WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTÉ

WHO/Smallpox/4
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ENGLISH ONLY

PRODUCTION METHOD OF SMALLPOX VACCINE AT THE MICHIGAN STATE LABORATORIES, LANSING, USA*

CONTENTS

* See also Ducor, D. H., (1947) Publ. Hlth Rep. (Wash.), 62, 565
Hornebrock, J. W. & Gerhard, W. H., (1951), Publ. Hlth Rep. (Wash), 66, 38

I. GENERAL DESCRIPTION

A. Definition. Smallpox vaccine consists of a glycerinated suspension of the vesicles of vaccinia from healthy vaccinated animals of the bovine species. The material is removed and prepared under aseptic conditions. The pulp, which consists of the whole vesicle and its contents, is ground and suspended in an aqueous solution of 50% glycerine containing 0.5% phenol as a preservative.

B. Properties. Smallpox vaccine is a grayish turbid suspension which may have a slight odour due to the preservative.

C. Use. For vaccination against smallpox.

II. GENERAL REGULATIONS (N.I.H. and M.D.H.)

A. No vaccine shall be prepared from any animal having a communicable disease other than vaccinia. Animals used for propagating smallpox vaccine must have responded negatively to a tuberculin test and prior to vaccination must have evidenced no ill health while in quarantine for at least seven days under daily veterinary inspection.

B. After the vaccine pulp has been removed from each animal, a necropsy shall be performed, permanent records of which shall be kept.

C. Each lot of smallpox vaccine shall be examined to determine its bacterial content, and a special examination shall be made of each lot to determine the absence of Cl. tetani. Permanent records of these examinations must be kept.

D. The finished product must be placed in sterile containers that comply with the requirements established by the USPHS. In the continental United States sealed capillary tubes are the only legal containers.

E. The smallpox unit is operated under strict isolation. No person is to enter it except those directly concerned with the manufacture of the vaccine. Persons engaged in the manufacture of the vaccine are not to frequent the barns, paddock, or tetanus toxoid unit.

F. Materials used in the smallpox unit should not be used by others, but be kept in the smallpox unit exclusively.

G. All calves used in smallpox vaccine production are to be slaughtered and autopsied within 48 hours after harvest of the vaccine. Under no circumstances may such an animal be removed from the premises alive.

H. The last two calves of a production season which has employed the same seed lot number are used as aphthous fever controls (foot and mouth disease). These animals are handled in the same manner as the other calves with the exception that they are inoculated over a smaller area and are kept alive for a two-week observation period following harvest of the vesicles. If the first two calves are used for controls it is wise to hold up subsequent production until disposition has been made of the controls.

III. MEDIA AND SOLUTIONS

A. (11 cc tubes). Thioglycollate agar.

B. Sterility test broth (Smith fermentation tubes).

C. Saline solution 0.85%.

D. 50% glycerine in distilled water.

E. 50% glycerine in distilled water containing 1% phenol.

F. Sterile distilled water (about 3 litres in 1-gallon jugs).

G. Pentobarbital sodium solution.

Pentobarbital sodium	50 g
Ethyl alcohol (95%)	100 g
Distilled water	900 g

Filter through a Seitz EK sterilizing pad and dispense in a sterile diaphragm stoppered 50 ml bottles. Pentobarbital is first dissolved in the alcohol.

H. Sterile soap solution

Ivory soap chips . . . . . 1 part v/v
Water . . . . . 2 parts

Dispense in 16 oz. French squares and sterilize by autoclaving. (The W/V relationship is approximately 55 g of soap to 425 ml H_2O.)

I. 95% ethyl alcohol

J. 1:500 solution of "Roccal"*

K. 1:1,000 solution of "Roccal"

L. Solution of rotenone

Rotone** . . . . . 1 oz.
Warm water . . . . . 1 gal.

IV. SEED VIRUS

A. Source of seed virus. The seed virus used by the Michigan Department of Health Laboratories was obtained from the Massachusets Antitoxin and Vaccine Laboratories. It may be identified as directly traceable to Mass strain No. 999, September 1937.

