Final Report

NASA Contract No. NAS 9-8780

HEM Research Inc.

5451 Randolph Road

Rockville, Maryland

Final Report, NASA 9-8780

In October, 1968, HEM Research Inc. undertook the task of preparing frozen cell stocks for the Lunar Receiving Laboratory, NASA Manned Spacecraft Center. The specific cells required under the contract were "Green" Monkey Kidney (C. aethiops), Human Embryonic Kidney (HEK), and Human Embryonic Lung (WI-38). The cells were to be shipped by HEM Research Inc; upon the request of the NASA technical monitor, under liquid or vapor phase nitrogen, to the Lunar Receiving Laboratory.

The Human Embryonic Kidney (HEK) and the Human Embryonic Lung (WI-38) were to be produced by HEM Research Inc. and the "Green" Monkey Kidney (GMK), by suggestion of the NASA technical monitor, was to be produced by Lederle Laboratories; Pearl River, New York. The reason for using the Lederle "Green" Monkey Kidney (GMK) was to reduce the time involved in screening the host tissue for latent simian agents. The ampoules of "Green" Monkey Kidney (GMK) prepared by Lederle Laboratories were from monkeys which had been held in quarantine for a minimum of eight weeks and given extensive serological testing before being sacrificed for use in tissue culture. The subsequent primary tissue culture cells as well as the passed cells were screened and held for virus CPE as required under the United States Public Health Service regulations for animals for vaccine production.

The statement of work covered the required number of units needed for each cell strain. All ampoules were to be packaged in one hundred 16 x 125mm^2 test tube equivalents. Our experience indicated that in converting all units needed for each strain to 16 x 125mm^2 tube equivalents, the following total number of ampoules were required.

"Green" Monkey Kidney (C. aethiops)
5000 screw cap tubes-50 ampoules
650 Leighton tubes-8 ampoules
350 four-ounce prescription bottles-18 ampoules
Total 76 ampoules

Human Embryonic Kidney (HEK)
9600 screw cap tubes-96 ampoules
1000 Leighton tubes-12 ampoules
550 four-ounce prescription bottles-33 ampoules
Total 141 ampoules

Human Embryonic Lung (WI-38)
6500 screw cap tubes-65 ampoules
750 Leighton tubes-9 ampoules
400 four-ounce prescription bottles-24 ampoules
Total 98 ampoules

In order to give reasonable assurance that the final tube equivalents might be obtained, we produced an overage of 10% for NASA's use and at least a test ampoule for check of the frozen material in our laboratory.

Work

The ampoules of "Green" Monkey Kidney (GMK) were sent by Lederle Laboratories in liquid nitrogen and received in our laboratory by November 1, 1968. Within a week the LR-40, liquid nitrogen container, purchased under the contract was received and the transfer of ampoules was made to this unit. The testing of this material for outgrowth could not be undertaken until after January 15, 1969 when the Laminar Flow Hood was received.

The only other equipment authorized for purchase under the contract was the PR-2 refrigerated centrifuge and it, with all needed accessories, was in our laboratory by November 15, 1968.

During the period through December 31, 1968 seven lots of Human Embryonic Lung (WI-38), totaling 93 ampoules, were produced with sterility tests completed. There were indications during this time that source tissue for Human Embryonic Kidney (HEK) might become a significant problem in satisfying the total requirements of the contract. To avoid that problem and any delay in meeting the target dates of delivery, Mr. Johansson, the President, and Mr. Johnson made trips domestically and abroad to intensify the search for available tissue. The efforts did, in fact, increase our line of supply of source tissue and production of Human Embryonic Kidney (HEK) ampoules in the quantities required became more of a reality.

The tests of each lot were set up to check for outgrowth, confluency, and general quality. All Human Embryonic Kidney (HEK) and Human Embryonic Lung (WI-38), as we had anticipated, were of good quality and reached confluency in five to seven days. Tubes were planted based on an ampoule yielding 100 tubes and with one feed between planting and confluency.

The "Green" Monkey Kidney (GMK), however, performed somewhat erratically with a much longer time involved in reaching confluency (nine to eleven days). The cells were consistently granular and generally inferior to freshly prepared "Green" Monkey Kidney (GMK) cells. Lederle Laboratories was contacted about this problem. Two ampoules of each lot

remained there and they agreed to do plating efficiencies on each lot to re-establish the actual tube equivalent in contrast to the expected 100 tube equivalent. An attached note from them dated June 6, 1968 supplies the information obtained from their plating efficiencies. Five of the nine lots supplied included ampoules with less than 100 tube equivalents. In view of this Lederle Laboratories, on June 23, 1968, supplied a supplemental shipment of 20 ampoules as compensation for the deficient lots. Also included in this report, is a copy of the Lederle test data (plating efficiencies) on the supplemental lots.

The methods employed in the preparation of Human Embryonic Kidney (HEK), primary and "Green" Monkey Kidney (GMK) are those methods conventionally used for such preparations with no new innovations employed. The specific technique applied is described in the section on tissue culture of Diagnostic Procedures for Viral and Ricketsial Infection "Trypsinization of Fresh Tissues." The Human Embryonic Kidney (HEK) tissue was dispersed by either the over-night trypsinization method or by the multiple extraction method dependent upon the time of arrival of the host tissue on any given day.

