-	-	-	-	-	-	-	S.A., Vaccine
27	-	-	-	-	-	-	-	-	-	-	-	Production 25
28	-	-	-	-	-	-	-	-	-	-	-	Seed ↓
29	-	-	-	-	-	-	-	-	-	-	-	Challenge 30
30	-	-	-	-	-	-	-	-	-	-	-	
37	-	-	-	-	-	-	-	-	-	-	-	Production Seed
38	-	-	-	-	-	-	-	-	-	-	-	Control 37
39	-	-	-	-	-	-	-	-	-	-	-	Tissue ↓
40	-	-	-	-	-	-	-	-	-	-	-	Culture 42
41	-	-	-	-	-	-	-	-	-	-	-	Fluid
42	-	-	-	-	-	-	-	-	-	-	-	
Significant Titer	10	20	20	10	20	20	20	10	10	10	10	

Day 0 0.2ml IP SA. Vacc., F.C. - nothing
7 0.2ml IP SA. Vacc.
14 0.2ml IP Prod Seed Check of Immunizations, Comb Tissue to Immunize.
28 Bled

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Figure 2

Rift Valley Fever (RVF) Vaccine Development
Vaccine Production Outline

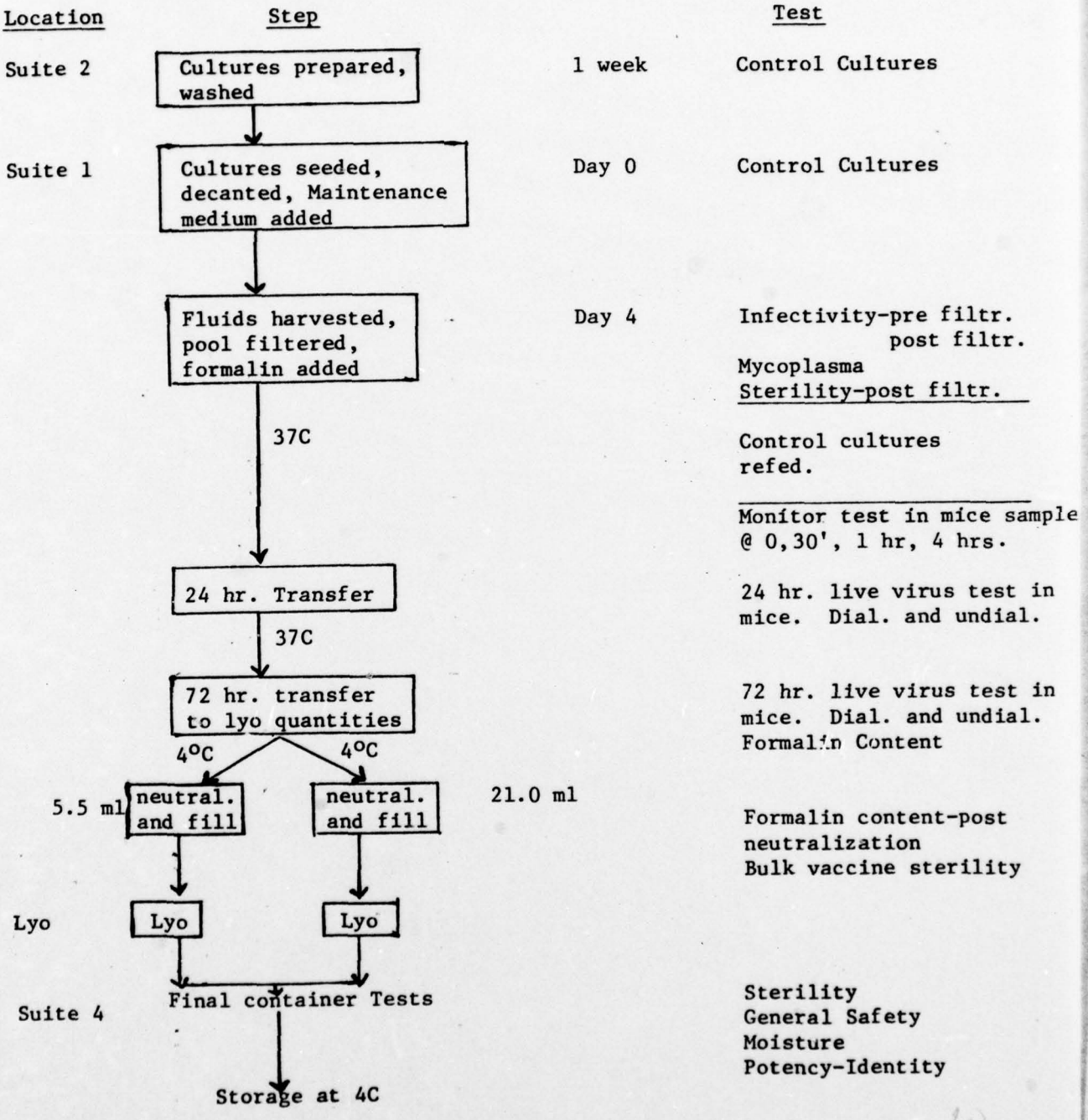

Table 5

Rift Valley Fever (RVF) Vaccine Development

RVF Vaccine (FRhL-2), Inactivated, Dried
Final Container Tests

Test	Vaccine Lot Number/Fill Size (ml)											
	1		2		3		4		5		6	
	5.5	21.0	5.5	21.0	5.5	21.0	5.5	21.0	5.5	21.0	5.5	21.0
Sterility	S[2]	S	S	S	S	S	S	S	S	S	S	S
General Safety	S	S	S	S	S	S	S	S	S	S	S	S
Formalin Content (%)	0.008	0.004	0.004	0.008	0.005	0.005	0.009	0.005	0.003	0.004	0.007	0.007
Moisture Content (%)	0.47	0.24	0.20	0.25	0.33	0.24	0.25	0.21	0.88	0.20	0.37	0.23
Potency, (ED_{50}, ml)	0.005	0.004	0.004	0.005	0.005	0.006	0.008	0.006	0.008	0.003	0.006	0.010
Potency, Ref. Vaccine[1]	0.004	0.001	0.003	0.003	0.004	0.001	0.007	0.003	0.006	0.005	0.003	0.004

[1] Reference Vaccine - GMK Vaccine, Lot 6, Run 2

[2] - S = Satisfactory

Table 6

Rift Valley Fever (RVF) Vaccine Development

Vaccine Production Status

Test or Procedure	Lot Number 7	8	9
Sterility (post filtr.)	CS[2]	CS	CS
Infectivity[1], (pre-filtr.)	7.6	6.97	6.6
(post-filtr.)	7.6, 6.9	6.7, 6.95	6.3
Monitor test in mice	CS	CS	P[3]
Safety test in mice, 24 hr.	CS	P	
Safety test in mice, 72 hr.	CS	P	
Bulk vaccine vol. (L.)	30	32.4	26.5
Bulk Vaccine Sterility (pre-lyo)	P		
Lyo, Run 1 (fill)	CS (5.5)		

1] As determined by the plaque method in Vero cell, $\log_{10}$/ml.

2] - CS = Completed satisfactorily

3] - P = In progress

Rift Valley Fever (RVF) HA Antigen

I. Introduction

As requested (Ref. SGRD-UIZ-A 9/12/78), work on preparing 5 liters of RVF HA antigen, BPL inactivated with a titer of 1:256 to 1:512 was begun. Thus far, 139 ml of antigen has been prepared in six sucrose-acetone extractions of Entebbe strain, RVF-infected mouse livers. After diluting 1:4, the batches of antigen had titers ranging from 1:1280 to 1:5120. One batch of antigen was prepared using the ZZ501 strain of RVF. The titer of this material was similar to that prepared with the Entebbe strain. The antigen is being maintained at -65°C prior to final dilution, safety testing and freeze-drying.