B. Maintenance of seed virus

1. Either lapine (rabbit) or calf passage virus may be used. A calf - calf - rabbit - calf - calf - rabbit sequence may be followed. However, a calf passage virus may be used exclusively if desired.

2. Rabbit passage restores the potency of a virus that seems to be losing its virulence.

3. The practice at the M.D.H. is to give the seed virus alternate rabbit - calf passages as in the following diagram:

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* A quaterniary ammonium disinfectant - Sterwin Chemicals; New York, N.Y.

** A proprietary rotenone concentrate - Jansen-Salsbery; Kansas City, Mo.

calf vaccine

rabbit
↓
calf ⟶ yields sufficient seed virus for one season
↓
rabbit
↓
calf ⟶ yields sufficient seed virus for one season
↓
etc.

C. Selection of rabbits for production of lapine virus

1. Select six large albino rabbits weighing from 7 to 10 lb. Females are usually more suitable than males.

2. Use only those possessing a smooth soft skin free from short tufts of guard hair.

D. Preparation of rabbits

1. Confine the rabbit in a dorso-ventral position on a rabbit table. The rabbit table is an oblong table 3-1/2 x 1-3/4 ft which permits fastening a limb to each corner of the table by means of a string.

2. Pluck the hair from the back and sides from the region of the shoulders back to the hips.

3. Wash the exposed skin surface thoroughly with soap and warm water. Wipe dry with a sterile towel.

4. Apply 95% alcohol to the skin, allow to dry, then rinse with sterile distilled water.

5. Dry with a sterile towel and drape with sterile cloths leaving only the bare skin exposed.

E. Vaccination. The inoculum consists of calf passage vaccine diluted with 50% glycerine to make a 1:8 suspension. The operator dons sterile gloves.

1. With a sterile 4-point scarifier (points set 1 mm apart) make 5 or 6 parallel scarifications in the skin running from the shoulders back to the hip region. The skin is broken without going deeply enough to draw blood.

2. Apply the virus suspension with a sterile spatula and thoroughly rub it into the scarified skin.

3. Allow the skin to dry, then remove rabbit to a clean cage containing food and water.

F. Harvest. On the fifth day after inoculation the vesicles are ready for harvest.

1. Confine the rabbit on the rabbit table in the same manner as previously described.

2. Sacrifice the rabbit by injecting 5 cc of a 5% solution of nembutal intravenously.

3. Wash the erupted area thoroughly with sterile soap solution and sterile distilled water. Repeat three times, then dry with a sterile towel.

4. Saturate the area with 95% alcohol. Cover with a sterile cloth for 10 minutes.

5. Remove the cloth and rinse the erupted area with sterile distilled water. Dry with a sterile towel and drape with sterile cloths leaving only the erupted area exposed.

6. Remove the vesicles by scraping with a sterile Volkmann spoon. The pulp is collected into a sterile Petri dish. Six good rabbits will yield about 20-30 g of pulp.

G. Preparation of seed suspension

1. Prepare a 1:4 suspension of the pulp with 50% glycerine by grinding in a Waring blendor.

2. Transfer a 10 cc sample with a pipette to a sterile vial containing 10 ml of 50% glycerine. This material is employed in the safety tests as described later in the procedure for vaccine production (see safety tests).

3. Following removal of the sample for safety tests, add sufficient 50% glycerine containing 1% phenol to make a final 1:8 suspension of the pulp in 50% glycerine, and which will then contain 0.5% phenol as a preservative.

4. Transfer the material to a sterile 4 oz. French square, label with its lot number, date of manufacture, volume, and concentration, and store in the freezer at -18°C.

5. Record production data on lapine virus record card.

After satisfactory completion and recording of the safety and potency tests the material is ready to be used as lapine seed virus to vaccinate a calf. The vaccine prepared from this calf is then used as the seed virus for the next season's production of smallpox vaccine provided all the necessary safety and potency tests have been satisfactorily completed and recorded. Preparation and testing of seed virus is the same as for regular vaccine and is described under vaccine production.

H. Standards for seed virus

1. Seed virus must contain not more than 2,000 organisms per ml as determined by plate count* (see bacterial count VI A).

