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ENGLISH ONLY

A STABLE DRIED SMALLPOX VACCINE

Description of the method used at the Lister Institute
of Preventive Medicine, Elstree, Herts, England^a

(Note: This vaccine is prepared from a partially purified suspension of vaccinia virus elementary bodies derived from sheep pulp. It is freeze-dried in 5.0 per cent. peptone and sealed in pure dry oxygen-free nitrogen at atmospheric pressure. 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. One batch has been exposed to 45°C for four 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.)

Extraction of virus from pulp

Crude sheep vaccinal pulp is extracted in an efficient mechanical homogenizer with 10 times its weight of McIlvaine's phosphate citric acid buffer 0.004 M with respect to the phosphate at pH 7.2 containing 10 per cent. (V/V) Arcton 113 (ICI trifluorotrchloroethane originally known as Arcton 63) and 0.4 per cent. (W/V) phenol. It is important not to overload the homogenizer. If necessary, the pulp should be extracted in two or more lots (each in 10 times its weight of buffer), to ensure efficient homogenization.

This extract is centrifuged horizontally at low speed (1500 r.p.m.) for five minutes in a size I international centrifuge. The deposit containing the Arcton 113 is discarded and the supernatant fluid is kept at 22°C over-night. It is then plated in nutrient agar to determine the bacterial count and is not used unless the bacterial count is less than 1000 organisms per ml and no pathogens are present. If the result of this test is satisfactory, the extract is titrated by pock count and accepted for drying if the titre is at least 1×10^9 i.u./ml.

^a Revision of method described in document WHO/Smallpox/7

Preparation of peptone solution

A 10 per cent. solution of bacteriological peptone^a 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 30 per cent. HCl. The peptone solution is sterilized by autoclaving for 15 minutes at 15 lb pressure.

Before drying the extract is mixed with an equal volume of 10 per cent. peptone. The mixture of virus and peptone is ampouled in 0.25 ml amounts and dried in an Edwards centrifugal freeze-drier.^b Pre-constricted ampoules^c as shown in the diagram, are used.

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 centrifuge is stopped shortly afterwards and drying is allowed to proceed for about five hours at a vacuum of 0.05 mm Hg.^d During this time heat may be 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 over-night, if necessary, without the application of heat.

Secondary drying and sealing

After primary desiccation, the ampoules are removed from the chamber and plugged lightly with sterile, non-absorbent cotton wool. They are then attached to the manifolds of the secondary drier and left for a further 18-20 hours at high vacuum over P₂O₅. They are sealed after filling with pure dry oxygen-free nitrogen at atmospheric pressure. If such nitrogen is unobtainable, the ampoules can be sealed under a vacuum of 0.01-0.03 mm Hg.

^a Difco peptone has been found to be the most suitable

^b The centrifugal freeze-drier used is manufactured by Edwards High Vacuum Ltd., Crawley, Sussex, England

^c The specifications of these ampoules are the property of Johnsen & Jorgensen, Flint Glass Ltd., London, England

^d The pressures are as measured in Pirani gauges

Vacuum testing

If pure oxygen-free nitrogen is unobtainable, the sealed ampoules are held at 4°C over-night, and are examined next day with a high frequency tester for retention of vacuum. Those failing to give a blue-green fluorescence are discarded. Ampoules filled with nitrogen are tested by immersing them in water and evacuating the container. Broken ampoules will fill with water when the vacuum is released.

Reconstitution

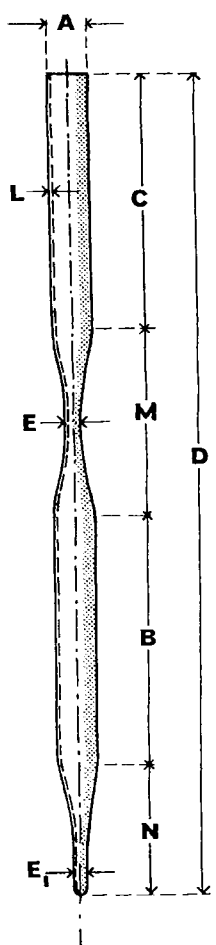
The dried material is reconstituted by adding 0.25 ml of 40 per cent. glycerol in buffer from the double ended ampoule.

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. Cross, R. M., Kaplan, C. & McClean, D. (1958) Bull. Wld Hlth Org. 19, 123
4. Cross, R. M., Kaplan, C. & McClean, D. (1957) Lancet, 2 March, p. 446

**TUBE FOR DOUBLE-ENDED AMPOULE FOR
RECONSTITUTING FLUID**

Constricted Reconstituting Tube - NEUTRAL

18.3.59	M.A.B.	DIM.	Size in millimetres
 <p style="text-align: center;">Scale Full Size</p>		Code No.	
		Size	
		A	4.9 / 5.6
		B	36.0 ± 1.0
		C	35.0 ± 2.0
		D	115.0 ± 2.0
		E	2.25 ± 0.25
		E ₁	2.0 ± 0.25
		L	0.55 ± 0.05
		M	26.0 ± 1.0
		N	18.0 ± 1.0

PRECONSTRICTED TUBE FOR AMPOULE TO CONTAIN
FREEZE-DRIED VACCINE

Freeze Dry Ampoule. Neutral
For Smallpox Vaccine - Printed

16.4.59	DM.	DIM.	Size in millimetres
		Code No.	
		Size	
		A	8.0 / 8.5
		B	65.0 ± 1.0
		C	85.0 ± 1.0
		D	110.0 ± 1.0
		E	2.75 / 3.5
		E ₁	6.0 ± 0.2
		F	3.5 ± 0.5
		I	0.55 ± 0.05
L	0.65 ± 0.05		
Die	909		

Scale Full Size

Length "D" from Ampoule m/c 122.0 ± 1.0 with Vacuum Released.