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REVLON HEALTH CARE GROUP
RESEARCH & DEVELOPMENT DIVISION
1 SCARSDALE ROAD, TUCKAHOE, NEW YORK 10707
(914) 779-6300

B0000174/ 1



RICARDO H. LANDABURU, Ph.D.
DIVISION DIRECTOR
PLASMA FRACTIONS RESEARCH & DEVELOPMENT

January 10, 1985

Mr. Clive Collins
Armour Pharmaceutical Co. Ltd.
Saint Leonards House
Saint Leonards Road
Eastbourne, Sussex
England

Dear Mr. Collins:

The following is a summary account of the experiment testing our heating conditions for Factor VIII in the elimination of HTLV III infectivity.

Nature of the Experiment - This is a test of the susceptibility of HTLV III virus to heat inactivation after the virus has been dispersed in samples of antihemophilic factor solution and then lyophilized. Two different formulations of commercial antihemophilic factor were used to test environments: Factorate$^{R}$, Generation I and Factorate$^{R}$, Generation II. As the first phase of a more extensive program, initial technical limitations in the processing of infected samples by the isolation laboratories have limited the test to aliquot scale of product rather than the commercially distributed size of container.

Implementation

1) A preliminary protocol was discussed with Dr. F. M. Prince of the New York Blood Center. His documentation of source material, inoculum and the preparation of infected test samples is contained in his account of 12/19/84. (attached)

2) On receipt of the vials of Revlon Health Care scientists a second protocol which documented the heating of the sample vials was initiated and completed. See memorandum, M.E.Hrinda to File-HTLV III Inactivation (attached). As a portion of this protocol the vials were re-labelled without disclosure to Dr. Prince of the individual heating conditions (blind). They were delivered to his laboratory on January 2, 1985. Moisture content in the dried Factor VIII was performed. Report is also attached.

3) Dr. Prince's final report should be referred to the coding of document (2) above. This report will be sent directly to you by Dr. Prince. I hope this serves to expedite your communication of results to the regulatory office. You should expect Dr. Prince's report during the week of January 21st. I would appreciate if you could contact me on the telephone upon receipt of this final report. Please do not hesitate to call me if you require any further information.

Very truly yours,

GRO-C

R. H. Landaburu, Ph.D.

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Attachments (5)

1. Protocol
2. Letter 12/19/84 RHL to Dr. Prince - Modification of Protocol
3. Dr. Prince's report 12/19/84
4. Memo M.E.Hrinda to File - HTLV III Inactivation - 12/26/84
5. Analytical Test Report - Moisture Content.

 



STUDIES OF AIDS RELATED VIRAL INFECTIVITY: ASSAY OF HTLV-III IN INFECTED PLASMA DERIVATIVES (FACTOR VIII, FACTOR IX) FOLLOWING HEAT TREATMENT

Purpose of Study:

The purpose of this study is to determine if a measured quantity of HTLV III added to liquid Factor VIII or to liquid Factor IX containing solution before lyophilization can be destroyed by heating the lyophilized preparation for different lengths of time.

Preparation of Experimental Inocula:

I. Factor VIII

Experimental vials of Factor VIII concentrate of two different characteristics (Factorate$^{R}$ Gen I, and Factorate$^{R}$ Gen II) which contain six logs of virus will be prepared as follows:

Gen II.

1. 10 vials of product will be reconstituted each with 20 ml of sterile water for injection by transferring the contents of the water vial to the product using a sterile syringe, releasing the vacuum and swirling the solution.
2. When fully reconstituted, the contents of the 10 vials will be asceptically transferred and pooled into a 500 ml sterile beaker or flask containing a mixing bar.
3. After thorough mixing, 2 aliquots of 20 ml each will be removed from the pool and placed into each of two fresh sterile vials of the same specification as product. These are fitted with sterile lyophilization stoppers, labelled, and maintained asceptically until further processed.
4. To the remaining pool of 160 ml (nominal) is added a concentrate of HTLV-III virus sufficient to infect each further 20 ml aliquot with six (6) logs of virus.
5. The infected pool is then distributed, 20 ml to each vial, to 8 additional fresh sterile vials which are subsequently sealed with sterile stoppers.
6. It will be expected that the flask (or beaker) will contain a residual small volume of infected product. (This is due to the partial specific volume of the vial solids.) This residual sample should be transferred to a sterile container and retained as a frozen sample for contingent examination as needed.

