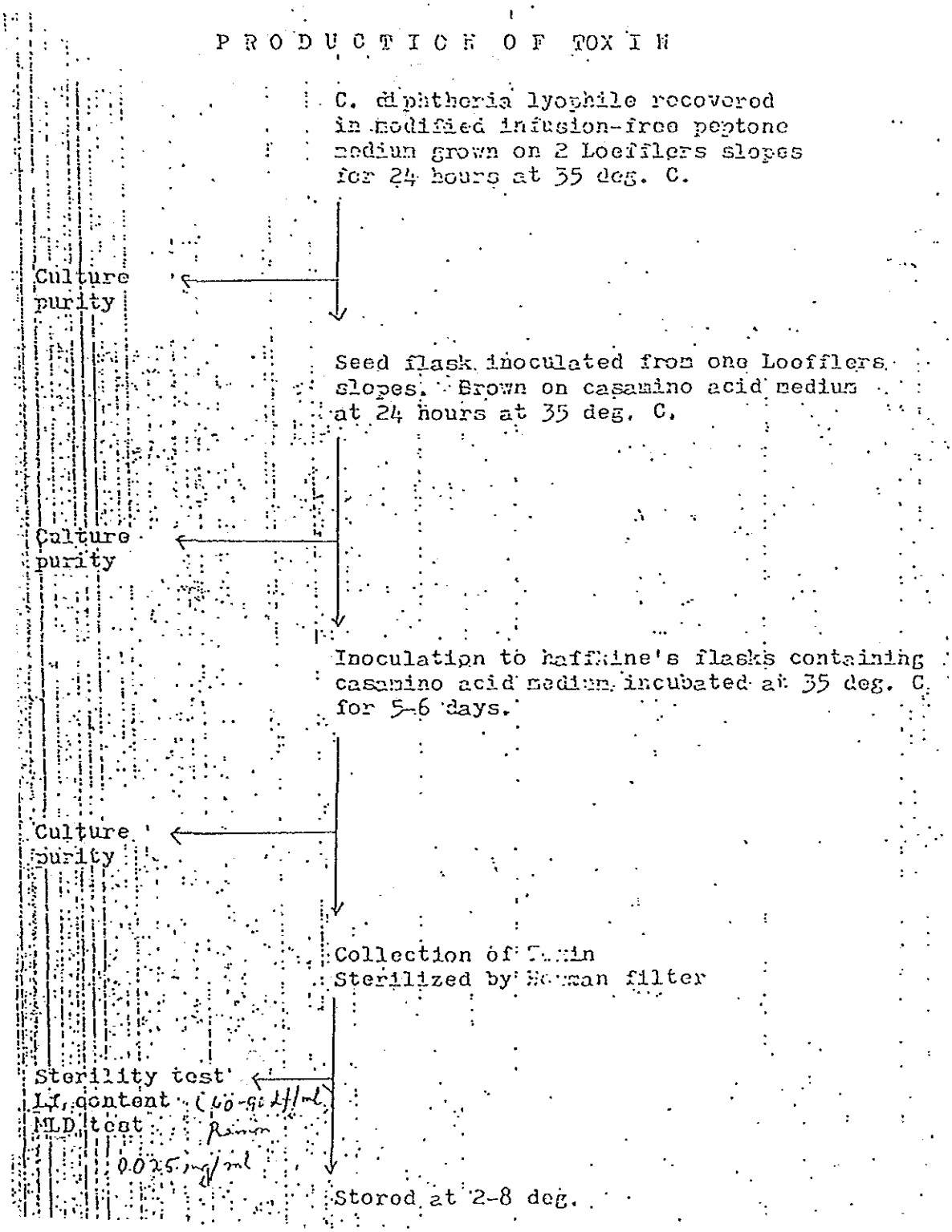