2. Seed virus must be free of harmful anaerobes as determined by the tetanus safety test (see tetanus safety test VI B).

3. Seed virus must have satisfactory potency as determined by rabbit inoculation (see potency test VI C).

V. VACCINE PRODUCTION

A. Preparation of calves for quarantine

1. Obtain female calves weighing from 350 to 400 lb. and having white abdomens. Examine the calves immediately after delivery for physical defects or any evidence of disease, especially skin diseases, hypoderma bovis larvae, and respiratory disease.

* If count is higher, incubate the virus suspension one half hour at 37°C and re-test. 50% glycerine is bactericidal at incubator temperature. However, this must be done with caution because the higher temperature also rapidly inactivates the virus.

2. Completely clip calves to be placed in quarantine with an ordinary horse clipper.*

3. Bathe the calf with Ivory bar soap and warm water, trim and clean the hooves. Dry the animal thoroughly with a Turkish towel. If lice are present sponge the entire body with a solution of rotenone. This preparation may be left on the skin and is not rinsed off. In the absence of rotenone, kerosene may be sponged over the body, but this must be removed by bathing with soap and water. Remove the calf to the quarantine room.

4. Keep record of each calf on form F433.

B. Quarantine and care of calves

1. Duration of quarantine and temperature records. Keep the calves in quarantine for at least one week, during which period rectal temperatures are taken and recorded twice daily - usually about 8 a.m. and at 4 p.m. Maintain the room temperature between 82°F and 85°F and record the calf and room temperatures on form F433.

2. Performance of tuberculin test.** Make the injection into the skin of the right caudal (tail) fold at the base of the tail. The fold is readily seen when the tail is lifted perpendicularly. Wipe the area clean with cotton and alcohol and inject 0.1 cc of tuberculin using a 35 minim syringe with a 1/4 inch, 25 gauge screw needle.

3. Reading the test. Judge the reaction on the third day (72 hours) or later. A positive reaction is indicated by a local swelling at the injection site which is firm and sometimes persists for days. Record results on form F117.

4. Disposition of calves that have a positive reaction to tuberculin. Calves with a positive reaction to tuberculin may not be used for vaccine production. A report should be made to the State Veterinary Authorities interested in tuberculosis eradication.

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* A Stewart clipmaster with an EH blade is used at M.D.H.

** Mammalian intradermic tuberculin obtained from the Bureau of Animal Industry, US Department of Agriculture, Washington, D.C.

5. Rejection of diseased animals. Reject calves that show any evidence of disease during the quarantine period. The most frequent ailment is respiratory in nature and varies in severity from a mild cold to pneumonia. These difficulties can be minimized if the room temperature is constantly maintained between 80°F and 85°F.

6. Feeding calves. Feed calves twice daily, once at 8 a.m. and again at 4 p.m. The ration consists of 1-1/2 quarts of rolled oats and about 1/2 of a pitchfork full of good-quality alfalfa hay per calf at each feeding. Provide a bucket of clean fresh water at each feeding.

7. Sanitation

(a) Place each calf in an individual stanchion equipped with a removable wooden-floor grating. Change calves to clean stanchions each morning. Clean soiled stanchions thoroughly with hot water and disinfect them with a reliable chlorine solution.*

(b) Remove the soiled floor gratings daily, scrub them with a brush and hot water and autoclave them one hour at 120°C.

(c) Wash the rooms down twice daily with a hose in order to eliminate dust, and use chlorine solution liberally on the walls and floors once a day applying the solution with a long-handled brush.

C. Preparation of the calf for vaccination

1. Secure the calf to the operating table by means of straps.

2. Use an Oster small-animal clipper with a No. 40 blade to clip the hair closely from the entire ventral surface and right side of the body. This would include the surfaces of the abdomen, the sterum, the axillae, the insides of the thighs and the right side to within a few inches of the spine.

3. Bathe the entire body with soap, brush and warm tap water. Clean the hooves with a brush.

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* HTH-15 Mathaison alkali works, N.Y.C. is used at M.D.H.

4. Use ordinary bar shaving soap to work up a lather, then with a straight razor closely shave an area that includes the abdomen right side, sterum, axillae, the inside surfaces of both thighs, and the udder. This shaven area is the inoculation site.