Included in this report are copies of the correspondence supplied by Lederle Laboratories describing the lot number, serological and tissue observations, and general quality of their lots. To provide some information on the Human Embryonic Kidney (HEK) Table 1 of our report provides the approximate gestation age of host fetus.

The Human Embryonic Lung (WI-38) was obtained from Dr. Leonard Hayflick, Stanford University. The material was received at the Passage 13

(p.13) level in two small plastic flasks ($75cm^2$). We sub-cultured this material to the Passage 17 level at which time we froze the contents of sixteen 32 ounce ($120cm^2$) prescription flasks. These flasks yielded 37 ampoules containing 6×10^6 cells per ampoule or the starting equivalent when thawed from frozen of three $60cm^2$ flasks. A typical scheme for production of a NASA lot is as follows:

2 ampoules

$6 \times 60cm^2$ flasks (p.18)

$6 \times 120cm^2$ flasks (p.19)

$12 \times 120cm^2$ flasks (p.20)

$24 \times 120cm^2$ flasks (p.21)

12 ampoules @ 2×10^7 cell/ampoule

This process, from thawing ampoules (p.18) to producing ampoules for NASA @ 2×10^7 cells per ampoule as (100 tube equivalents) covered nineteen days. The Human Embryonic Lung (WI-38) ampoules shipped were produced from $240 \times 120cm^2$ flasks and handled on four separate days with the following lot numbers being made and containing the following number of ampoules:

Lot Number	Number of Ampoules
01394	12
01417	13

Lot Number	Number of Ampoules
01418	12
01419	11
01420	12
01364	9
01392	11
01393	13
01483	11
01484	13

The total number of ampoules supplied under the contract were delivered in two shipments. Each shipment was accompanied by a representative from HEM Research Inc. The first shipment was made on April 18, 1969, and the second shipment was made on June 25, 1969. For both shipments the cells were delivered under liquid and vapor phase nitrogen, as requested under the contract, to the Lunar Receiving Laboratory by our representative. The transfer of ampoules from the transport container to the Lunar Receiving Laboratory repository was made by our representative and a technician assigned to the Lunar Receiving Laboratory. The transport container for each shipment had sufficient liquid nitrogen remaining to fill repositories at the Lunar Receiving Laboratory (eliminating the doubt of insufficient nitrogen during transit).

The equipment purchased under the contract is in good repair and in our laboratory for use in filling the contract needs for 1969-1970, in the event that we were awarded the new contract. Upon the completion of the

contract, this equipment is available for disposal at the request of the contract administrator.

This equipment includes:

(1) Laminar Flow Hood, Model No. Microvoid 4B, Recirculator.

(2) Centrifuge, International Refrigerator, Model No. PR-2 with six place free-swinging head and centrifuge cups.

* (3) Linde Liquid Nitrogen Repository, Model No. LR-40.

*The container listed under Item No. 3 is presently at the Lunar Receiving Laboratory. We have two LR-40's from the Lunar Receiving Laboratory stored at our laboratory. The first of these two units was bought here from Houston to transport the shipment of June 25, 1969. It was found to be faulty (did not hold liquid nitrogen for the expected period). The second unit was sent and was also found to be faulty.

Table 1

Human Embryonic Kidney

Lot Number	Number of Ampoules	Gestation
01585	10	30 weeks
01591	11	28 weeks
01650	18	33 weeks
01659	9	30 weeks
01696	11	24 weeks
01697	12	32 weeks
01713	10	36 weeks
01792	10	36 weeks
01893	14	28 weeks
01908	9	28 weeks
01933	27	36 weeks

Table 2

Human Embryonic Kidney Suspension Sterility

Lot Number	Blood Agar	Thioglocyllate	Saborauds	PPLO	Test Tubes
01585	-----	-----	-----	----	-----
01591	-----	-----	-----	----	-----
01650	-----	-----	-----	----	-----
01659	-----	-----	-----	----	-----
01696	-----	-----	-----	----	-----
01697	-----	-----	-----	----	-----
01713	-----	-----	-----	----	-----
01792	-----	-----	-----	----	-----
01893	-----	-----	-----	----	-----
01908	-----	-----	-----	----	-----
01933	-----	-----	-----	----	-----

All sterilities observed for fourteen days, the samples above represent an inoculum of concentrated cell suspension prior to freezing.

