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REGISTRATION
31 JAN 1985

Copies

Ricardo H. Landaburu, Ph.D.
Revlon Health Care Group
Research and Development Dir
1 Scarsdale Road
Tuckahoe, New York 10707

Dear Ricardo:

I enclose the results of our first HTLV-III inactivation study. Disappointingly the HTLV-III stock which had previously titered $10^{5.3}$ $TCID_{50}$/ml gave a titer of only $10^{2.5}$ to $10^{3.0}$ when tested at a 1:4 dilution. Thus we were unable to show a >5 log kill as had been hoped. The most that can be concluded from the study is that the combined effect of lyophilization and heating inactivated $\geq$2.5-3.0 logs.

A new virus stock has been prepared for use in the next experiment. This is now being titrated. Please give me a call when I return to the lab on February 4.

With all best wishes.

Sincerely yours,

GRO-C

Alfred M. Prince, M.D.

cc: Mr. Clive Collins.

THE LINDSLEY F. KIMBALL RESEARCH INSTITUTE
of The New York Blood Center

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Revlon Health Care HTLV-III Study No. 1 12-19-84

Title: Effect of Heating in the dry state of HTLV-III diluted in Factorate Gen I and Gen II on Infectivity

Sponsor: Dr. Ricardo Landaburu, R.H.C.

Investigator: Alfred M. Prince, M.D. NYBC

Materials:

1. Factorate Gen I Lot No. Y 70702
2. Factorate Gen II Lot No. X 50909
3. Sterile Water for Injection (RHC)

The above were delivered by Dr. Landaburu and stored at 4°C.

4. HTLV-III Virus Stock 84-01 05-27-84.

This is unconcentrated supernatant fluid from H-9/HTLV-III Producer cells cultivated in RPMI 1640 medium with 20% fetal calf serum. The stock was frozen in alcohol/dry ice and is stored at -70°C. The infectivity titer, based on inoculation of serial dilutions into H-9 cell cultures, and reverse transcriptase assays on supernatants taken 14 days after inoculation was $10^{5.3}$ $TCID_{50}$/ml.

Details of Procedure:

1. Hydration of Product

a. Gen I Bring vial and water for injection (WFI) to 37°. Hydrate 1 vial with 54 ml water.

b. Gen II Bring vials and WFI to room temperature. Hydrate each vial with 20 ml WFI.

2. Preparation of Control Samples for Moisture determination

4 ml of Gen I and Gen II each added to a 10 ml screw capped vial. They are labelled: Gen I Control for moisture 12-19-84; Gen II Control for Moisture 12-19-84.

3. Lyophilization Apparatus

A Virtis Model 6211-0260 7.8 liter Drum Manifold cooled with alcohol and dry ice and fitted with Quickseal valves and stoppering adaptors was attached to a Welch duoseal vacuum pump. A Pall 0.22u filter cartridge was placed in the vacuum line between pump and manifold. The stoppering adaptors permit stoppering under vacuum.

4. Preparation of Infected Samples

a. Gen I 5 ml HTLV Stock is added to 15 ml Gen I and 4 ml aliquots are distributed to 4 10 ml screw capped vials.

b. Gen II 7 ml of HTLV-III Stock is added to 21 ml Gen II and 4 ml aliquots are added to 6 vials.

5. Preparation of non-lyophilized assay samples

a. HTLV Stock is diluted in 1:4 RPMI 1640 20% fetal calf serum and frozen in three 1 ml aliquots by swirling in alcohol and dry ice. Aliquots are labled: HTLV-III 1:4 -70 12-19-84 and held in HTLV-III box I in -70° Revco. This is to control for possible inactivation by factorate.

b. One vial each of Gen I and Gen II infected samples are shell frozen in alcohol and dry ice Labled: Gen I or Gen II and HTLV-III -70° for assay 12-19-84 and stored at -70 with above sample.