5. Scrub the inoculation site thoroughly at least six times with a sterile hand brush and sterile soap solution. The soap solution is melted by heating in a steam bath just before use. Rinse thoroughly with lukewarm tap water after each scrubbing. After the last scrubbing rinse with sterile tap water.

6. Rinse with 95% alcohol. Allow the alcohol to dry and rinse with sterile distilled water using about three one-gallon jugs for this purpose.

7. Wipe the inoculation site dry with a sterile towel. Drape the animal with sterile cloths leaving only the inoculation site exposed.

D. Vaccination procedure

1. The seed suspension is prepared by mixing 5 to 10 ml of stock seed with 10 to 15 ml of 50% glycerine giving a total volume of 25 ml. Dispense in a 4-oz. French square and place in the freezer until used.

2. The operator dons a sterile gown and sterile rubber gloves.

3. A 4-point scarifier containing 4 needles 1/2 cm apart is used. This is a T-shaped instrument consisting of a needle block and a handle. The needles are readily replaced by loosening the screws which hold them fast.

4. Hold the scarifying instrument perpendicularly to the skin and with slight pressure draw parallel lines in the skin about 1/2 cm apart following the long axis of the body. The skin must be definitely broken, but do not go deeply enough to draw blood.

5. The seed suspension is applied to the entire inoculation area following scarification. Care is taken to rub material well into the scarified skin using a sterile spatula.

6. Allow the calf to remain on the table about 30 minutes and then remove the animal to the incubation room for 6 days.

E. Incubation. The incubation room is a duplicate of the quarantine room with the exception that it is equipped with ruby glass windows to avoid direct sunlight, which has an inhibiting effect on the development of the pox lesion.

1. Sanitation

(a) Calf. Keep the calf clean at all times. Remove faecal soilage by hosing with warm water. Twice each day spray the vaccinated area with a 1:1,000 solution of "Roccal" for its germicidal effect.

(b) Room. Keep the calf on a floor grating which is removed daily, scrubbed and sterilized in the autoclave. Scrub the floor of the incubation unit daily with a chlorine disinfectant (HTH-15), and keep the walls, window ledges, etc., absolutely free of dust by washing the room down twice a day with a hose.

2. Feeding. Feed calves the same as when they are in quarantine except that the hay and oats are previously autoclaved for one hour at 120°C.

3. Temperature. Take rectal temperatures each morning and afternoon and record them on form F433. Record maximum and minimum room temperatures on the same form. The thermostat should be adjusted to give a room temperature between 82°F and 85°F.

F. Collection of the pulp. Collect the virus from the calf on the sixth day of incubation.

1. Place the calf on the table as previously described and anaesthetize deeply by administering intravenously a 5% solution of nembutal at the rate of 1 ml per 10 to 14 lb. of body weight. Operator dons sterile gown and rubber gloves.

2. Cleanse the vaccinated area by scrubbing alternately with sterile soap solution and rinsing with warm tap water. Repeat 13 times.

3. Use sterile hand brushes for each scrubbing. Discard the brushes if their use softens and ruptures the vesicles, and employ the gloved hands instead.

4. After the last soap washing rinse the calf with sterile tap water. Follow this by rubbing 95% alcohol over the area to remove all traces of soap. Rinse again with sterile tap water and dry with a sterile towel. Spray Roccal 1:500 on the area, then cover with sterile towels for 15 minutes. Rinse with sterile distilled water and dry.

5. Sacrifice the calf by inserting a trocar into the carotid artery. Dispose of the blood down the floor drain of the operating room.

6. When the calf is dead, rinse the vaccinated area with three or four jugs of sterile distilled water. Dry the area with a sterile towel and drape it with sterile cloths leaving only the vaccinated site exposed.

7. The operator changes clothes and dons another sterile gown and another pair of sterile rubber gloves. Collect the pulp by scraping the erupted skin surface with a sterile Volkmann spoon. Place the harvested pulp in a sterile tared glass dish. Remove the pulp to the laboratory, weigh it, and place it in the freezer.