Table 3

Human Embryonic Kidney Tissue Sterility

Lot Number	Blood Agar	Thioglocyllate	Saborauds
01585	-----	-----	-----
01591	-----	-----	-----
01650	-----	-----	-----
01659	-----	-----	-----
01696	-----	-----	-----
01697	-----	-----	-----
01713	-----	-----	-----
01792	-----	-----	-----
01893	-----	-----	-----
01908	-----	-----	-----
01933	-----	-----	-----

Table 4

Test (Quality Control) HEK NASA Lots

Lot Number	Planted	Fed	Confluent
01585	3/10/69	3/13/69	3/15/69
01591	3/10/69	3/13/69	3/15/69
01650	3/10/69	3/13/69	3/15/69
01659	3/10/69	3/13/69	3/15/69
01696	3/10/69	3/13/69	3/15/69
01697	3/10/69	3/13/69	3/15/69
01713	5/12/69	5/15/69	5/17/69
01792	5/12/69	5/15/69	5/18/69
01893	5/12/69	5/15/69	5/17/69
01908	5/12/69	5/15/69	5/18/69
01933	5/12/69	5/15/69	5/17/69

Table 5

WI-38 Suspension Sterility

Lot Number	Blood Agar	Thioglocyllate	Saborauds	PPLO
01392	-----	-----	-----	----
01393	-----	-----	-----	----
01394	-----	-----	-----	----
01417	-----	-----	-----	----
01418	-----	-----	-----	----
01419	-----	-----	-----	----
01420	-----	-----	-----	----
01483	-----	-----	-----	----
01484	-----	-----	-----	----

Table 6

Test (Quality Control) WI-38 NASA Lots

Lot Number	Planted	Fed	Confluent
01392	2/3/69	2/6/69	2/8/69
01393	2/3/69	2/6/69	2/8/69
01394	2/3/69	2/6/69	2/8/69
01417	2/3/69	2/6/69	2/8/69
01418	2/3/69	2/6/69	2/8/69
01419	2/3/69	2/6/69	2/8/69
01420	2/3/69	2/6/69	2/8/69
01483	2/3/69	2/6/69	2/8/69
01484	2/3/69	2/6/69	2/8/69

The following media used in growing both the Human Embryonic Kidney (HEK) and the Human Embryonic Lung (WI-38) were:

Planting Media

Eagles BME (in Hanks BSS with 0.35 grams $NaHCO_3$/liter)	*90%*
Fetal Bovine Serum	*10%*
Penicillin-Streptomycin 100 micrograms/ml	
Neomycin 60 micrograms/ml	

Feeding Media

Eagles BME (in Earles BSS with 2.2 grams $NaHCO_3$/liter)	*90%*
Fetal Bovine Serum	*10%*
Penicillin-Streptomycin 100 micrograms/ml	
Neomycin 50 micrograms/ml	

Maintenance Media

Eagles BME (in Earles BSS with 2.2 grams $NaHCO_3$/liter)	*98%*
Fetal Bovine Serum	*2%*
Penicillin-Streptomycin 100 micrograms/ml	
Neomycin 50 micrograms/ml	

April 18, 1969

Included in the first shipment to the Lunar Receiving Laboratory, NASA Manned Spacecraft Center, Houston, Texas by HEM Research Inc., Rockville, Maryland, 20852 - under Contract No. NAS 9-8780 - are the following ampoules:

"Green" Monkey Kidney (C. aethiops)

MN53 - 158	10 Ampoules	
MN54 - 81	9 Ampoules	
MN57 - 135	11 Ampoules	
MN58 - 142	9 Ampoules	
MN56 - 180	10 Ampoules	Total: 49 Ampoules, "Green" Monkey Kidney

Human Embryonic Kidney (HEK)

01585	9 Ampoules	
01591	10 Ampoules	
01650	17 Ampoules	
01659	9 Ampoules	
01696	10 Ampoules	
01697	11 Ampoules	Total: 66 Ampoules, Human Embryonic Kidney (HEK)

Human Embryonic Lung (WI-38)

01394	12 Ampoules	
01417	13 Ampoules	
01418	12 Ampoules	
01419	11 Ampoules	
01420	12 Ampoules	Total: 60 Ampoules, Human Embryonic Lung (WI-38)

SHIPMENT DATE: April 18, 1969

June 25, 1969

Included in the second shipment to the Lunar Receiving Laboratory, NASA Manned Spacecraft Center, Houston, Texas by HEM Research Inc., Rockville, Maryland, 20852 - under Contract No. NAS 9-8780 - are the following ampoules:

"Green" Monkey Kidney (C. aethiops)

MN49 - 124	10 Ampoules	
MN54 - 97	7 Ampoules	
MN58 - 158	9 Ampoules	
MN61 - 124	15 Ampoules	
MN66 - 102	5 Ampoules	
MN66 - 124	6 Ampoules	
MN71 - 100	9 Ampoules	Total: 61 Ampoules, "Green" Monkey Kidney

Human Embryonic Kidney (HEK)

01713	10 Ampoules	
01792	10 Ampoules	
01893	14 Ampoules	
01908	9 Ampoules	
01933	27 Ampoules	Total: 70 Ampoules, Human Embryonic Kidney (HEK)

Human Embryonic Lung (WI-38)

01364	9 Ampoules	
01392	11 Ampoules	
01393	13 Ampoules	
01483	11 Ampoules	
01484	13 Ampoules	Total: 57 Ampoules, Human Embryonic Lung (WI-38)

SHIPMENT DATE: June 25, 1969

Enclosures:

Letters from Lederle Laboratories regarding information on monkeys used for tissue culture.