II. Processing

RVF infected mouse livers were collected and extracted according to our procedures (Thomas, et al, J. Biol. Stand. 6: 51-58, 1978). The status of this work to prepare 5 liters of HA antigen is summarized in Table 7 .

III. Conclusion

Initial work on preparing 5 liters of RVF HA antigen has progressed well. One or two extractions remain to be done for this effort. The ZZ501 strain did not produce any increase in HA titer.

Table 7

Sucrose-Acetone Extraction
of RVF Infected Suckling
Mouse Livers

Lot No.	Date Extracted	RVF Strain	Volume of antigen (ml)	HA Titer
1	10/30/78	Entebbe	16	1:5120
2	11/2/78	"	22	1:20,480
3	11/6/78	"	21	1:20,480
4	11/13/78	"	27	1:10,240
5	11/16/78	ZZ501	21	1:20,480
6	11/27/78	Entebbe	29	ND*
7	11/30/78	"	24	ND

* Not done

Rift Valley Fever (RVF) SA-51 Virus Strain

I. Introduction

Experiments were started on the high-titer SA-51 strain of RVF virus to determine its potential as a vaccine seed.

II. Experimental Studies

A. Passage history

The passage history of the SA-51 virus is outlined in Table 8.

B. Preparation of the second tissue culture passage

First passage fluid from FRhL-2 cells infected with SA-51 virus was diluted (10^{-3}) and inoculated into certified FRhL-2 cells. Virus infected fluids were obtained after four days of incubation when the cytopathic effect (CPE) was estimated to be 100 percent. Aliquots of this fluid have been stored at -65C.

Titrations of the second passage virus by CPE and plaque techniques yield titers ranging from 8.4 to 9.3 $\log_{10}$, average 8.7. The plaque size varies from 0.5 to 2.0 mm, average 1.0 mm, on day 4.

Additional experiments are in progress to prepare and test a trial vaccine in mice.

Table 8

Passage History of the RVF SA-51 Virus

Passage	Host		Location	Date
Isolation from sheep blood to mice presumably followed by 4 passages in sheep (4th passage in a lamb)			S. Africa S. Africa	1951 1951
4th passage	Lamb ($S_1M_1S_4$)	Received at	Plum Island	5/11/78
1st passage	FRhL-2 ($S_1M_1S_4FRhL\text{-}2_1$)	Passed at Received at	USAMRIID TSI-GSD	6/6/78
2nd passage	FRhL-2 ($S_1M_1S_4FRhL\text{-}2_2$)	Passed at	TSI-GSD	8/28/78

VEE Vaccine Development

I. Introduction

VEE vaccine, inactivated, dried was prepared on 4/23/73, tested and submitted as MNLBR 109, August 1974. At the time of manufacture, it was requested by USAMRIID that one-half of the inactivated and neutralized vaccine be stored in bulk at -20°C for possible use in a combined vaccine. A letter of 8 September 1977 from Col. Barquist requested that the 2 liters of stored vaccine be processed in final containers and tested. This was accomplished on 20 October 1977. Testing of the vaccine was finished this year.