G. Autopsy

1. Autopsy the calf immediately except in the case of a calf that is to be held for a two-week observation period for aphthous fever (foot and mouth disease) as specified by the National Institutes of Health Regulations.

2. Make a systematic gross inspection of the carcass and all the organs. Excise and incise with a sharp knife the following lymph nodes:

prescapular, prefemoral, inguinal, iliac, tibial, mesenteric, hepatic, renal, mediastinal, bronchial, and mandibular.

3. Record the autopsy findings on form F117. Discard the vaccine from any animal showing evidence of sepsis or communicable disease.

4. Aphthous fever control calves are similarly autopsied following their two-week observation periods.

H. Preparation of the vaccine

1. Transfer the pulp to a sterile Waring blendor cup and add a sufficient volume of 50% glycerine to make a 1:2 suspension W/V.

2. Carry out grinding in the cold by placing the Waring blendor cup in the freezer compartment. Grind for 4-10 minute periods at low speed with a 15-minute rest between each grinding period. This will prevent excessive heating of the vaccine during the grinding process.

3. Remove a 10 ml sample after the fourth grinding period and transfer it to a sterile vial containing 10 ml of 50% glycerine. Label it with the lot number and date and store at room temperature. This unpreserved sample is used for the safety tests as required by National Institutes of Health Regulations.

4. Add sufficient 50% glycerine containing 1% phenol to make a 1:4 pulp suspension (see calculations below). Grind again for 10 minutes using the high-speed setting on the motor.

5. Run the material through the screening apparatus which contains a 100-mesh wire screen (see photograph) and collect the screened material in sterile plasma bottle. Drape the screening assembly with phenol-saturated gauze during the screening process.

6. Seal the bottles with sterile rubber stoppers, label with lot number, volume, date of preparation and store in the freezer at -18°C.

7. Record preparation data on form F117.

8. Calculations

Example:

If net weight of pulp is 100 g

(a) Add 100 ml of 50% glycerine to make 1:2 suspension W/V.

(b) Weight of pulp x 2 - 10 = amount of phenolized glycerine which must be added to make a 1:4 suspension after removal of the 10 ml sample.

Thus: (100 x 2) - 10 = 190 ml of 50% glycerine plus 1% phenol needed to make a 1:4 suspension.

(c) The amount of preservative is 1% of 190 or 1.9 ml of phenol.

(d) The total amount of diluent (50% glycerine) is (weight of pulp x 3) - amount of preservative. Thus: (100 x 3) - 1.9 = 298.1 ml of diluent.

VI. SAFETY AND POTENCY TESTS

Before vaccine virus can be released for filling, tests must be conducted to show that the bacterial count is satisfactory, that it is free of Cl. tetani and that it is of sufficient potency.

A. Bacterial count

1. One ml of bulk vaccine is diluted with 19.0 ml of phy. saline. Of this dilution 1 ml is cultured in each of not less than 5 agar pour plates. The average colony count may not exceed 50 per plate. (N.I.H. regulations)

2. Heat the tubes of veal infusion or thioglycollate agar in a steam cabinet until the agar is melted. Allow the agar to cool to about 45°C before pouring.

3. Pour the agar from each tube into a Petri dish containing a vaccine dilution, mix by swirling dish gently, let them stand till the agar is hard. Invert the plates and place them in a 37°C incubator for 48 hours.

4. Count the colonies and record the results on form F132.

5. If necessary re-plate samples at intervals until the count is 500 bacteria or less per ml.

B. Test for Cl. tetani. Make test for Cl. tetani on the ground glycerinated pulp which was removed as a sample before phenol was added to the vaccine. Test samples from each calf separately. Store the samples at 10°C or higher for at least seven days or until the bacterial count is below 1,000 per ml.

1. Plant 2 ml of virus suspension in each of four Smith fermentation tubes containing at least 25 ml of broth as made for sterility testing. (Just before they are to be used, heat the tubes to 100°C for 30 minutes. Tilt them to remove the air from the closed arm and cool to at least 40°C before inoculation.)

2. Incubate all tubes at 37°C for nine days, and inspect daily for signs of growth in the media.

3. Inoculate a mouse subcutaneously with 1 ml of the unfiltered broth from each Smith tube showing growth or gas in the closed arm 24 to 48 hours after its appearance.