October 28, 1968

June 6, 1969

June 23, 1969

LEDERLE LABORATORIES

Lederle

A Division of AMERICAN CYANAMID COMPANY

PEARL RIVER, NEW YORK 10965

AREA CODE 914 735-5000

October 28, 1968

HEM Research Inc.
Rockville, Maryland 20852
Purchase Order No. 000312

PRIMARY CELL BANK

Record of Shipping Data and Culturing Procedures:

Primary Cell Derivation Cercopithecus monkey kidney tissue

Average % Viable 70 - 80%

Vol. of Suspension/Ampoule 4 cc, Cells/ml 7.5×10^6

No. of Ampoules Shipped 100, Date Shipped 10/28/68

Shipped Via Mercury Air Freight to JFK DAX to destination

Storage Temperature Upon Receipt Liquid nitrogen

Method of Recovery Thaw rapidly by agitating in water bath at 37 - 40°c

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Culture Medium (Growth) Hanks lactal w/10% C. S. (pH) 7.4

Culture Medium 1st Renewal (Growth) Earles lactal w/2% C.S. (pH) 7.2

Culture Medium (Maintenance) Earles lactal maint. (pH) 7.4

Recommended Dilutions per Ampoule Upon Thawing:

1. Roller tubes - 1 ampoule + 130 ml growth medium #1 to fill 128 tubes.
2. Plastic culture flasks (Falcon, 75 CM^2) or milk dilution bottle - 1 ampoule + 150 ml growth medium #1 to fill 6 cultures.
3. Blake culture bottles - 1 ampoule + 300 ml growth medium #1 to fill 3 Blakes.
4. Povitsky culture bottle (5 L.) - 1 ampoule + 300 ml growth medium #1 to fill one Povitsky.

Procedures for Thawing and Resuspending:

1. Upon receipt, ampoules should be stored at liquid nitrogen temperatures or thawed as soon as possible. Use of protective gloves and face mask are desirable as a safety precaution.

2. Remove ampoule from low temperature storage vessel (liquid nitrogen) and place immediately into a thaw bath between 37°c and 40°c. The bath should not exceed 40°c in temperature. Thaw rapidly and remove from bath as soon as all the ice has melted.

3. Wipe ampoule with disinfectant solution and take into sterile area for decapping.

4. Remove neck of ampoule by scoring glass with a three cornered file and breaking between several layers of gauze which has been dipped in the disinfectant solution and squeezed dry.

5. Contents of ampoule can now be removed with a sterile five or ten milliliter hypodermic syringe equipped with a 12 gauge canula and dispensed into a culture flask containing growth medium #1* at the appropriate volume recommended on the cover sheet.

6. Following an initial incubation period of four days, growth medium #1 is evacuated and the cultures are renewed with growth medium #2*.

7. Between the 8th to 10th day or when the monolayer is confluent, the cultures are renewed with maintenance medium*.

*See the attached formulation sheets.

William F Daly

William F. Daly
Department 472
Extension 3331

WFD:em

HEM Research Inc.
Rockville, Maryland 20852

Purchase Order No. 000312
October 28, 1968

Index of Ampoule Position in
LNR-35 (Lederle Labs PP&E #47304)

Position	Monkey Number	No. of Ampoules
1A	MN61-124	9
1B	MN61-124	7
	MN49-124	2
2A	MN57-135	2
	MN56-180	2
	MN58-142	5
2B	MN49-124	9
3A	MN57-135	1
	MN56-180	4
	MN54-81	4
3B	MN56-180	4
	MN58-142	5
4A	MN54-81	6
	MN53-158	3
4B	MN57-135	9
5A	MN53-158	8
	MN58-158	1
5B	MN58-158	9
6A	MN58-158	1
	MN54-97	8
6B	MN56-180	1
		100

DESCRIPTION OF TISSUE PREPARATION

Cell Bank No.	Animal Isolation Date	Initiation of Tissue Preparation	Trypsinization Method	Date Cells Frozen	No. of Ampoules
MN58-158	5.3.68	7.10.68	16 hours	7.11.68	11
MN53-158	3.25.68	6.26.68	16 hours	6.27.68	11
MN54-81	3.25.68	6.26.68	16 hours	6.27.68	10
MN57-135	5.3.68	7.2.68	16 hours	7.3.68	12
MN58-142	5.3.68	7.10.68	16 hours	7.11.68	10
MN56-180	4.9.68	6.12.68	16 hours	6.13.68	11
MN49-124	2.2.68	6.19.68	16 hours	6.20.68	11
MN61-124	6.7.68	7.17.68	16 hours	7.18.68	16
MN54-97	3.25.68	7.24.68	16 hours	7.25.68	8

MEDIA WORKSHEET # 1

SOLUTION: Hanks Lactal W/10% Calf Serum

DATE: October 28, 1968

LOT NO.:

VOLUME:

CONTROL NO.:

INGREDIENTS PER LITER	QUANTITY Gms	WEIGHT USED	CHECK LIST
UNIT I			
NaCl	8		
KCl	0.4		
$MgSO_4 \cdot 7H_2O$	0.2		
Na_2HPO_4	0.047		
Glucose	1.0		
KH_2PO_4	0.06		
UNIT II			
$CaCl_2$	0.14		
Demin. Dist. H_2O	100 ml		
UNIT III			
Phenol Red	0.015		
UNIT IV			
1N NaOH	2 ml		
Streptomycin (active base)	100,000 γ		
Neomycin (active base)	100,000 γ		
$NaHCO_3$	0.35		
Lactal Hydrolysate	5.0		
Demin. Dist. H_2O	1,000 ml		
Calf Serum	100 ml		
Eagles Vit (100x)	100 ml		

FILTRATION DATA:

REMARKS:

Reagent grade used throughout.

