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ORGANISATION MONDIALE DE LA SANTÉ

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A STABLE DRIED SMALLPOX VACCINE

CORRIGENDUM

Page 3, line 14: delete "(1956) Bull. Wld Hlth Org. 16, 2"
insert "(1957) Bull. Wld Hlth Org. 16, 63-77"

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PREPARATION D'UN VACCIN ANTIVARIOLIQUE DESSECHE STABLE

CORRIGENDUM

Page 3, Bibliographie : dans la référence N° 2,
remplacer "(1956) Bull. Org. mond. Santé, 16, 2"
par "(1957) Bull. Org. mond. Santé, 16, 63-77"

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WHO/Smallpox/7
18 January 1957

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A STABLE DRIED SMALLPOX VACCINE

Summary of the method of preparation[a] based on methods described by Collier[1]

(Note: This vaccine is prepared from a partially purified suspension of vaccinia virus elementary bodies derived from sheep pulp in 5.5 per cent. peptone freeze-dried and sealed in vacuo. Repeated batches of the vaccine have been shown to retain satisfactory potency after exposure to 45°C for at least eight weeks, and 37°C for at least three months. For full details reference should be made to Collier's article. One batch has been exposed to 45°C for two years, after which time it still produced 100 per cent. successful primary vaccinations.[2,3] In series production the conservative claim of retention of potency for one month at 37°C is made, but in practice this period may be expected to be considerably prolonged.)

Twenty five g of crude sheep pulp are ground in a mortar with 80 ml McIlvaine's phosphate-citric acid buffer, 0.004 M, PO_4 pH 7.2, and 1.0 g powdered neutral glass. The crude suspension is centrifuged at low speed (1000 g), the supernatant kept and the deposit re-extracted in buffer. This is repeated twice and the three supernatants are pooled. The virus is sedimented by centrifugation in an angle centrifuge. The speed and duration of centrifugation necessary to sediment the virus depend on the radius of rotation of the centrifuge head and the angle of inclination of the tubes (at 40° from vertical 2500 g for 60 minutes should be enough). The resulting deposit is resuspended in 15 ml of the same buffer, containing 0.5 per cent. phenol. This suspension is clarified by low-speed horizontal centrifugation for two minutes. The supernatant is saved, the deposit

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[a] The description given here is modified from the original in Collier's paper in order to include modifications in procedure introduced since his paper was written.

The pressures given in Collier's paper were measured by McLeod gauge. Those given here are measured by Pirani gauge.

resuspended in a further 15 ml of buffer, and clarified again. The pooled supernatants constitute the final elementary body suspension (E.B.S), which is then incubated at 22°C for 48 hours to reduce bacterial contamination. The E.B.S. is then plated to determine the bacterial count, and titrated in eggs for virus content. It is not used unless the bacterial count is less than 1000 organisms per ml and the virus titre more than 5×10^9 i.u./ml. After passing these tests, one vol. of E.B.S. is diluted 10 times with 5.5 per cent. peptone, made up as follows:

A 5.5 per cent. solution of bacteriological peptone is made in distilled water. The pH is adjusted to 8.0 with 40 per cent. NaOH, after which the solution is heated to 90°C, and filtered while hot. The pH is then changed to 7.4 with 50 per cent. HCl. The peptone solution is sterilized by autoclaving for 15 minutes at 15 lb pressure. The suspension is then ampouled in 0.25 ml amounts and dried in an Edwards centrifugal freeze-drier.[a]

The ampoules are closed with caps made of a layer of cotton wool between two layers of gauze. Such caps maintain sterility without interfering with the passage of water vapour.

Primary drying. The ampoules are placed in the primary chamber. The centrifuge is started and evacuation begun.

"Snap-freezing" occurs about 15 minutes later, when the vacuum has reached 1-2 mm Hg. The rotor is stopped shortly afterwards, and drying is allowed to proceed for about five hours at a vacuum of 0.05 mm Hg. During this time, heat is supplied to the drying heads, the total input of watts being approximately equal to the number of ml of material being dried. Drying can be satisfactorily carried out overnight, if necessary, without the application of heat.

Constriction, secondary drying and sealing. After primary desiccation, the ampoules are removed from the chamber, and constricted at the necks in a blow-lamp

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[a] The centrifugal freeze-drier used is manufactured by Edwards High Vacuum Ltd., Crawley, Sussex, England.

flame to facilitate subsequent sealing. No ampoule is allowed to remain in contact with the atmosphere for more than two or three minutes during this process; those not actually being constricted are kept in glass desiccators over P_2O_5. They are then attached to the manifolds, and left for a further 18-20 hours at high vacuum over P_2O_5. They are sealed under a vacuum of 0.01-0.03 mm Hg.

Vacuum testing. The sealed ampoules are held at 4°C overnight, and are examined next day with a high-frequency tester for retention of vacuum, those failing to give a blue-green fluorescence being discarded.

Reconstitution. The dried material is reconstituted by adding 40 per cent. glycerol in buffer to the original volume.

REFERENCES

1. Collier, L. H. (1955) J. Hyg. (Lond.), 53, 76-101

2. Cockburn, W. C., Cross, R. M., Downie, A. W., Dumbell, K. R., Kaplan, C., McClean, D. & Payne, A. M. M. (1956) Bull. Wld Hlth Org. 16, 2

3. McClean, D. (1957) Lancet in press