4. Nine days after planting, inoculate a mouse subcutaneously with 1 ml of unfiltered broth from each Smith tube regardless of whether or not there is any visible growth.

5. Observe the mice daily for six days for symptoms of tetanus. If any die within three days without showing symptoms of tetanus, repeat the test immediately by inoculating 0.1 ml of broth from the same tube. If mice are repeatedly killed by 0.1 ml of broth, guinea-pigs may be substituted. Discard the entire lot of vaccine if symptoms of tetanus appear in any animals inoculated with the sample.

6. Record results of the test on form F132.

C. Determination of the potency. Potency tests are performed on the bulk vaccine and on vaccine-filled in capillaries. Test for potency by rabbit inoculation according to the technique of Force and Leake as given in Hygienic Laboratory Bulletin number 149, 1927.

1. Dilution of the bulk vaccine

(a) Shake the bottle of vaccine thoroughly and make serial dilutions in sterile saline as follows:

0.5 ml vaccine plus 49.5 ml saline equals 1:100 dilution
1 ml of 1:100 plus 9 ml saline equals 1:1,000 dilution
1 ml of 1:1,000 plus 2 ml saline equals 1:3,000 dilution
1 ml of 1:1,000 plus 9 ml saline equals 1:10,000 dilution
1 ml of 1:10,000 plus 2 ml saline equals 1:30,000 dilution

(b) Rotate the tubes between the palms to disperse the vaccine before mixing with a pipette. Use a separate pipette for each dilution and mix up and down 10 times. Bulk vaccine is tested in the following dilutions:

1:1,000, 1:3,000, 1:10,000, 1:30,000

2. When testing vaccine from capillaries, the vaccine must first be pooled into a single container and the dilutions made from this pool using not less than 0.1 ml to make the first dilution.

3. Preparation of the rabbit

(a) Remove the hair from the back and sides of a 7 to 10 lb. albino rabbit by plucking.

(b) With a stencil having four apertures 2.5 x 5 cm, mark four equal areas on each side of the rabbit. Use a moistened indelible pencil, taking care not to stretch the skin.

4. Inoculation of the rabbit

(a) Keep on hand 10-inch lengths of glass tubing of an inside diameter of about 3 mm which have been plugged with cotton at both ends and sterilized. Break off squarely at the centre, making two pipettes.

(b) With the sharp end of one of the pipettes thus formed, scarify one of the marked-off areas taking care to avoid cutting, until an even redness without bleeding results. Further scarification will of course take place as the vaccine is inoculated.

(c) By means of a rubber bulb attached to the pipette used for scarification draw up 0.2 ml of the dilution of vaccine to be tested. Holding the end of the pipette closely against the skin of the rabbit, rub within the marked area, releasing the vaccine gradually to avoid loss outside the area of inoculation.

(d) In the case of bulk vaccine start with the highest dilution using the same pipette for the four dilutions. The vaccine under test is inoculated on the left side and control vaccine, the potency of which is known, is inoculated in corresponding dilutions on the right side.

(e) Vaccine from capillaries of finished vaccine taken at random shall be incubated at 35°C to 37°C for at least 18 hours. The heated vaccine is tested at the same time as the unheated vaccine but only at a dilution of 1:1,000. The control vaccine is tested in the same manner as the lot of production vaccine.

(f) As soon as the inoculation is finished, place the rabbit in a cage containing food to attract its attention and prevent it licking the inoculated sites.

5. Interpretation and recording of potency tests. Record all results on form F119.

(a) After five days inspect the rabbit and record the amount of eruption in each rectangle. This is expressed as the percentage of the area covered by a confluent eruption of vesicles and by the actual number of vesicles if discrete lesions are formed.

(b) A satisfactory vaccine should yield 80-100% confluency in the 1:1,000 dilution; no more than a 20% reduction in the 1:3,000 dilution; at least one discrete lesion in the 1:30,000 dilution, with an approximately proportionate number of discrete lesions in the 1:10,000 dilution.

(c) Vaccine falling below these standards may be retested. If repeated tests show the vaccine to be of substandard potency it must be discarded.