II. Processing

A. Thawing and filling

VEE vaccine, inactivated and neutralized (C-84), contained in two bottles, was placed in a 37°C water bath and thawed on 10/17/77. A total of 1950 ml was pooled and the pH was adjusted to 7.0 with sterile HCl. A pre-drying sample was removed and the remainder was distributed in 5.5 ml aliquots in 20 ml serum bottles.

B. Drying and labelling

Three trays of vaccine were freeze-dried in the Hull drier and were removed and capped on 10/20/77. Eighteen bottles of vaccine were discarded due to improper sealing along with five double-fill bottles. Bottles of vaccine were sent to the Quality Compliance Department for final container testing (ie. sterility, general safety and residual moisture content). Labels were placed on the bottles of vaccine on 10/21/77 and 270 bottles of vaccine were placed at -20°C. This part of the C-84 vaccine is labelled Lot C-84-1A.

C. Testing

Final container testing on Lot C-84-1A is summarized in Table 9. As shown, the results indicate a potency (0.009 ml, ED_{50}) similar to that obtained with Lot C-84-1 vaccine (0.006 ml, ED_{50}) as measured by challenge of the immunized guinea pigs with Trinidad strain VEE. Potency, as determined by hemagglutination-inhibition and plaque reduction, of the guinea pigs sera and mouse challenge are also shown. The mouse potencies were equivocal but, as has been seen in the past, they are not as sensitive as the guinea pig in measuring potency of VEE vaccines.

III. Conclusion

It is concluded that storage of the inactivated and neutralized vaccine in bulk at -20°C for 4½ years had no adverse effect on VEE vaccine, inactivated.

An addendum to IND 914 (MNLBR 109) was prepared and forwarded to USAMRIID on 9/22/78.

Table 9

Final Container Test on VEE Vaccine,
Inactivated, Dried Lot C-84-1A (10/20/77)[1]

Test	Code of Federal Regulations 1977	Result	Lot C-84-1 (Comparison)
Bacterial sterility - 20 bottles	610.12	Passed[2]	Passed
General safety: guinea pigs	610.11	Passed[3]	Passed
adult mice		Passed[3]	Passed
Residual moisture content (duplicate)	610.13	0.1%[4]	0.66%
Guinea pig potency via challenge	MNLBR 109 (IND 914) Aug. 74, p. 15[5]	0.009 ml, ED_{50}	0.006 ml, ED_{50}
HAI		0.038 ml, ED_{50}	0.017 ml, ED_{50}
Plaque-Neut.		0.013 ml, ED_{50}	0.03 ml, ED_{50}
Mouse potency via challenge	MNLBR 109 (IND 914) Aug 74, p. 15[5]	0.12 ml, ED_{50}	0.005 ml, ED_{50}

1] Second half of C-84-1 vaccine, manufactured 4/23/73 and maintained at -20°C in bulk liquid form for 4½ years. The bulk vaccine was thawed at 37°C in a water bath and the pH was adjusted to 7.0 prior to filling and drying.

2] Quality Compliance Department, 11/4/77 - Final Container Book #24, P 10.

3] Quality Compliance Department, 10/28/77 - Test #1623.

4] Quality Compliance Department, 11/10/77.

5] Submission for Lot C-84-1, VEE Vaccine, Inactivated, Dried.

WEE Vaccine Development

I. Introduction

Four WEE vaccine preparations were employed in potency testing using guinea pigs and rats as the test animals. The protocols were provided by USAMRIID and involved bleedings to 90 days post-immunization followed by challenge.

II. Vaccines

Four vaccines were tested. Two were prepared by Merrell-National Laboratories and two by USAMRIID. The first vaccine produced by Merrell-National was designated MNLBR106 Lot 1 and employed B11, WEE virus; the second vaccine by Merrell-National was made in Duck Embryo Cells using strain 1344 WEE virus. The two vaccines from USAMRIID were labelled Lot 1-1974 and Lot 2-1974.

III. Methods

Testing was done on sera obtained from immunized animals by Plaque Reduction Neutralization Tests in Vero cells, using the 80% reduction method. The Reed and Muench was used in computing all results shown in Table 10.

IV. Results

A. Guinea Pig Potency Test

An evaluation of the four vaccines tested was made by comparing them in three ways; protection and challenge, significant antibody responses (four-fold or greater) by Plaque Reduction Neutralization Tests (PRNT) and antibody responses of any magnitude, i.e. $\geq$1:10 by PRNT.

As noted in Table 10 by protection and challenge, USAMRIID Lot 2-1974 afforded the best protection, followed by USAMRIID Lot 1-1974, MNLBR106 Lot 1 and finally Merrell-National's vaccine made from Duck Embryo Cells infected with strain 1344 WEE virus.

When PRNTs were performed on sera from animals immunized with the four vaccines (Table 10), no discernible differences were noted among the preparations where only significant rises were considered as an indication of protection. The criterion of 80% reduction of plaques rendered the tests much less sensitive.