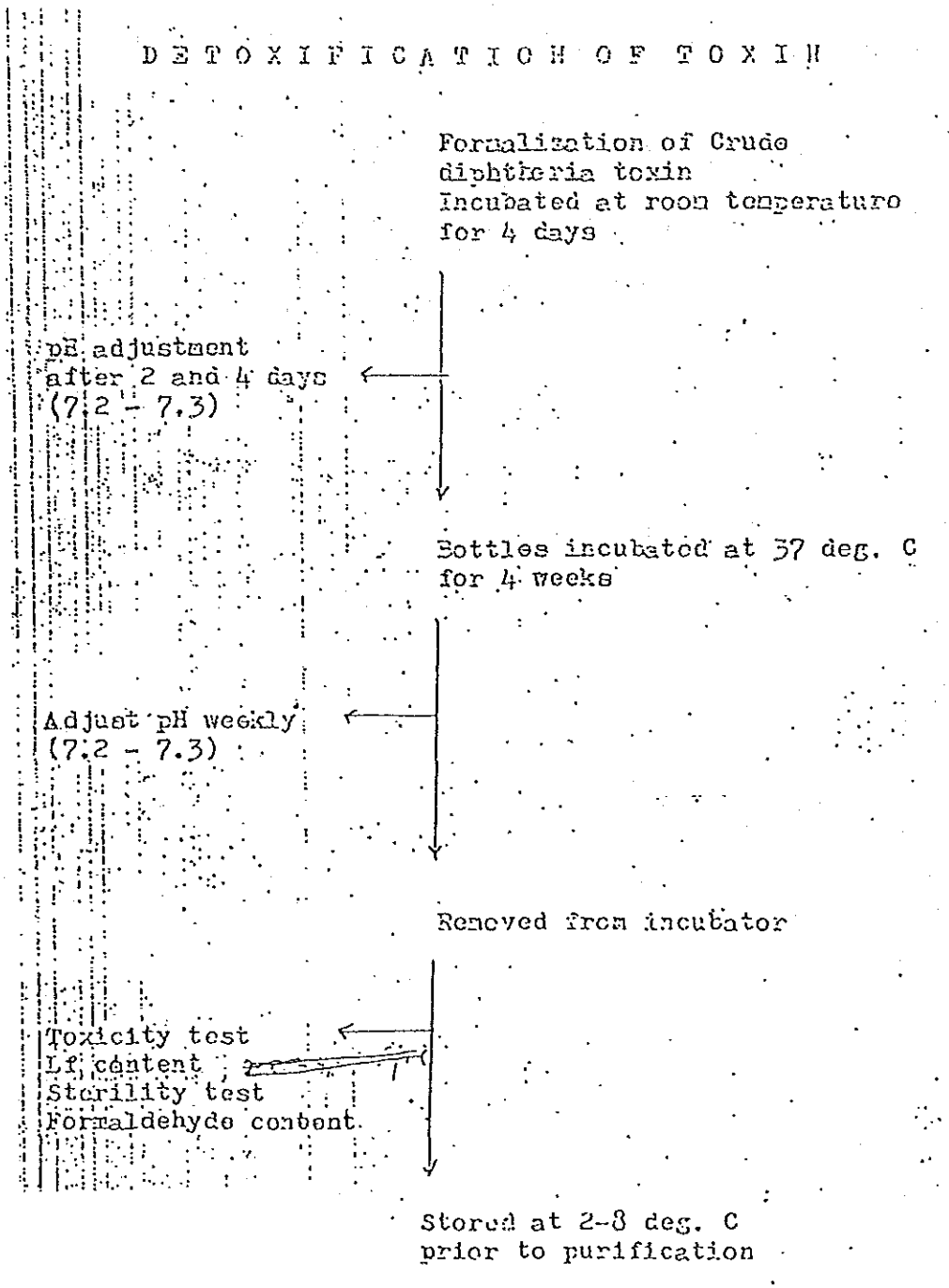