STERILITY	ON TEST	OFF TEST	STERILITY	ON TEST	OFF TEST
Thioglycollate					
Sabouraud's					
Trypticase Soy					

BOOK NO.:

PAGE NO.:

SIGNATURE:

LPR 5772 10/60

MEDIA WORKSHEET # 2

SOLUTION		DATE
Lactal W/2% Calf Serum		October 28, 1968
LOT NO.	VOLUME	CONTROL NO.

INGREDIENTS PER LITER	QUANTITY Gms	WEIGHT USED	CHECK LIST
Sodium Chloride NaCl	7.175		
Potassium Chloride KCl	0.400		
Calcium Chloride (Anhydl) $CaCl_2$	0.200		
Magnesium Sulfate $MgSO_4 \cdot 7H_2O$	0.200		
Sodium Phosphate Monobasic - $NaH_2PO_4 \cdot H_2O$	0.125		
Dextrose	1.000		
Sodium Bicarbonate	1.100		
Phenol Red (Water Soluble)	0.015		
Lactal Albumin Hydrolysate	5.000		
Streptomycin Sulfate (active base	100,000 γ		
Neomycin Sulfate (active base)	100,000 γ		
Make up with Demineralized Distilled water to	1000 ml		
Calf Serum	20 ml		
Eagles Vit. (100X)	10 ml		

FILTRATION DATA:

REMARKS:

Reagent grade used throughout.

STERILITY	ON TEST	OFF TEST	STERILITY	ON TEST	OFF TEST
Thioglycollate					
Sabouraud's					
Trypticase Soy					

BOOK NO.	PAGE NO.		SIGNATURE

MEDIA WORKSHEET # 3

SOLUTION		DATE
Lactal Maint.		October 28, 1968
LOT NO.	VOLUME	CONTROL NO.

INGREDIENTS PER LITER	QUANTITY Gms	WEIGHT USED	CHECK LIST
Sodium Chloride NaCl	7.175		
Potassium Chloride KCl	0.400		
Calcium Chloride (Anhydl) $CaCl_2$	0.200		
Magnesium Sulfate $MgSO_4 \cdot 7H_2O$	0.200		
Sodium Phosphate Monobasic - $NaH_2PO_4 \cdot H_2O$	0.125		
Dextrose	1.000		
Sodium Bicarbonate	1.600		
Phenol Red (Water Soluble)	0.015		
Lactal Albumin Hydrolysate	5.000		
Streptomycin Sulfate (active base)	100,000 ɣ		
Neomycin Sulfate (active base)	100,000 ɣ		
Make up with Demineralized Distilled water to	1000 ml		

FILTRATION DATA:

REMARKS:

Reagent grade used throughout.

STERILITY	ON TEST	OFF TEST	STERILITY	ON TEST	OFF TEST
Thioglycollate					
Sabouraud's					
Trypticase Soy					

BOOK NO.	PAGE NO.		SIGNATURE

LEDERLE LABORATORIES
American Cyanamid Company

October 28, 1968

Pre-Tested Green Monkey Tissue

Preparation

Monkey No.	MN 49-124
Isolation Date	2/2/68
Date Cell Growth Initiated	6/20/68
Date Cell Suspension Frozen	6/20/68

Tests

(10% of total cell suspension tested)

Cell sheets (3)

7/11/68 Examination for hemadsorption viruses - Satisfactory

Pooled fluids from vessels above taken 7/11/68 and tested in

Rhesus Monkey Kidney*	- 10 ml.	Tested 7/15/68	Satisfactory 7/29/68
Cercopithecus Monkey Kidney*	- 10 ml.	7/25/68	Satisfactory 8/8/68
Subculture	- 10 ml.	8/15/68	Satisfactory 8/29/68
Rabbit Kidney*	- 10 ml.	7/31/68	Satisfactory 8/14/68
Human Amnion*	- 10 ml.	7/26/68	Satisfactory 8/9/68

* Primary Cell Cultures

Frances W. Bingham

LEDERLE LABORATORIES
American Cyanamid Company

October 28, 1968

Pre-Tested Green Monkey Tissue

Preparation

Monkey No.	MN 53-158
Isolation Date	3/25/68
Date Cell Growth Initiated	6/27/68
Date Cell Suspension Frozen	6/27/68

Tests

(10% of total cell suspension tested)

Cell sheets (4)

7/17/68 Examination for hemadsorption viruses - Satisfactory

Pooled fluids from vessels above taken 7/17/68 and tested in

Rhesus Monkey Kidney*	- 10 ml.	Tested 7/24/68	Satisfactory	8/7/68
Cercopithecus Monkey Kidney*	- 10 ml.	7/25/68	Satisfactory	8/8/68
Subculture	- 10 ml.	8/15/68	Satisfactory	8/29/68
Rabbit Kidney*	- 10 ml.	8/5/68	Satisfactory	8/19/68
Human Amnion*	- 10 ml.	8/9/68	Satisfactory	8/23/68