Comparing the fate of the animal with his antibody level as measured by the PRNT shows that guinea pigs whose sera reduce plaques to

any discernible degree survive, even those animals with PRNT titers of 1:10. Table 10 reveals that Lot 2 gave, by far, most protection, while the remaining three showed little difference when comparisons were made. This agrees with protection and challenge results where USAMRIID Lot 2-1974 was shown to be the most potent vaccine.

In summary it can be stated that protection and challenge is a more sensitive potency test in guinea pigs than the PRNT and any discernible reduction in the number of WEE plaques by serum from an animal given a WEE vaccine will result in survival of the vaccinee.

B. Rat Potency Test

The rat appears to be the least promising of the three animals used in evaluating four WEE vaccines. The back titration did not give the expected straight line regression in deaths as the challenge virus was diluted. At 10^{-1} one of seven animals died while at 10^{-5} four of six animals died. At no concentration were we able to cause one hundred percent death of the animals.

During the test proper only one rat died. This animal had been given the DEC WEE vaccine diluted 1:5.

Table 11 shows results of the PRNT. The USAMRIID Lot 2-1974 was the only vaccine producing any significant antibody rises.

Table 10

Evaluation of Four (4) WEE Vaccines in Guinea Pigs by Three Tests

Test	ED_{50} of WEE Vaccine			
	MNLBR106 Lot 1	MNL DEC	USAMRIID Lot 1-1974	USAMRIID Lot 2-1974
Protection & challenge	1:50	1:45	1:70	>1:125
PRNT with significant antibody rises	1:9	1:10	1:7	1:10
PRNT with post immunization titers of 10 or greater	1:17	1:12	1:15	1:74

Table 11

WEE Plaque Reduction Rat Potency Test

Serum from	Geometric Mean Titers B11 virus day -21	day 0	day 30	day 60	day 90	Significant antibody rises
B11 vaccine MNLBR106						
Undil	<10	<10	<10	<10	<10	*0/6 (0%)
1:5	<10	<10	<10	<10	<10	0/6 (0%)
1344 vaccine DEC						
Undil	<10	<10	<10	<10	<10	0/7 (0%)
1:5	<10	<10	<10	<10	<10	0/7 (0%)
B11 vaccine Lot 1-1974 USAMRIID						
Undil	<10	<10	<10	<10	<10	0/6 (0%)
1:5	<10	<10	<10	<10	<10	0/6 (0%)
B11 vaccine Lot 2-1974 USAMRIID						
Undil	<10	<10	<10	12	22	1/2 (50%)
1:5	<10	<10	<10	12	12	3/7 (43%)

* Rises/total rats immunized.

Chikungunya Vaccine Development

I. Introduction

A. Tests for safety, sterility, purity and potency were completed for Lot 1 Chikungunya Vaccine, Inactivated, Dried. A submission form was written summarizing the production and test results. This was followed by a detailed "Procedures Manual" prepared for the production of the vaccine in diploid cells.

B. Preliminary experiments with Chikungunya vaccine suggest that a neutralizing antibody titer of 1:10 would protect a mouse, inoculated intracerebrally, from live virus.

All work on Chikungunya vaccine was stopped June 1978.

II. Experimental-Safety and Potency Tests Completed in 1978

A. MAP Test

The mouse antibody production test completed this year demonstrated that the S-27 seed virus was free of detectable mouse agents.

B. Identity Test of the S-27 Virus

A standard neutralization test was performed to demonstrate the identity of the vaccine seed virus by comparing it with a known prototype Chikungunya virus. Rabbit antiserum capable of neutralizing Chikungunya virus, strain 168, 10,000-fold also neutralized the S-27 virus to the same degree.

C. Test for Live Virus in the Vaccine

The formalin-inactivated virus fluid, prior to freeze-drying, was inoculated into newborn mice by the intraperitoneal and intracerebral routes to demonstrate that the vaccine was free of live virus. All of the test mice survived showing the vaccine to be safe.

D. Potency Test

The potency of S-27 Chikungunya virus vaccine was compared with a reference vaccine made with the 15561 Chikungunya virus (Harrison, et al., J. Immunol. 107:643,1971). The parallel testing of these vaccines in mice demonstrated that the S-27 vaccine was as potent as the reference vaccine.

III. Experiments to Demonstrate Antibody Response in Mice Vaccinated With S-27 Chikungunya Vaccine

A. Antibody Response

Groups of weanling mice were vaccinated with undilute and various dilutions of Chikungunya virus vaccine. The animals were bled after 7, 14 and 21 days. The individual mouse sera were tested for neutralizing antibody by the plaque-reduction test. The data in Table 12 show that neutralizing antibodies appear in the sera by day 7, but the response is greater on days 14 and 21 following the administration of two doses.

B. Correlation of Antibody Response With Protection

The data in Table 12 were used to calculate an ED_{50} potency value based on measurable antibody titers of 1:10, or greater, leading to protection. The calculated potency values from the antibody data and from live virus challenge tests are shown below:

Data Source	ED_{50}*	
	Day 7 (1-dose)	Day 14 (2-dose)
Antibody Response in Sera from exsanguinated mice	0.33 ml	0.01 ml
Mice surviving live virus challenge	0.33 ml	0.01 ml

* In ml per dose

The data may be interpreted to show indirectly that a neutralizing antibody titer in mice of 1:10, or greater, would result in survival of the animal if challenged with live virus.