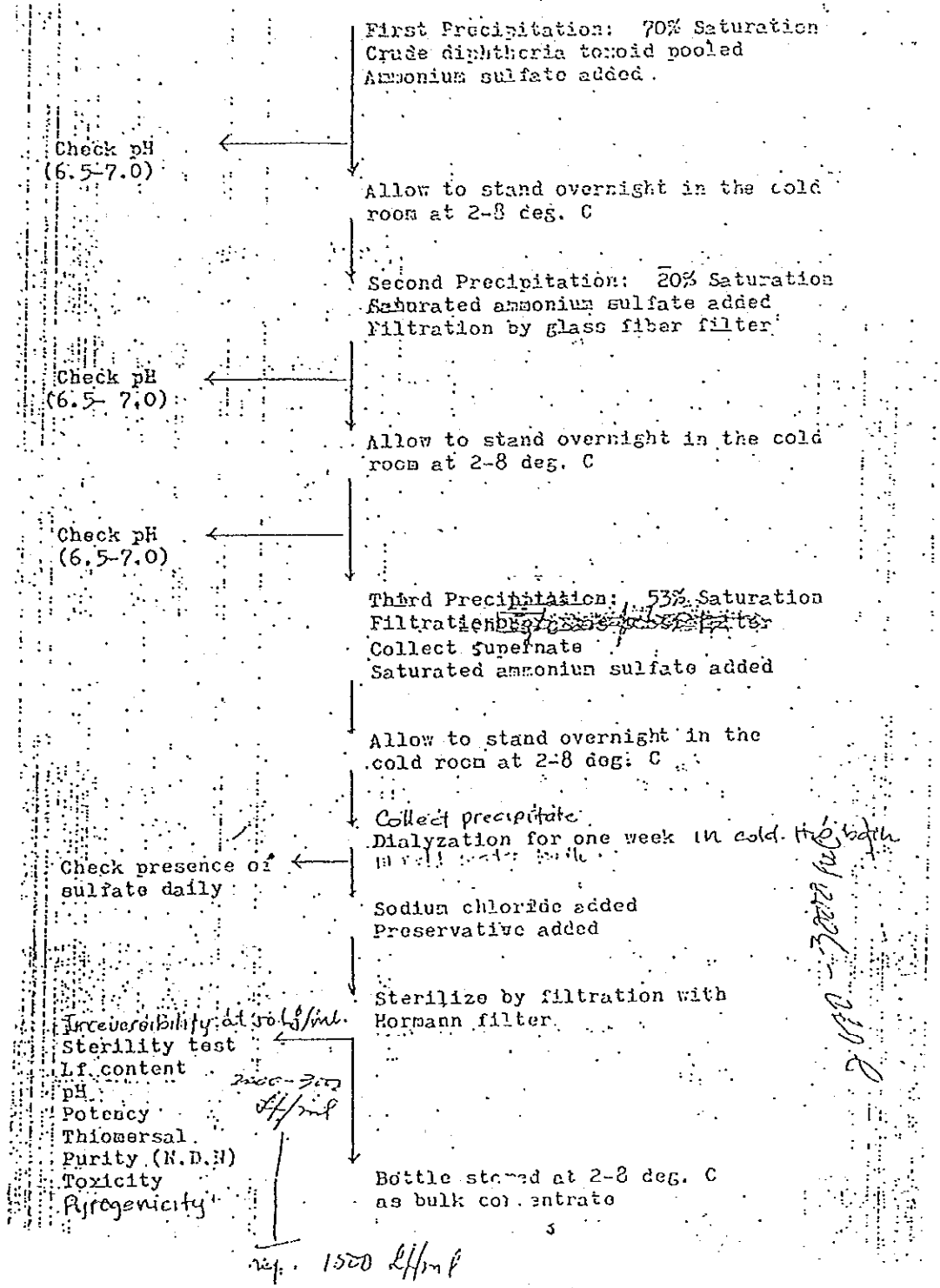
18. ジフテリアトキソイド静置培養法