* Primary Cell Cultures

Frances W Bingham

LEDERLE LABORATORIES
American Cyanamid Company

October 28, 1968

Pre-Tested Green Monkey Tissue

Preparation

Monkey No.	MN 54-81
Isolation Date	3/25/68
Date Cell Growth Initiated	6/27/68
Date Cell Suspension Frozen	6/27/68

Tests

(10% of total cell suspension tested)

Cell sheets (2)

7/17/68 Examination for hemadsorption viruses - Satisfactory

Pooled fluids from vessels above taken 7/17/68 and tested in

Rhesus Monkey Kidney*	- 10 ml.	Tested 7/24/68	Satisfactory	8/7/68
Cercopithecus Monkey Kidney*	- 10 ml.	7/25/68	Satisfactory	8/8/68
Subculture	- 10 ml.	8/15/68	Satisfactory	8/29/68
Rabbit Kidney*	- 10 ml.	8/5/68	Satisfactory	8/19/68
Human Amnion*	- 10 ml.	8/9/68	Satisfactory	8/23/68

* Primary Cell Cultures

Frances W. Bingham

LEDERLE LABORATORIES
American Cyanamid Company

October 28, 1968

Pre-Tested Green Monkey Tissue

Preparation

Monkey No.	MN 54-97
Isolation Date	3/25/68
Date Cell Growth Initiated	7/25/68
Date Cell Suspension Frozen	7/25/68

Tests

(10% of total cell suspension tested)

Cell sheets (3)

8/15/68 Examination for hemadsorption viruses - Satisfactory

Pooled fluids from vessels above taken 8/15/68 and tested in

Rhesus Monkey Kidney*	- 10 ml.	Tested 8/15/68	Satisfactory 8/29/68
Cercopithecus Monkey Kidney*	- 10 ml.	8/15/68	Satisfactory 8/29/68
Subculture	- 10 ml.	9/4/68	Satisfactory 9/18/68
Rabbit Kidney*	- 10 ml.	8/21/68	Satisfactory 9/4/68
Human Amnion*	- 10 ml.	9/16/68	Satisfactory 9/30/68

* Primary Cell Cultures

Frances W. Bingham

LEDERLE LABORATORIES
American Cyanamid Company

October 28, 1968

Pre-Tested Green Monkey Tissue

Preparation

Monkey No. MN 56-180

Isolation Date 4/9/68

Date Cell Growth Initiated 6/13/68

Date Cell Suspension Frozen 6/13/68

Tests

(10% of total cell suspension tested)

Cell sheets (4)

7/5/68 Examination for hemadsorption viruses - Satisfactory

Pooled fluids from vessels above taken 7/5/68 and tested in

Rhesus Monkey Kidney*	- 10 ml.	Tested 7/8/68	Satisfactory	7/22/68
Cercopithecus Monkey Kidney*	- 10 ml.	7/8/68	Satisfactory	7/22/68
Subculture	- 10 ml.	7/25/68	Satisfactory	8/8/68
Rabbit Kidney*	- 10 ml.	7/31/68	Satisfactory	8/14/68
Human Amnion*	- 10 ml.	7/8/68	Satisfactory	7/22/68

* Primary Cell Cultures

Frances W. Bingham

LEDERLE LABORATORIES
American Cyanamid Company

October 28, 1968

Pre-Tested Green Monkey Tissue

Preparation

Monkey No.	MN 57-135
Isolation Date	5/3/68
Date Cell Growth Initiated	7/3/68
Date Cell Suspension Frozen	7/3/68

Tests

(10% of total cell suspension tested)

Cell sheets (4)

7/26/68 Examination for hemadsorption viruses - Satisfactory

Pooled fluids from vessels above taken 7/26/68 and tested in

Rhesus Monkey Kidney*	- 10 ml.	Tested 8/5/68	Satisfactory	8/19/68
Cercopithecus Monkey Kidney*	- 10 ml.	7/29/68	Satisfactory	8/12/68
Subculture	- 10 ml.	8/15/68	Satisfactory	8/29/68
Rabbit Kidney*	- 10 ml.	8/5/68	Satisfactory	8/19/68
Human Amnion*	- 10 ml.	8/9/68	Satisfactory	8/23/68

* Primary Cell Cultures

Frances W. Bingham

LEDERLE LABORATORIES
American Cyanamid Company

October 28, 1968

Pre-Tested Green Monkey Tissue

Preparation

Monkey No. MN 58-142

Isolation Date 5/3/68

Date Cell Growth Initiated 7/11/68

Date Cell Suspension Frozen 7/11/68

Tests

(10% of total cell suspension tested)

Cell sheets (4)