Table 12

Neutralization Antibody Response in Mice to One- and Two-Doses of Lot 1 Chikungunya Vaccine, Inactivated, Dried

Vaccine Dilution Inoculated (IP)	Day Bled (post-vaccination) Day 7 (1-dose) (Reciprocal Serum Dilution/mouse*)[1]	Day 14 (2-dose)	Day 21 (2-dose)
Undilute	17,40,56,63, 0 (NA)[2]	Not done	Not done
1:3	0,0,0,0,0,0	10,42,45,47, 57,70	40,46,46,176, 180,184
1:9	0,0,0,0,0,0	10,24,25,45, 46,176	0,0,44,173, 180,180
1:27	0,0,0,0,0,0	0,0,12,47, 49,181	0,0,0,12,46, 46
1:81	Not done	0,0,0,0,0,49	0,0,0,12,46, 46

* = [1] Antibody titer expressed as the reciprocal of a serum dilution causing an 80% reduction in plaque numbers of S-27 virus fluid containing 40 or 190 p.f.u. 0 = <1:10 (lowest test dilution):

NA = [2] Serum not available.

Q Fever Program

I. Introduction

A submission for Q Fever Skin Test Antigen, MNLBR 110, Lot 1 was prepared as an addendum to Q Fever Vaccine, Phase 1, Inactivated, Dried, Lot 5, NDBR 105 and forwarded to USAMRIID for approval.

Rocky Mountain Spotted Fever

I. Introduction

A vaccine submission including laboratory data from Rocky Mountain Spotted Fever Vaccine, Inactivated, Dried, MNLBR 108, Lots 1, 2 and 3 was prepared and submitted to USAMRIID for approval.

Tissue Culture

I. Introduction

During 1978, two new Production Seed Stocks were prepared at Passage 10; FRhL-2 and IMR-91. Five Production Lots of FRhL-2 and one of MRC-5 were stabilized, frozen and certified. Two previously prepared Lots of MRC-5 and one of IMR-90 were also certified for vaccine use. A total of 931 ampules of frozen FRhL-2 were processed into 8235 cultures for preparing ten lots of RVF vaccine.

Physically, we have made improvements in the tissue culture laboratories this year by replacing the stainless steel hoods with laminar flow hoods, adding one inverted microscope and one LR-310 liquid nitrogen freezer. Additional equipment to be delivered, as yet, includes four production roller devices, automatic filler and ampule sealer, additional laminar flow equipment, karyology laboratory equipment and additional liquid nitrogen processing and storage equipment. Preliminary plans for new tissue culture facilities have been drawn up. Frozen ampule production capacity should increase from approximately 400 to 1200 ampules per lot as equipment is put on-line this coming year.

II. Process Studies

A. Production Cells

1. Primary Duck Cells

Five ampules of primary DEC, Lot 1 were shipped to USAMRIID.

2. WI-38 Diploid Cell Line

No work was done.

3. FRhL-2 Diploid Cell Line

One ampule of Passage 5 FRhL-2 was obtained from the ATCC through Dr. Petricianni and was processed into a Production Seed at Passage 10. The passage history and logistics are summarized in Table 13. Five of the ampules have been used to prepare Production Lots and six have been shipped to USAMRIID.

Five Production Lots of FRhL-2 were prepared and tested as shown in Table 14. Lot 14 was prepared from a Passage 10 ampule obtained from ATCC while Lots 15, 16, 17, and 18 were derived from the new production seed (above). Chromosome analyses, by Metpath, Inc., were completed on these

lots and three previously prepared lots as indicated in Table 15. Production lots prepared this year (i.e. 14-18) were grown in media containing Reheis Fetal Calf Serum while the three previous lots (i.e. 10, 12 and 13) were grown in the presence of fetal calf serum from MBA. There appears to be a higher incidence of polyploidy and breaks and/or gaps in cells processed with MBA serum. FRhL-2 cells prepared from the new production seed (Lots 15-18) were harvested at Passage 16, eliminating one passage. This was made possible by the vigor of the new seed allowing us to start production with three 150 cm^2 flasks/ampule instead of one flask/ampule as needed with Passage 10 cells from ATCC. Cells harvested from Lots 15-18 (new production seed) were smaller and the cell-sheets were more compact than seen with Lot 14 (ATCC P10 seed). One additional production lot (Lot 19) was started.

Lots 10, 12, 13, 15 and part of 16 were used (931 ampules total) to prepare 8235 cultures for ten lots of RVF vaccine. Cultures were satisfactory and only 18 cultures (0.2%) were discarded (from one lot) due to mold contamination. A compartment-type thawing bath was devised to thaw 12 ampules, in isolation, at one time for this project. Additionally, a holding-box to isolate each of the thawed ampules was made of 2 inch sections of heavy-walled vacuum tubing placed in a disposable cardboard storage container. These devices aided the conduct of the work.

A total of 44 ampules were processed into 404 cultures for assorted experiments and viral seed passages.

Shipment of frozen ampules to USAMRIID included 130 of Lot 10 FRhL-2 and 70 of Lot 15.