7. The 10 filled vials (2 non-infected; 8 infected) are frozen at a temperature below -40°C and then lyophilized and sealed under vacuum with aluminum flip top seals. The lyophilization sequence will be determined by the choice of equipment and performance on a dummy run.
8. The actual sequence of lyophilization is to be fully documented. The sealed evacuated vials are to be stored at a temperature of 2-10°C pending distribution and during transfer.
9. The exterior surfaces of the fully sealed experimental vials will be disinfected using an agent and procedure to be devised and agreed upon, such that the vials may be handled with appropriate caution outside of the containment environment. Documentation and labelling of the vials will include assurance that the agreed exterior sterilization has been performed.
10. Six (6) of the 8 infected vials will be transferred by courier to RHC R&D. (The two non-infected, 2 of the 8 infected lyophilized vials as well as the frozen sample will be retained at the NYBC).
11. The six infected vials will be handled and treated as per separate heating protocol and returned to the NYBC on a coded basis for reconstitution and virus assay.
12. One of the two non-infected vials is to be assayed at NYBC for residual moisture content of the lyophilized cake.

Gen I - Each step will be conducted identically with that noted for the Generation II product, but because of the product format differences the following modifications are required. These are noted stepwise:

1. The vials are to be reconstituted with sixty (~~60~~ 54) ml of sterile W.F.I. Product and W.F.I. must be at 37°C for proper reconstitution.
2. Pooling container volume should be increased to ~~1000~~ 600 ml.
3. Size of aliquot is increased to ~~60~~ ml. 27
4. Nominal volume remaining is 480 ml.
5. Size of aliquot is increased to 60 ml.

6.-11. No Change.

II. Factor IX - Although the vial sizes differ, the Factor IX product is reconstituted with 20 ml of sterile W.F.I. and inocula preparation is identical with that noted for Gen II Factorate$^{R}$.

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NOT TO BE DUPLICATED

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REVLON HEALTH CARE GROUP
RESEARCH AND DEVELOPMENT

LIST OF EXPERIMENTAL MATERIALS & SERVICES

To Be Provided by RHC R&D

A. 10 vials Factorate$^{R}$-Gen I (500 u/vial nominal)
20 vials WFI (sterile) 30 ml each
Clean non-sterile glass vials (10), stoppersd and aluminum seals.

B. 10 vials Factorate$^{R}$-Gen II (500 u/vial nominal)
10 vials WFI (sterile) - 20 ml each.
Clean non-sterile glass vials (10), stoppers and aluminum seals.

C. 10 vials Factor IX concentrate, Prothar$^{R}$ (500 u/vial nominal
10 vials WFI (sterile) - 20 ml each.
Clean non-sterile glass vials (10), stoppers and aluminum seals.

(Each of the above item groups to be packaged as unit experiments and adequately labelled for identification)

D. Facilities and regulated water bath in Tuckahoe to conduct heating protocol.

To Be Provided by New York Blood Center

A. Viral inoculum, containment facility, viral assay.

B. Sterilization facilities for glassware etc.

C. 500 ml and 1000 ml containers for pooling syringes etc.

D. Lyophilizer for contaminated samples; lyophilization method and moisture testing.

E. Reagents and equipment to sterilize container exteriors.

F. Real-time documentation, Final report.

REVLON HEALTH CARE GROUP
RESEARCH & DEVELOPMENT DIVISION
1 SCARSDALE ROAD, TUCKAHOE, NEW YORK 10707
(914) 779-6300

cc: Ms. A. Bessler
Dr. E. Neiss
Dr. M. Rodell
Dr. C. G. Smith

B0000176/1

RICARDO H. LANDABURU, Ph.D.
DIVISION DIRECTOR
PLASMA FRACTIONS RESEARCH & DEVELOPMENT

December 19, 1984

Dr. F. Prince
The New York Blood Center
310 E. 67th Street
New York, N.Y. 10021

Dear Dr. Prince:

This is to further confirm our conversation today, on the modification to be introduced into the protocol for evaluation of the effect of heating in the HTLV-III. These modifications which are the consequence of the need to adopt the experiment to the N.Y.B.C. virus stock available at this time as well as the evidence that your lyophilization equipment in the P-2 area does not fit the Armour vials, contemplates the separation of the study in two faces; the second one gathering the information requested by protocol would be performed as described in such document for the remainder of the evaluation as soon as the virus stock and the lyophilization attachment are available. This is seen by us to occur as early as the beginning of February.

This first phase of the study contemplates the evaluation of Generation II Factor VIII at three different heating conditions and of Generation I Factor VIII at one heating condition. We understand that measurements will permit the validation of survival of 5 log/ml of HTLV-III to different heat exposures. We further understand that a final report of results will be issued in early February.

Our protocol with you is therefore changed only for the first phase of the study in the level HTLV-III per ml of product. Reconstitution of product will be as indicated in the protocol, however filling of lyophilization vials will be done in 10 ml vials (acceptable in your equipment) at a level of infectivity of $10^{4.7}$ to $10^{5}$ particle logs per ml. Dry vials will be sent to Tuckahoe for heating steps and returned to you as soon as these maneuvers are completed. The disposition of vials for moisture content and of duplicate vials remains as per protocol.