8/2/68 Examination for hemadsorption viruses - Satisfactory

Pooled fluids from vessels above taken 8/2/68 and tested in

Rhesus Monkey Kidney*	- 10 ml.	Tested 8/5/68	Satisfactory	8/19/68
Cercopithecus Monkey Kidney*	- 10 ml.	8/5/68	Satisfactory	8/19/68
Subculture	- 10 ml.	8/28/68	Satisfactory	9/11/68
Rabbit Kidney*	- 10 ml.	8/5/68	Satisfactory	8/19/68
Human Amnion*	- 10 ml.	9/16/68	Satisfactory	9/30/68

* Primary Cell Cultures

Frances W. Bingham

LEDERLE LABORATORIES
American Cyanamid Company

October 28, 1968

Pre-Tested Green Monkey Tissue

Preparation

Monkey No.	MN 58-158
Isolation Date	5/3/68
Date Cell Growth Initiated	7/11/68
Date Cell Suspension Frozen	7/11/68

Tests

(10% of total cell suspension tested)

Cell sheets (4)

8/2/68 Examination for hemadsorption viruses - Satisfactory

Pooled fluids from vessels above taken 8/2/68 and tested in

Rhesus Monkey Kidney*	- 10 ml.	Tested 8/5/68	Satisfactory 8/19/68
Cercopithecus Monkey Kidney*	- 10 ml.	8/5/68	Satisfactory 8/19/68
Subculture	- 10 ml.	8/28/68	Satisfactory 9/11/68
Rabbit Kidney*	- 10 ml.	8/5/68	Satisfactory 8/19/68
Human Amnion*	- 10 ml.	9/16/68	Satisfactory 9/30/68

* Primary Cell Cultures

Frances H. Bingham

LEDERLE LABORATORIES
American Cyanamid Company

October 28, 1968

Pre-Tested Green Monkey Tissue

Preparation

Monkey No. MN 61-124

Isolation Date 6/7/68

Date Cell Growth Initiated 7/18/68

Date Cell Suspension Frozen 7/18/68

Tests

(10% of total cell suspension tested)

Cell sheets (6)

8/8/68 Examination for hemadsorption viruses - Satisfactory

Pooled fluids from vessels above taken 8/8/68 and tested in

Rhesus Monkey Kidney*	- 10 ml.	Tested 8/9/68	Satisfactory	8/23/68
Cercopithecus Monkey Kidney*	- 10 ml.	9/4/68	Satisfactory	9/18/68
Subculture	- 10 ml.	9/18/68	Satisfactory	10/2/68
Rabbit Kidney*	- 10 ml.	8/9/68	Satisfactory	8/23/68
Human Amnion*	- 10 ml.	9/16/68	Satisfactory	9/30/68

* Primary Cell Cultures

Frances W. Bingham

LEDERLE LABORATORIES

Lederle

A Division of AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK 10965
AREA CODE 914 735-5000

June 6, 1969

Mr. Fred Johnson
H.E.M. Research, Inc.
5451 Randolph's Rd.
Rockville, Maryland 20852

Dear Fred:

The following cultivability studies were recently done on our retention samples of the frozen cercopithecus monkey kidney tissue (100 ampoules) purchased by your firm in October, 1968 under our P.O. #000312.

Monkey #	Days to Confluency	Max. dilution level for confluency (Ampoule:Growth Medium)	Estimated Recovery
MN53-158	10	1:140	100
MN54-81	10	1:130	100
MN57-135	10	1:130	100
MN58-142	10	1:130	100
MN54-97	10	1:100	70
MN49-124	10	1:100	70
MN58-158	10	1:100	70
MN56-180	10	1:80	60
MN61-124	10	1:80	60

It is apparent from the above that the cell recovery on several numbers was lower than expected and therefore we plan to ship you twenty additional ampoules in the near future to make up for your loss.

I am sorry for any inconvenience this may have caused.

Yours truly,

William F. Daly
Head, Normal Tissue Culture

WFD:em

LEDERLE LABORATORIES

Lederle

A Division of AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK 10965
AREA CODE 914 735-5000

June 23, 1969

Mr. Fred Johnson
HEM Research Inc.
5451 Randolph's Road
Rockville, Maryland 20852

PRIMARY CELL BANK

Record of Shipping Data and Culturing Procedures:

Primary Cell Derivation Cercopithecus monkey kidney tissue

Vol. of Suspension/Ampoule 4cc, Cells/ml 7.5×10^6

No. of Ampoules Shipped 20, Date Shipped 6/23/69

Shipped Via Hand carried by Dr. D. McCoy

Storage Temperature Upon Receipt Liquid nitrogen

Method of Recovery- Thaw rapidly by agitating in water bath at 37-40°C.

Culture Medium (Growth) Hanks lactal w/10% C. S. (pH) 7.4

Culture Medium 1st Renewal (Growth) Earles lactal w/2% C. S. (pH) 7.2

Culture Medium (Maintenance) Earles lactal maint. (pH) 7.4

Cultivability Data:

Monkey #	LNR Position	No. of Ampoules	Maximum Dilution for Confluency (Ampoule:Growth Media)	Days to Confluency	Estimated Recovery
MN 71-100	1-B	9	1:130	10	100
MN 66-124	1-A	6	1:130	10	100
MN 66-102	2-B	5	1:130	10	100

Procedures for Thawing and Resuspending:

1. Upon receipt, ampoules should be stored at liquid nitrogen temperatures or thawed as soon as possible. Use of protective gloves and face mask are desirable as a safety precaution.