4. Primary Chick Embryo Cells

No work was done.

5. MRC-5 Diploid Cell Line

One lot of MRC-5 (Lot 5) was produced and two previous lots (3 and 4) were tested and certified for use in vaccine work. The status of these lots is given in Table 16. All testing is complete with the exception of the chromosome analysis on Lot 5. Chromosome analyses on Lots 3 and 4 are shown in Table 17. Both lots were processed in the presence of MBA fetal calf serum and the incidences of polyploidy and breaks and/or gaps was high as was seen with FRhL-2 preparations. MRC-5, Lot 5 was processed in the presence of Reheis FCS and it should be interesting to compare the chromosome analysis on it to the other two lots. A total of 5 ampules were processed into 70 cultures for experimental use and one ampule of production seed (Pass 17) was shipped to USAMRIID.

6. IMR-90 Diploid Cell Line

Testing on Lot 1, IMR-90 was completed this year as in Table 18. The chromosome analysis from Metpath, Inc. is given as Table 19. As above, for FRhL-2 and MRC-5, the association of MBA FCS in the media and a high incidence of polyploidy and breaks and/or gaps is present.

7. IMR-91 Diploid Cell Line

Passage 5 IMR-91 was obtained from The Institute for Medical Research, Camden, N.J. and was processed to a Cell Seed at Passage 10. The history is shown in Table 20.

8. FCL-7 Diploid Cell Line

No work was done.

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

A total of 19 ampules, representing dog #140 was shipped to USAMRIID.

B. Experimental

Little work in the experimental area was done this year with the production requirements for RVF taking priority for time and space. A heat-sealable ampule (Cooke Engineering) was tested for use in our system and was found to be acceptable. The major advantage of these ampules, made of polypropylene, is that they are non-shatterable. One production lot (FRhL-2 Lot 17, Pass 16) was later processed in these ampules. Of the 37 ampules used for testing, one was found to have a cracked seam upon thawing, possibly a flaw in the manufacturing process.

IMR 90 (Pass 19) cells, which had been frozen by our "in situ" method (Thomas et al., Cryobiol. 13: 648, 1976) and stored at -65°C for one year were tested and viable cultures were produced within a week. This system has been used very successfully this past year for short-term storage of other cells used in safety and assay tests (i.e. Vero, KB, MRC-5, FRhL-2, CV-1).

III. Cell Inventory

A summary of the inventory and use of ampules for the year is in Table 21. As shown, 231 ampules of certified cells were shipped to USAMRIID and 1210 ampules were used here for testing and vaccine work. Of the various cells maintained for test purposes, 8 ampules were shipped to USAMRIID and 27 ampules were used here for testing purposes. Fewer ampules of these cells are normally used since the cells are passed several times after freezing before use in safety and assay tests.

IV. Conclusion

The certified cell system has worked well under actual production and use conditions. In addition to the production of vaccines, it has given us a "clean substrate" for use in preparing viral seeds.

The circumstantial evidence that various fetal calf serums can cause higher incidences of polyploidy and breaks and/or gaps in normal diploid cells bears watching. Consideration to screening serum for these effects should be given in addition to the usual tests for sterility and growth-promoting ability before purchase.

Table 13

Passage of FRhL-2 to
Prepare Production Seed
Cells

Passage no.	Date	Days	No. bottles	Note
5	5/29/73			Frozen ampule from ATCC
6	1/27/78	0	1 x 75 cm^2	
7	1/30/78	3	3 x 75 cm^2	
8	2/2/78	3	9 x 75 cm^2	
9	2/6/78	4	27 x 75 cm^2	
10	2/9/78	3	40 x 150 cm^2 + 1 x 75 cm^2	---Held for 4 weeks-normal
Harvest*	2/14/78	5	40 x 150 cm^2	

*	Total area harvested	6000 cm^2
	Total cells	6324 x 10^6
	Viability (% aqueous/isotonic)	94/98
	No. amps frozen **	100
	Sheeting - 1 amp	3 x 150 cm^2/4 days (Lots 15 - 18)
	Bulk sterility	Passes
	30-day hold harvest fluids	Sterile

** Room temperature conditioning with 7½% DMSO.

Table 14

Certification of Five Lots of
Male Fetal Rhesus Lung Diploid Cells
FRhL-2 in 1978