DETOXIFICATION OF TOXIN
Formalization of Crude diphtheria toxin Incubated at room temperature for 4 days
pH adjustment after 2 and 4 days (7.2 - 7.3)
Bottles incubated at 37 deg. C for 4 weeks
Adjust pH weekly (7.2 - 7.3)
Removed from incubator
Toxicity test
Lf content
Sterility test
Formaldehyde content
Stored at 2-8 deg. C prior to purification

PURIFICATION OF TOXOID
(Ammonium Sulfate Fractional Method)



19. 百日せきワクチンファーメンター培養法

STANDARD OPERATING PROCEDURE FOR PERTUSSIS VACCINE

Name of Product- Pertussis Vaccine

Description. Pertussis Vaccine is a bacterial vaccine containing two strains of Bordetella pertussis at a concentration of approximately 32 opacity units per milliliter. The original liquid medium and the bacterial growth is harvested, centrifuged and suspended in buffered saline solution with pH 7.0, and containing merthiolate at a final concentration of 1:10,000.

Strains of culture employed:

Two phase I strains of B. pertussis are used in vaccine production. These are B. pertussis strain 509 (agglutinogens 1,2,6 and 7) from the culture collection of the National Institute of Public Health, Bilthoven The Netherlands and B. pertussis strain 134 (agglutinogens 1;3; and 7) obtained from Dr. Pillemer (Lederle), The Netherlands. They were selected as they gave high level of protection to mice and are stable phase I organism.

Source. Culture of Phase I strains of B. pertussis are obtained from the Institute of Public Health and Environmental Hygience, Bilthoven, The Netherlands.

Characteristics. All strains of B pertussis used in vaccine production should be characterized by the following:

1. Typical morphology and staining characteristics- small coccoid Gram-negative rods, with a tendency to short chain formation.
2. Typical growth on Bordet-Gengou blood agar medium-pearly white mucoid growth with hemolytic activity on blood medium exhibited in 48 to 72 hours incubation.
3. No growth on plain agar medium.

Lyophilization. The lyophilized culture of a strain of B Portussis is revived by aseptically opening the ampoule and the content suspended in 0.5 ml of sterile distilled water and transferred to a fresh slant of Bordet-Gengou blood agar medium by means of a sterile capillary pipette fitted with small rubber bulb. The suspension is then spread on the blood agar slant. This is incubated at 35°C for 72 hours after which sub-cultures are made on the same medium. Twelve slants are sufficient for one lyophilization. The subcalture are then incubated for 72 hours at 35°C after which each tube is examined microscopically and from the cultures showing typical growth and absence of contamination, growth is scraped off by means of a sterile loop and transferred to an empty sterile culture tube. The collected growth is then diluted with about 16 ml of 10% skim milk and then dispensed in 48 ampoules by means of sterile capillary pipette, each ampoule to contain 0.3 ml. The filled ampoules are then placed on the centrifugal freeze-dryer. The lyophilizing process takes 7-8 hours, after such period each ampoule is vacuum sealed and then tested for vacuum with a vacuum tester, properly labelled as to strain number and date lyophilized, and the ampoules stored at 5°C to 10°C until they are needed for vaccine production. The next day, 2 lyophilized culture tubes are selected at random and sterility test is done the same way as in reviving a lyophile. Lyophilized cultures of B portussis may remain viable up to 5 years.

Technique of Portussis Vaccine production

1. Preparation of Culture Media:

A. Bordet-Gengou blood agar

Formula:

Pooled potatoes	125.0 g
Glycerine, C.P. Merck's	10.0 ml
Bacto agar	30.0 g
Proteose peptone, Difco	10.0 g
Sodium Chloride C.P.	5.3 g

Distilled water q.s. ad - - - - - - - - - - - - 1,000.0 ml

Sterile citrated sheep blood - - - - - - - - - 200.0 ml

N.B . Use only glass or stainless containers.

Boil gently washed sliced, peeled potatoes in one-half the volume of distilled water and glycerine until soft. Strain through four thicknesses of gauze. To the extract add salt, peptone and agar and heat in pan of water to saturate the agar. Add remaining water and bring up to volume. Adjust to pH 7.3 - 7.4.

Dispense in 22.5 ml amounts in 25 mm x 200 mm culture tubes and sterilise in the autoclave for 1 hour at 15-lbs. pressure. Allow the medium to cool some what before removing from autoclave. Immediately place the test tubes of the medium in a water bath at 45°C. The water bath employed should be such that a large volume of water at 45°C can be used for rapid cooling and holding of the medium at 45°C. It has been possible to cool tubes of medium to this temperature using running water at 45°C in about one hour time.

Add 4.5 ml of blood to each tube of the medium aseptically. Rotate a test tube continously to ensure thorough mixing.

The tubes are place in inclined or slanting position to solidify. Care should be taken to keep the stopper free from blood and agar.