B0000176/2

We at Revlon understand that the depletion of your stock since we first discussed the experiment is a normal laboratory occurance and are very appreciative of your willingness to respond to our needs at this time of the year and to be so receptive to the requirements set within our industry.

Very truly yours,

GRO-C

R. H. Landaburu, Ph.D.

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

310 East 67 Street, New York, N.Y. 10021 (212) 570-3000

B0000177/1

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. GRO-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 $4^{o}C$.

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 $-70^{o}C$. 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 $37^{o}$. 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.

GRO-C

12-21-84

B0000178/1

REVLON HEALTH CARE GROUP INTEROFFICE MEMORANDUM

DATE: December 26, 1984 C.C.

TO: File - HTLV III Inactivation

FROM: Dr. M. E. Hrinda GRO-C

SUBJECT: HEATING OF LYOPHILIZED FACTOR VIII CONTAINING HTLV III VIRUS

The purpose of this memorandum is to provide a supplementary protocol under which heat treatment of lyophilized Factorate® product samples may be conducted. The source of the experimental product vials containing HTLV III Virus was Dr. A. Prince of the New York Blood Center who prepared the samples from commercial product (Factorate® "Generation I" and Factorate® Generation II"). The vials provided by Dr. Prince are described in his letter received on December 26, 1984 with the vials. A copy is attached to this file. The exterior of the vials received on this date was decontaminated with a 1% solution of SDSD (Sodium Dodecyl Sulfate) at the NYBC prior to receipt at these laboratories via RHC scientists acting as informed messengers.

Received from Dr. Prince were:

1. Four vials; source Factorate® "Generation II" containing HTLV III.
2. Two vials; source Factorate® "Generation I" containing HTLV III.
3. One vial as per (1) above without HTLV III for moisture analysis.
4. One vial as per (2) above without HTLV III for moisture analysis.

The disposition of the vials in groups (1) and (2) is as follows:

1. One each of the vials is to be heated at $60^{\circ}C \pm 0.5^{\circ}C$ for four (4) different time periods.

   A vial designated 1A for 10 hours
   A vial designated 1B for 30 hours
   A vial designated 1C for 48 hours
   A vial designated 1D for 72 hours

2. One each of the vials is to be heated at $60^{\circ}C \pm 0.5^{\circ}C$ for two (2) different time periods.

   A vial designated 2A for 10 hours
   A vial designated 2B for 30 hours

This method and procedure for conducting the heat treatment is adopted from that described in EMP #2/1981 and EMP #3/1982 (see reference document EMP #3/1982, Phase C-Pasteurization of Lyophilized vials). The following has been modified and simplified for this present use.

Rx-5527-B

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PROTOCOL AND DATA LOG - HEAT TREATMENT OF VIALS

Experimental vials which have been lyophilized and sealed may be promptly subjected to pasteurization. If refrigerated, the vials are allowed to warm to ambient temperature. The following vials are to be heated:

1A, 1B, 1C, 1D, 2A, 2B

Time Initiated 11:00am 12/26/84 Date 12/26/84

Investigator GRO-C

2. The vials are inspected and if labels have been affixed and are easily attached, labels are removed and replaced with waterproof tags. Waterproof tags are added to the vials' caps even if the original identification labels appear secure. An additional label is attached to each vial by a length of nylon twine so that the label can remain outside the water bath after the vial is submerged.

Time Completed 11:10 a.m. Date 12/26/84

Investigator GRO-C

Witnessed as Completed by GRO-C

Date 12/26/84

3. Prior to the date of heating the experimental vials, a validation experiment is to be conducted on the thermometers to be used to calibrate the water bath for the pasteurization. This may be done at an additional site using a water bath of known calibration. A calibrated glass thermometer, and at least one electronic probe will be required.* The temperature of calibration is 60°C ± 0.5°C. The characteristics of the electronic probe will be determined and recorded in a laboratory notebook. This will comprise a calibration curve for each probe (to be tagged and identified by number) in the range 50°C to 70°C as referenced to the calibration bath and glass thermometer. The investigator will use these curves to assist monitoring the pasteurization as described below.

Calibration:

Water Bath No. 12509 Location 19256
Thermometer (glass) 60.2°C (If applicable)
Thermometer (electronic) _____ (If applicable)
Notebook Reference A03107

Time 3:12 pm. Date 12/21/84

Investigator GRO-C

*If a second probe is available, it will be used to record bath temperature as noted below.