Item	Lot 14 Pass 17 [1]	Lot 15 Pass 16 [2]	Lot 16 Pass 16 [2]	Lot 17 Pass 16 [2]	Lot 18 Pass 16 [2]
1. Surface area harvested (cm^2)	107,850	107,850	107,850	97,950	107,850
2. Total cells (X 10^9)/cell pack (ml)	9.5/33	14/42	12.5/40	10.9/36	12.2/42
3. Cells/cm^2 (X 10^5)	0.9	1.4	1.2	1.1	1.1
4. No. ampules frozen	364	365	349	348	389
5. Cells/ampule (X 10^6)	26	36	34.8	31.1	31.2
6. Percent viability (aqueous trypan blue)	96	96	95	91	95
7. Bulk sterility - CFR 610.12	S [3]	S	S	S	S
8. 2-week hold of cell samples after harvest	S	S	S	S	S
hemadsorption (G. pig RBC)	S	S	S	S	S
9. 30-day hold of harvest fluids	S	S	S	S	S
PPLO-CFR 610.30	S	S	S	S	S
10. PPLO: frozen-thawed cells (3X) - CFR 610.30	S	S	S	S	S
11. Sheetability: 1 amp - 1 roller - 700 cm^2 (no.)	3 days (21)	3 days (20)	3 days (20)	4 days (21) [4]	4 days (23) [4]
(antibiotic-free) 1 amp - 2 rollers	4 days	5 days	4 days	4 days	4 days
1 amp - 10 x 75 cm^2 plastics	2 days	2 days	3 days	4 days [4]	2 days
1 amp - 20 x 75 cm^2 plastics	4 days	3 days	3 days	3 days	3 days
12. Hemadsorption - sheetability test (G. pig RBC)	S	S	S	S	S
13. PPLO: sheetability test - CFR 610.30	S	S	S	S	S
14. M. tuberculosis (Lowenstein-Jensen)	S	S	S	S	S
15. Tissue Culture safety - CFR 630.13	S	S	S	S	S
16. Embryonated egg safety (allantoic) CFR 610.13 (4)	S	S	S	S	S
17. Oncogenicity (new-born hamsters)	S	S	S	S	S
18. Karyology	S	S	S	S	S

[1] Old P10 production seed from ATCC

[2] New P10 production seed (prepared at The Salk Inst. - Gov. Serv. Dis.)

[3] S = test satisfactory

[4] No CO_2 gassing

Table 15

Chromosome Analyses On Eight Lots
of FRhL-2 (Metpath, Inc.)

FRhL-2 Lot No. Passage No.	No. of chromosomes [3]								Polyploidy	Breaks and/ or gaps
	35	38	39	40	41	42	43	44		
	(No. cells)								(%)	(%)
Lot 10 Pass 18 [1]					5	94	1		5	8
Lot 12 Pass 18 [1]			1		5	91	2	1	5	5
Lot 13 Pass 18 [1]	1				5	94	0		4	2
Lot 14 Pass 18 [1]					5	89	6		0	1
Lot 15 Pass 17 [2]					8	384	8		0	1.5
Lot 16 Pass 17 [2]					0	100	0		0	3
Lot 17 Pass 18 [2]					1	99	0		0	0
Lot 18 Pass 18 [2]					1	98	1		0	0

[1] From P10 Production Seed obtained from ATCC thru Dr. Petricianni. One pass beyond freeze-down of production lot. Lots 10,12,13 grown on MBA serum. Lots 14 thru 18 grown on Reheis serum.

[2] Lots 15→18 from P10 Production Seed developed from P5 seed from Dr. Petricianni. Lots 15 & 16 one pass beyond production freeze-down; Lots 17 & 18 two passes beyond production freeze-down.

[3] Lot 15 - full test of 400 counts and 50 karyotypes; Rest of lots - monitor test of 100 counts and 20 karyotypes.

Table 16

Status of Three Lots of Human
Male Fetal Lung Diploid Cells
MRC-5-Passage 23

		Lot 3	Lot 4	Lot 5
1.	Surface area harvested (cm^2)	108,000	107,100	108,000
2.	Total cells (X 10^9)/ cell pack	9.8/34 ml	11.3/48 ml	16.2/45 ml
3.	Cells/cm^2 (X 10^5)	0.9	1.1	1.5
4.	No. ampules frozen	347	385	386
5.	Cells/ampule (X 10^6)	27	32	42
6.	Percent viability (aqueous/isotonic)	94/98	94/98	92/96
7.	Bulk sterility - CFR 610.12	S [1]	S	S
8.	2-week hold of cell samples after harvest	S	S	S
	hemadsorption (G. pig RBC)	S	S	S
9.	30-day hold of harvest fluids	S	S	S
	PPLO-CFR 610.30	S	S	S
10.	PPLO: frozen thawed cells (3X) - CFR 610.30	S	S	S
11.	Sheetability: rollers-700 cm^2 (no.)	2 days(19)	2 days (11)[2]	4 days(21)[3]
	(antibiotic-free) 2 rollers/1 amp	3 days	4 days	ND
	10 x 75 cm^2 plastics/1 amp	3 days	2 days	2 days
	20 x 75 cm^2 plastics/1 amp	4 days	3 days	4 days
12.	Hemadsorption-sheetability test (G. pig RBC)	S	S	S
13.	PPLO: sheetability test - CFR 610.30	S	S	S
14.	M. tuberculosis (Lowenstein-Jensen)	S	S	S
15.	Tissue culture safety - CFR 630.13	S	S	S
16.	Embryonated egg safety (allantoic) - CFR 630.13 (4)	S	S	S
17.	Oncogenicity (new born hamsters)	S	S	S
18.	Karyology	S	S	ND

[1] S = Test satisfactory; ND = test not done

[2] 4 days for 9 additional rollers without CO_2 gassing

[3] No CO_2 gassing

Table 17

Chromosome Analyses on Two Lots
of MRC-5, Passage 24*
(Metpath, Inc.)

No. of chromosomes	Lot No.	
	3-Full test (no. cells)	4-Monitor test
41	2	
44	2	1
45	21	11
46	373	87
47	2	
70		1 **
Polyploidy (%)	15	4
Breaks and/or gaps (%)	7.5	4

* One passage beyond production freeze-down.