After solidification, tubes of finished medium are incubated for 48-72 hours at 35°C.

Each tube is examined macroscopically for evidence of contamination and stored at 5-10°C until needed.

B. Composition of 1 liter B_2 medium

Casamino acids - - - - - - - - - - - - 6 g

L-Glutamic acid - - - - - - - - - - - 5 g

NaCl - - - - - - - - - - - - - - - - - 2.5 g

KH_2PO_4 - - - - - - - - - - - - - - - 0.5 g

$MgSO_4.7H_2O$ - - - - - - - - - - - - 0.1 g

$CaCl_2.2H_2O$ - - - - - - - - - - - - 0.01 g

$FeSO_4.7H_2O$ - - - - - - - - - - - - 0.0125 g

C. B_2 medium (Main Stock Solution)

Formula: 60 liters

Casamino Acids (Difco, technical)	800 g
L-Glutamic Acid	2,000 g
Sodium Chloride, NaCl	1,000 g
Potassium phosphate, monobasic, KH_2PO_4	200 g
Magnesium Sulphate, $MgSO_4.7H2O$	40 g
Calcium Chloride, $CaCl2.6H_2O$	4 g
Ferrous Sulphate, $FeSO_4.7H_2O$	5 g
Glutathione	4 g
Copper Sulphate, $CuSO_4$	.2 g
Yeast dialyzate	20,000 ml

Dissolve the components mentioned above in the order given in 38 liters distilled water of about 60°C. Heat under stirring to 75°C. Adjust the pH (measured at 20°C) with 10N NaoH. Add 20,000 ml yeast dialysate and adjust the volume with triple distilled water to 60 liters.

Filter the solution through Seitz filter press (11 K5 and 11 EKB-sheets) Dispense the sterile filtrate in 5,12 liter bottles (11 liters per bottle).

Keep the concentrated medium in the cold room at about 4°C.

D. Yervey Medium

Formula: 5 liters

Casamino acids (Difco, technical) . . . . . 70 g

Potassium chloride, KCl . . . . . 1.0 g

potassium phosphate, monobasic, KH_2PO_4 . . . . . 2.5 g

Magnesium chloride, $MgCl_2$ . . . . . 10.5 g

Nicotinic acid . . . . . 0.1 g

Glutathione . . . . . 0.05 g

Starch . . . . . 5.0 g

Dissolve 5 g starch in 250 ml cold distilled water. Add 250 ml hot distilled water and mix thoroughly. Add the starch solution to the rest of the quantity of distilled water to which all the other components are already added. Adjust the pH to 6.8 (requiring approximately 6.0 ml 5N NaoH). Sterilize the medium for 10 minutes at 120°C and readjust the pH to 6.8.

Dispense the medium in 200 ml portions in 500 ml flasks. Plug the flasks with cotton plugs and sterilize for 15 minutes at 116°C.

E. Casamino acids solution

Formula: 2 liters

Casamino acids . . . . . 200 g

Distilled water . . . . . 2 liters

Dissolve the casamino acids while heating, adjust the pH to 7.2 with 5N NaoH. Filter through filter-paper and dispense the clear fluid in 320 ml portions in 500 ml bottles. Sterilize for 30 minutes at 110°C.

F. Starch Solution

Formula: 2 liters

Starch . . . . . 60 g

Distilled water . . . . . 2 liters

Mix the starch under stirring with cold water, and stir until a smooth suspension is obtained. Heat under constant stirring and boil for a half a minute. Dispense in 400 ml portions in 500 ml bottles. [illegible] with cotton and sterilize for 30 minutes at 110°C.

G. Preparation of B_2 Medium

To make 40 liters B 2 medium the following components are added:

Main stock solution	6 liters
Starch solution	2 liters
Casamino acids solution	1.6 liters
Distilled water	30.4 liters

Mix all the above components in the order given in a fermentor. Sterilize for 20 minutes at 110°C.