VII. MISCELLANEOUS INFORMATION

A. Hydrogen ion concentration

1. Ph of smallpox vaccine is approximately 7.1
2. Ph of 50% glycerine is approximately 6.1
3. Ph of 50% glycerine plus 1% phenol is approximately 6.0

B. Storage. Vaccine may be stored indefinitely from the date of manufacture provided the filled product is tested within nine months of issue. Smallpox vaccine in the bulk container is stored at -18°C at M.D.H.

C. Datings. Distributed vaccine has a three-month expiration date from the date of issue in the winter months (October through March) and a two-month expiration date from the date of issue in the summer months (April through September) if kept continuously below 5°C (41°F).

D. Production schedule

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
(1) harvest	(1) inoculate calf	(1) potency test (2) inject mice from tubes of 9 days before	(1) harvest	(1) inoculate calf		
(2) autopsy calf (3) grind and screen vaccine (4) tetanus safety test, inoculate Smith and meat tubes	(2) discard mice from previous week's test (3) bring in new calf, clip and bathe (4) TB test, 2 calves	(3) bacterial counts-pour plates	(2) autopsy calf (3) grind and screen vaccine	(2) bring new calf, clip and bathe		
Check inoculated tubes and mice every day						

For 3 calves/wk: add harvest on Tuesday and vaccinate on Wednesday

SMALLPOX VACCINE POOLING PROCEDURE

1. Assemble bottles of acceptable lots of vaccine (oldest harvests with the lowest bacterial counts).

2. Prepare: (a) a sterile screening apparatus;
   (b) a sterile one-gallon Pyrex bottle;
   (c) a sterile stopper for the bottle.

3. Materials: (a) bottle (at least 100 ml) of 50% glycerine;
   (b) bottle (at least 100 ml) of 50% glycerine with 1% phenol.

4. If ice is present in the vaccine, permit it to thaw at room temperature. Usually 30 minutes is sufficient for ice to melt. DO NOT ALLOW VACCINE TO BECOME WARM.

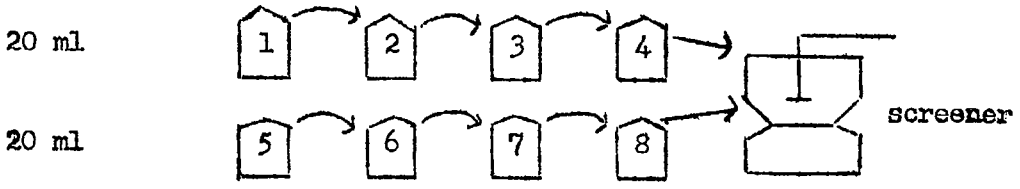
5. Place screener over the mouth of the one-gallon bottle. Use aseptic precautions. Cover with a cloth soaked in 5% phenol.

6. Aseptically decant vaccine from the bottles into the hopper of the screener:

   A. Fill the screener not more than 2/3 full at any time.
   B. Avoid overheating. Operate the screener at the slowest possible speed.
   C. Reason for screening: to remove any calf-hairs or tissue which may have passed through the mesh in previous screening.

7. Rinsing the vaccine bottles:

   A. For each bottle, 10 ml of 50% glycerine - 0.5% phenol are used.
   B. In actual practice, a more thorough rinse may be achieved by decanting larger volumes from bottle to bottle.
   EXAMPLE: eight bottles are to be pooled
   8 x 5 ml = 40 ml of 50% glycerine
   8 x 5 ml = 40 ml of 50% glycerine plus 1% phenol
   80 ml of 50% glycerine plus 0.5% phenol
   Add 20 ml of 1st rinse to bottles No. 1 and 20 ml to No. 5
   Swirl and decant into bottles No. 2 and No. 6 respectively:



   The second rinse is performed in the same manner

8. Remove specimens (1 ml each) for safety (bacterial count and potency test).

9. Label the vaccine bottle: Pool No. and date.

10. Return vaccine bottle to freezer until tests are completed.

11. Record data on form F119.
    Bacterial count may be given on "Remarks" section of "Pooling Record".

SIGNATURE _____