6. Lyophilization of Samples

Samples are shell frozen by rapid swirling in alcohol and dry ice bath and placed on the lyophilizer at 4:00 p.m. 12-19-84. At 4:00 p.m. 12-21-84 (48 hours) samples will be stoppered under vacuum, sealed with screw caps (since crimper does not fit these vials) and heat shrinkable plastic seals, and then surface decontaminated by immersion in 1% sodium dodecyl sulfate for 15 min.

1 vial of Gen I and Gen II and HTLV III will be retained at 4°C in the P-3 refrigerator labeled: Gen I (or Gen II) and HTLV-III lyophilized not heated 12-19-84.

2 vials labled: Gen I and HTLV-III Surface decontaminated with 1% SDS 12-19-84 and 4 vials labeled: Gen II and HTLV-III Surface decontaminated will be held at 4°C for pick up by RHC personnel on 12-26-84. These are to be returned to NYBC on 2 January 1985 for assay.

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January 3, 1985

RHC Study 1

Procedure for Titration

1. Hydrate each lyophilized vial with 4.0 ml H_2O for injection. Dissolve at room temp. and hold on ice.

2. Centrifuge 3 x 10^8 H-9 cells and suspend in 20 ml 30% conditioned infection medium (RPMI 1640,20% FCS, 2 µg/ml polybrene). Hold for 1 hour (1.5 x 10^7/ml).

3. Make dilutions of samples in infection medium.

4. Putout 0.1 ml cells to wells of 24 well plates and 1.0 ml to 6 T-25 flasks.

5. Put out virus dilutions to plates (2 wells/dilution) and 1 ml of undiluted heat treated samples to flasks. (Remainder of samples frozen in HTLV box I.)

6. Hold 1 hr. 37°C.

7. Feed with 30% conditioned infection medium. 1.5/well, 14 ml/flask.

Schedule Mon. Jan. 7 Feed by mixing and removing 0.75 from wells. Feed 0.75 ml infection medium (= 2 µg polybrene/ml). For flasks remove 7.5 and add 7.5.

Fri. Jan.11 Feed as above.

Mon. Jan.14 Feed as above.

Fri. Jan.18 Harvest for reverse transcriptase determination 0.75 ml supernate to 0.25 ml 30% PEG.

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RHC Study No. 1 RESULTS

SAMPLE	HTLV-III TITER ($TCID_{50}$/ml)
HTLV-III stock 84-01 diluted 1:4 in RPMI 1640 20% fetal calf serum frozen 12/19/84	$10^{2.5}$
HTLV-III diluted 1:4 in Gen I frozen, not lyophilized	$10^{3.0}$
HTLV-III diluted 1:4 in Gen II frozen, not lyophilized	$10^{2.5}$
HTLV-III diluted 1:4 in Gen I lyophilized, not heated	$<10^{1*}$
HTLV-III diluted 1:4 in Gen II lyophilized, not heated	$<10^{1}$
HTLV-III diluted 1:4 in Gen I lyophilized, heated IA	$<10^{0**}$
HTLV-III diluted 1:4 in Gen I lyophilized, heated IB	$<10^{0}$
HTLV-III diluted 1:4 in Gen I lyophilized, heated IC	$<10^{0}$
HTLV-III diluted 1:4 in Gen I lyophilized, heated ID	$<10^{0}$
HTLV-III diluted 1:4 in Gen II, lyophilized, heated IIA	$<10^{0}$
HTLV-III diluted 1:4 in Gen II, lyophilized, heated IIB	$<10^{0}$

* No infectivity in 2 cultures (1.5ml) inoculated with 0.1 ml undiluted material.

**No infectivity in 15 ml cultures inoculated with 1.0 ml undiluted material.

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CONCLUSIONS

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1. Lyophilization alone inactivated $\geq$1.5-2.0 $\log_{10}$ HTLV-III infectivity

2. Lyophilization plus each of the heating conditions inactivated $\geq$2.5-3.0 $\log_{10}$ of HTLV-III infectivity.

3. Higher inactivation process efficacy could not be demonstrated in this experiment due to the unexpectedly low titer of the virus stock employed.

GRO-C

Alfred M. Prince, M.D.
24 January, 1985