H. Yeast dialyzate. The yeast dialysate used in B_2 medium is prepared as follows:

Bregers Yeast (Fleischmann pure dry yeast type 2019)	150.0 g
Distilled water in cellulose casing	240.0 ml
Distilled water in dialyzing cylinder	600.0 ml

Introduce portions of the weighed amount of dry yeast powder and distilled water alternately into a collusose casing (20 inches long of size 4"), one end of which has been previously securely tied. After all the yeast powder has been added, the open end of the casing is tied loosely leaving enough air space to massage the mixture into a homogenous paste. The casing is moistened from time to time with distilled water during homogenizing process. It is rolled with the hands until all of the mixture is uniform in consistency. The air bubbles are worked up in the casing and the materials packed down as tightly as possible. The end of the excess casing is then washed free of any excess powder, and placed in the dialyzing cylinder, (Pyrex, 2-liter capacity) containing 600 ml of distilled water. The cylinder is placed in a water bath at one time. At end of the dialyzing period the casing are removed from the cylinder and the dialyzates are pooled in glass stoppered 2 liter bottles, and stored in the cold room with temperature of 5°C-10°C until it is used in the preparation of B_2 medium. The dialyzate is preserved with Chloroform in a final concentration of 0.5%. [91]

I. Buffered Saline Solution

Formula:

Sodium phosphate, monobasic, NaH_2PO_4 . . . . 28.81 g of $NaH_2PO_4.H_2O$-33.0 g

Sodium phosphate, dibasic, Na_2HPO_4 . . . . . 125.0 g

Distilled water . . . . . 1,000.0 ml

Add 20 ml buffer to each 1,000 ml of 0.85% salt solution to make buffered saline solution, adjust to pH 7.0.

Autoclave at 121°C for 30 minutes.

J. Thioglycollate broth (Dehydrated medium from Baltimore Biological Laboratory) for determination of bacterial contaminations.

Dissolve 29.5 grams of the powder in a liter of freshly distilled water.

Carefully adjust to pH 7.4. Filter through filter paper.

Dispensed 300 ml amounts in 500 ml Erlenmeyer flasks.

Autoclave 20 minutes at 123°C(17 lbs. pressure). Final pH should be 7.0 to 7.2.

K. Trypticase soy broth (dehydrated medium from baltimore Biologicals Laboratory for determination of fungal contaminants.

Suspend 30 grams of the powder in a liter of freely distilled water. Mix throughly and then warm gently until solution is complete. If desired, 0.5 to 1.0 gram of agar may be added per liter of broth. Heat to boiling to dissolve the medium completely.

Carefully adjust to pH 7.3.

Dispense 300 ml amounts in 500 ml Erlenmeyer flasks.

Sterilize in the autoclave at not over 123°C 17 lbs. pressure for 20 minutes.

Final pH should be 7.3 + 0.2.

L. Skim Milk Powder (Bacto) for lyophilization of B. pertussis culture.

Dissolve 10 grams of skim milk powder in 100 ml distilled water and dispense in 20 ml amounts in 25 mm x 250 mm culture tubes. Sterilize for 15 minutes at 121°C. Store at 5°C to 10°C until it is needed.

II. Preparation of the Vaccine

Preparation of seed culture. At each vaccine preparation two strains of B. pertussis culture are revived. About one week before the main culture should be seeded, four 500 ml. Erlenmeyer flasks with 200 ml Verwey medium,, are inoculated with two lyophilized cultures of both strains 509 and 134 and incubated at 35°C during three to four days in a gyratory shaker.

The cultures are checked after initiation of growth with a Gram-smear for purity, and the pure culture kept at 5°C.

Twenty-four hours before inoculation of the main culture a volume of 5 ml of two pure cultures is transferred in duplicate in 200 ml. Verwey medium in 500 ml Erlenmeyer flask, and placed on the shaker at 35°C after 24 hours the culture is Gram-stained to check the purity. A sterile transfer assembly is placed aseptically on the seed culture that has been sel selected for seeding the main culture.

Sterilization of the fermentor

Fill the fermentor with distilled water in such a way that the pH and PC2-electrode are immersed in the water over at least a few centimeters. Close the rubber tube on the KCL reservoir of the pH electrode by means of a tube clamp or adjust the pressure on the jacket of the lectrode to 1 kg cm^{-2} with an air-pump. Fill the PC2 electrode with distilled water, close the ingoing tube but keep the outgoing tube open. Place the long pipes in a high position so that the lower ends are above the liquid level. Place a container just below the fermentor and cover the fermentor with a suitable cover. Switch on the recorder by pressing "MAINS" and "REC". Start the driving unit (at about 20 $\overset{\circ}{}$ $min.^{-1}$). Switch on the temperature contol; switch the steam and water selector or position "ST".