2. Remove ampoule from low temperature storage vessel (liquid nitrogen) and place immediately into a thaw bath between 37°C and 40°C. The bath should not exceed 40°C in temperature. Thaw rapidly and remove from bath as soon as all the ice has melted.

3. Wipe ampoule with disinfectant solution and take into sterile area for decapping.

4. Remove neck of ampoule by scoring glass with a three cornered file and breaking between several layers of gauze which has been dipped in the disinfectant solution and squeezed dry.

5. Contents of ampoule can now be removed with a sterile five or ten milliliter hypodermic syringe equipped with a 12 gauge canula and dispensed into a culture flask containing growth medium #1* at the appropriate volume recommended on the cover sheet.

6. Following an initial incubation period of four days, growth medium #1 is evacuated and the cultures are renewed with growth medium #2*.

7. Between the 8th to 10th day or when the monolayer is confluent, the cultures are renewed with maintenance medium*.

*See the attached formulation sheets.

William F. Daly

WFD:em

MEDIA WORKSHEET # 1

SOLUTION: Hanks Lactal W/10% Calf Serum

DATE: June 23, 1969

LOT NO.

VOLUME

CONTROL NO.

INGREDIENTS PER LITER	QUANTITY Gms	WEIGHT USED	CHECK LIST
UNIT I			
NaCl	8		
KCl	0.4		
$MgSO4 \cdot 7H_2O$	0.2		
Na_2HPO_4	0.047		
Glucose	1.0		
KH_2PO_4	0.06		
UNIT II			
$CaCl_2$	0.14		
Demin. Dist. H_2O	100 ml		
UNIT III			
Phenol Red	0.015		
UNIT IV			
1N NaOH	2 ml		
Streptomycin (active base)	100,000 ɣ		
Neomycin (active base)	100,000 ɣ		
$NaHCO_3$	0.35		
Lactal Hydrolysate	5.0		
Demin. Dist. H_2O	1,000 ml		
Calf Serum	100 ml		
Eagles Vit (100x)	100 ml		

FILTRATION DATA:

REMARKS:

Reagent grade used throughout.

STERILITY	ON TEST	OFF TEST	STERILITY	ON TEST	OFF TES
Thioglycollate					
Sabouraud's					
Trypticase Soy					

BOOK NO. | PAGE NO.

SIGNATURE

MEDIA WORKSHEET # 2

SOLUTION		DATE
Lactal W/2% Calf Serum		June 23, 1969
LOT NO.	VOLUME	CONTROL NO.

INGREDIENTS PER LITER	QUANTITY Gms	WEIGHT USED	CHECK LIST
Sodium Chloride NaCl	7.175		
Potassium Chloride KCl	0.400		
Calcium Chloride (Anhydl) $CaCl_2$	0.200		
Magnesium Sulfate $MgSO_4 \cdot 7H_2O$	0.200		
Sodium Phosphate Monobasic - $NaH_2PO_4 \cdot H_2O$	0.125		
Dextrose	1.000		
Sodium Bicarbonate	1.100		
Phenol Red (Water Soluble)	0.015		
Lactal Albumin Hydrolysate	5.000		
Streptomycin Sulfate (active base	100,000 γ		
Neomycin Sulfate (active base)	100,000 γ		
Make up with Demineralized Distilled water to	1000 ml		
Calf Serum	20 ml		
Eagles Vit. (100X)	10 ml		

FILTRATION DATA:

REMARKS:

Reagent grade used throughout.

STERILITY	ON TEST	OFF TEST	STERILITY	ON TEST	OFF TEST
Thioglycollate					
Sabouraud's					
Trypticase Soy					

BOOK NO.	PAGE NO.		SIGNATURE

LPR 5772 10/60

MEDIA WORKSHEET # 3

SOLUTION		DATE
Lactal Maint.		June 23, 1969
LOT NO.	VOLUME	CONTROL NO.

INGREDIENTS PER LITER	QUANTITY Gms	WEIGHT USED	CHECK LIST
Sodium Chloride NaCl	7.175		
Potassium Chloride KCl	0.400		
Calcium Chloride (Anhydl) $CaCl_2$	0.200		
Magnesium Sulfate $MgSO_4 \cdot 7H_2O$	0.200		
Sodium Phosphate Monobasic - $NaH_2PO_4 \cdot H_2O$	0.125		
Dextrose	1.000		
Sodium Bicarbonate	1.600		
Phenol Red (Water Soluble)	0.015		
Lactal Albumin Hydrolysate	5.000		
Streptomycin Sulfate (active base)	100,000 ɣ		
Neomycin Sulfate (active base)	100,000 ɣ		
Make up with Demineralized Distilled water to	1000 ml		

FILTRATION DATA:

REMARKS:

Reagent grade used throughout.

STERILITY	ON TEST	OFF TEST	STERILITY	ON TEST	OFF TEST
Thioglycollate					
Sabouraud's					
Trypticase Soy					

BOOK NO.	PAGE NO.		SIGNATURE