4. If available, one of the two electronic probes is retained to monitor the experimental bath temperature. The primary probe is inserted through the stopper of a reference (dummy) vial similar to that used for the innoculation experiment. The probe is inserted into the lyophilized cake and the vial sealed with vacuum replenished if possible. This vial probe will be used to monitor cake temperature. A record of the temperature of this vial (and bath temperature if possible) will be attached to the permanent record of this protocol.

5. After the above preliminary steps and on the day of experiment, a covered water bath of suitable dimensions is set at 60°C and the temperature is verified to be 60°C ± 0.5°C using thermometers described in step 3.

Water Bath No. 6750 Location 19-256

Temperature 60.0°

Time 10:50 a.m. Date 12/26/84

Investigator GRO-C

Witnessed by GRO-C Date 12/26/84

6. The experimental vials 1A, 1B, 1C, 1D, 2A, 2B as well as the dummy vial containing electronic probe are placed in the water bath and weighted so as to remian fully immersed. The cover of the bath is secured. The cake temperature is 60°C ± 0.5°C; the time is noted. The vials are to remain at 60°C for time periods noted in this protocol.

Immersion Time 11:15 a.m.

Bath Temperature 60.0°C Cake Temperature 23°

Time to reach 50°C 11:18
Time to reach 55°C 11:19
Time to reach 60°C ± 0.5°C 11:20

Date 12/26/84

Investigator GRO-C

Witnessed as completed GRO-C Date 12/26/84

Investigator N/A Date N/A

7. Upon removal from the bath and cooling, the vials are identified with permanent labels, verified against tags used for heating. They are placed in metal or plastic containers suitably identified and labelled with biohazard labels and stored under refrigeration at +5°C (approx.) until returned to Dr. Prince at NYBC for viral assay.

Removal of Vials from Bath:

1A
Time 9:20 pm
Date 12/26/84
Investigator GRO-C
Witnessed as Completed GRO-C

1B
Time 5:20 pm
Date 12/27/84
Investigator GRO-C
Witnessed as Completed GRO-C

1C
Time 11:20 a.m.
Date 12/28/84
Investigator GRO-C
Witnessed as Completed GRO-C

1D
Time 11:20 a.m.
Date 12/29/84
Investigator GRO-C
Witnessed as Completed GRO-C

2A
Time 9:20 pm
Date 12/26/84
Investigator GRO-C
Witnessed as Completed GRO-C

2B
Time 5:20 pm.
Date 12/27/84
Investigator GRO-C
Witnessed as Completed GRO-C

8. Transter of Heated Vials for Assay:

Date of Transfer 2 JAN 85
Transferred By GRO-C
Witness of Identity GRO-C
Receipt by or for Dr. Prince GRO-C
Date 01/02/05

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REVLON HEALTH CARE GROUP
RESEARCH & DEVELOPMENT DIVISION
ANALYTICAL TEST REPORT – FORMULATIONS RX-5167-C

| ITEM | DATE SUBMITTED |
|---|---|
| GEN I, GEN II CONTROLS | 1-8-85 |
| EXPERIMENT # LYOPHYLIZED | LOT # — |
| ANALYTICAL # 853028 | PROJECT # (OR NAME) |
| STORAGE STATION | AGE |
| REQUESTED BY DR. C. REHM/Dr. Lamontanaro | DEPARTMENT |

☐ EXPT. FORM ☐ CLINICAL ☐ STABILITY ☐ RELEASE

Formulation
Ingredient % or Amt

| TEST REQUESTED | THEORY | TEST RESULTS |
|---|---|---|
| MOISTURE CONTENT | | |
| (1) GEN I | CONTROL | |
| Sample Wt = | | 42.53 mg |
| Total moisture in the vial = | | 0.9444 mg |
| % moisture (w/w) = | | 2.22 % |
| (2.) GEN II | CONTROL | |
| Sample Wt = | | 61.55 mg |
| Total moisture in the vial = | | 0.8902 mg |
| % moisture (w/w) = | | 1.45 % |

Package:

Requester Comment:

| INGREDIENT | DATE | ANALYST BOOK REF. | METHOD # | ANALYTICAL CHEMISTRY DISPOSITION |
|---|---|---|---|---|
| MOISTURE | 1-8-85 | 403/06-29,30 | KS-82-02 | ☐ Released for Pre-clinical Use<br>☐ Released for Clinical Use<br>☑ Analyzed as shown above<br>☐ Rejected (see comments below) |

SIGNATURE GRO-C DATE 1/8/85

COMMENTS MOISTURE DETERMINED BY AQUATEST I TITRATOR

APPROVED GRO-C DATE 1/8/84

DISTRIBUTION: White & Canary – ORIGINATOR Pink – ANAL. CHEM. FILE Blue – ANAL. CHEM/SECT. FILE
Blue Short Copy – RECEIPT - ORIGINATOR detach & retain

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