Switch on the PO_2 and p^H controller. Check whether the water and the closed-circuit valves are closed. Set the temperature controllers on 110°C, (glass fermentors) or on 120°C(fermentor with stainless steel vessel). Open the steam valve as far as possible. Open the valve of the drain a little. Allow the temperature in the fermentor to increase till 100°C, while all tubes clamps on the tubes are completely open. As soon as the temperature is reached, screw all clamps down until only a little steam escapes from them. Apply a clamp to the tube of the outgoing air and screw it down until just open.

Raise the temperature now to 110°C(0,41 kg cm^{-2}), or 120°C (1 kg. cm^{-2}). Adjust, as soon as the sterilization temperature is reached, the drain of the filter assembly in a such a way, that the condensate can escape. Take care that the pressure in the glass fermentor never exceeds 0,5 kg. cm^{-2}. Maintain the sterilization temperature during at least 10 mins. After this time switch off the driving unit. Close the clamp on the tube for emptying the fermentor, and let the stainless steel pipe come down until it just reaches the bottom of the fermentor. Take off the normal tube clamp from the tube of the sampling system, at the side of the small air filter, and place an artery clamp instead. The procedure for collecting a sterile cotton wool filter to the sampling system is as follows:
Open the artery clamp a little and flush one end of the filter with the escaping steam, keeping it at a square angle (take care that no steam reaches the cotton wool, making it wet). Close the clamp, and connect the filter with the rubber tube; now close the lower clamp on the sampling system. Open now the clamp at the side of the air filter. Pull the sampling pipe in the fermentor down until the right position, and start the driving unit again.

Sterilize another 10 minutes, and then close all tubes by means of their tube clamps. Close the steam valve and switch the steam/water selector to "W". Take away the clamps on the tube of the outgoing air line, between fermentor and panel. Adjust the reduction valve for air on the panel to

0.5 kg. cm^{-2}. Press the button "air perm". Open the air valve on the panel completely, and add a clamp on the rubber tube, coming from the air outlet of the control unit. Connect the air tube with the air filter of the fermentor, and take away the clamp on this tube as soon as the pressure in the fermentor reaches 0.1 kg. cm^{-2}. The full air pressure is now applied to the filter and this should let the air pass instantaneously. As soon as full passage of air through the filter is reached, turn the reduction valve back to 0.3 kg. Cm^{-2}, and adjust the air flow rate to at least 2 liter $min.^{-1}$ by means of the needle valve.

pH calibration and measurement:

The digital read-out, should be switched to the corresponding channel. The electrode is brought into a buffer of accurately known value, near to pH 7.0. Also the Pt-sensor should be brought into a second buffer solution of accurately known value of about pH 4.0. The procedure is repeated until the readings at both pH values are correct without further adjustment. The pH-reading on the panel (fermentor) is then adjusted to this value by means of the asymetry potential potentiometer. Sample taken from the fermentor is then brought and measured keeping the sample on the same temperature in fermentor.

PO_2 - probes calibration

The PO_2-electrodes are kept fully assembled, and filled with distilled water. Before use the membrane should be tested by applying a slight over-pressure, by means of a syringe, on the ingoing tube while closing the other one.

Calibration is done as follows:

The control panel should be switched on at least half an hour before calibration. The digital read-out, should be switched to the corresponding channel. As soon as, after sterilization, the temperature of the fermentor has reached 35°C, the distilled water in the electrode is exchanged for

half saturated $KHCO_3$solution (saturated $KHCO_3$ solution, diluted with an equal volume of distilled water). By means of a syringe of 10 ml this solution is brought through the ingoing tube in the electrolyte reservoir of the electrode. Simultaneously the distilled water is expelled from the electrolyte reservoir, and taken up in a small glass beaker.