** 2 cells with multiple breaks

Table 18

Testing of Lot 1 Human Female
Fetal Lung Diploid Cells
IMR-90, P21

Item	Status
1. Surface area harvested (cm^2)	108,000
2. Total cells (X 10^9)/ cell pack	14.6/48 ml
3. Cells/cm^2 (X 10^5)	1.4 (PDL 36.7)*
4. No. ampules frozen	366
5. Cells/ampule (X 10^6)	37.7
6. Percent viability (aqueous/isotonic)	94/97
7. Bulk sterility - CFR 610.12	S*
8. 2-week hold of cell samples after harvest	S
hemadsorption (G. pig RBC)	S
9. 30-day hold of harvest fluids	S
PPLO-CFR 610.30	S
10. PPLO: frozen-thawed cells (3X)-CFR 610.30	S
11. Sheetability: rollers-700 cm^2 (no.)	3 days (20)
(antibiotic-free) 2 rollers/1 amp	5 days
10 x 75 cm^2 plastics/1 amp	3 days
20 x 75 cm^2 plastics/1 amp	5 days
12. Hemadsorption - sheetability test (G. pig RBC)	S
13. PPLO: sheetability test - CFR 610.30	S
14. M. tuberculosis (Lowenstein-Jensen)	S
15. Tissue culture safety - CFR 630.13	S
16. Embryonated egg safety (allantoic) CFR 630.13 (4)	S
17. Oncogenicity (new born hamsters)	S
18. Karyology	S

*S = Test satisfactory; ND = not done; PDL = population doubling.

Table 19

Chromosome Analysis on Lot 1
IMR - 90, Passage 22 *
(Metpath, Inc.)

No. of chromosomes	No. of cells
44	2
45	16
46	378
47	4
Polyploidy (%)	5
Breaks and/or gaps (%)	7

* One passage beyond production freeze-down.

Table 20

Passage of Male Fetal Human
Lung Diploid Cells
IMR-91

Passage	Date	Days	No. of bottles	Comment
5[1]	8/4/78		1 x 25 cm^2	PDL - 10.7
6	8/11/78	7	1 x 75 cm^2	
7	8/14/78	3	3 x 75 cm^2	
8	8/17/78	3	4 x 150 cm^2	
			1 x 75 cm^2	frozen-3 amps
9	8/22/78	5	12 x 150 cm^2	
10	8/28/78	6	36 x 150 cm^2	
Harvest[2]	8/31/78	3	--	99 ampules[3] PDL 21

[1] Received from The Institute for Medical Research, Camden, N.J.

[2] 663 x 10^6 cells total

18.4 x 10^6/flask

1.2 x $10^5/cm^2$

[3] 6.5 x 10^6/amp 98% viable

1 amp sheets 3 x 150 cm^2 in 5 days

PDL = population doubling

Table 21

Cell Inventory and Use
1978

Item #	Cell	Lot # *	Pass	Date Frozen	Ampule Cell Count (X 10^6)	Viability (%)	No. Amps Jan. 78	Amps Shipped	Amps Used	Current Inventory	Use
1	FRhL-2	PS	10	2/14/78	6.4	94-98	--	6	5	89	Certified Cells for Vaccine Prep.
		8-OPS	17	11/23/76	8.0	75	292	-	8	284	
		10	17	7/19/77	21.2	90-91	214	130	80	4	
		12	17	9/14/77	23.6	86-87	319	--	307	9	
		13	17	9/29/77	23.0	85-88	273	--	270	3	
		14	17	2/22/78	26.0	96-98	--	--	57	307	
		15	16	3/22/78	38.0	95-100	--	70	245	50	
		16	16	7/12/78	34.8	95-100	--	--	161	188	
		17	16	9/13/78	31.1	91-100	--	--	37	311	
		18	16	9/20/78	31.2	95-100	--	--	39	350	
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	323	--	7	315	
4	IMR-91	MS	10	8/31/78	5.2	100	--	--	1	98	
5	MRC-5	PS	17	6/7/77	7.0	100	33	1	--	32	
		3	23	12/9/77	27.0	94-98	313	--	13	300	
		4	23	12/21/77	32.0	94-98	343	--	--	343	
		5	23	1/11/78	42.0	92-96	--	--	34	352	

Table 21

Cell Inventory and Use
1978

(continued)

Item #	Cell	Lot #	Pass	Date Frozen	Ampule Cell Count (X 10^6)	Viability (%)	No. Amps Jan. 78	Amps Shipped	Amps Used	Current Inventory	Use
6	DEC (Duck)	1	Primary	2/26/75	152.0	93	11	5	--	6	
7	(Dog Kidney)	(Dow Chem.)	Primary	4/5/77 (rec'd)	--	--	1527	19	--	1508	
8	BSC-1	--	76	2/14/75	14.0	84-87	25	4	--	21	Test Cells
9	CV-1	--	29	12/21/76	1.3	85	16	--	5	11	
			36	10/20/78	--	--	--	--	--	81	
10	KB	--		3/18/75	14.0	89-98	55	--	3	52	
11	LLC-MK2	--	264	2/11/75	4.0	78	36	4	--	32	
12	RK13	--	73	6/16/75	9.0	83	44	--	4	40	
13	Vero	--	122	4/24/75	2.0	82	71	--	15	56	

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

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