Both tubes of the electrolyte reservoir are now closed by pulling them over the small metal rods in the top of the electrode. The electrode is now kept overnight for equilibration. Thereafter the fermentor is brought on the conditions of the culture with regard to stirrer speed, air flow rate and temperature. As soon as the PO_2-reading indicates that equilibrium has been reached, the span is adjusted to give the desired value.

It is advisable not to switch on the stirrer during the first 5 minutes: the foam will die out, and a better escape of O_2 will be obtained. As soon as the reading is constant (switch off the stirrer from time to time, and switch on after the foam layer has disappeared) the zero point of the electrode (the point at which the PO_2 as zero) has been reached.

Introduction of B2 medium and sterilisation

Empty the fermentor by closing the outgoing air line thus causing a little over pressure in the fermentor. Through the long stainless steel pipes the fluid is pressed out. Open the outgoing air tube again. Place the tube in a high position, above the expected liquid level, and do the same with the sampling tube. Check whether the stirrer is turning free.

Fill the fermentor with medium through the long pipe. Repeat the fermentor sterilisation procedure with the following exception: between the sampling and the small cotton wool filter a clamp is applied during the whole sterilisation period, in order to avoid the wetting of this filter.

Also the influent air tube on the D-F filter is closed, and the drain of the filter assembly is adjusted so that condensate can escape. As soon as, after this second sterilization period, the pressure has reached 0.1 kg. cm^{-2}, the clamp on the ingoing air line is taken away, also the condensate drain is closed. When the temperature in the fermentor has come down to 100°C, the water valve on the panel is opened and the flow adjusted to 0.5 l $min.^{-1}$. Switch the steam/water selector on position "W". Set the controller on the desired temperature and apply 500 watts heating by bringing the selector switch into the right position. Take away the clamp on the pH-electrode. Check, after the fermentor has cooled down completely, if all gland-nuts on the fermentor are tightened properly.

Cultivation

Prior to seeding, sample of medium is taken for pH and extinction measurement. About 10 ml of phenolum liquifactum is brought into the last washing bottle. The distilled H_2O in the pO2 electrode is replaced for KH_2CO_3 solution and the electrode is calibrated. The amount of inoculum for a 40 liter culture is about 100 ml of a broken culture. Temperature is controlled at 39°C, the air flow rate is 5 l $min.^{-1}$ and the pH and PO2 are recorded. The seeding of the fermentor is performed through a short inoculation pipe. To this end the rubber tube of the seeding system-mounted on an Erlenmeyer flasks or a bottle with inoculum is connected with the tube on inoculation pipe. The procedure is to be performed under strict asepsis using a gas flame. The cotton wool filter on the inoculum bottle is then connected with compressed air system. By opening the clamp in the seeding outfit the prescribed amount of inoculum is pressed into the fermentor. Directly hereafter, the tube of the seeding system is closed with two clamps next to each other. The rubber is then cut off in between these two clamps. The ends of the tube and the scissors are disinfected subsequently with alcohol

Immediately after seeding the culture conditions such as stirrer speed, air flow rate, pH control are established. A sample is taken from the fermentor after seeding and every 24 hours for extinction and pH determination and microscopic examination. As soon as the culture has started growing, when the pH is rising considerably, the switch governing the automatic stopping of the culture can be actuated. The panel should be programmed to achieve that the heating is switched off at a pH of 8.2. This occurs normally after 50-70 hours. The culture can be processed further as soon as the pH has reached 8.2. The end point of the culture is related to the pH reached during growth. If this pH is reached outside the normal working hours and automatic cooling has been started, the culture is processed directly on the next day.

Usually the bacterial yield is from $60-80^9$ cells/ml. After completion of the cultivation the cells and the culture fluid have to be separated from each other.

The Measurement of Extinction for Determination of the Bacterial Cond:

The more ornament of extinction for determination of the bacterial concentration is done in a special calibrated spectrophotometer.

The culture fluid is diluted before measurement with saline in such a way that the measured extinction is between 0.25 and 0.740, because the interval is considered as the most reliable one for measurement. It is advisable to use standard dilutions of 1:5 and 1:10 etc.

Harvest of crude vaccine

After completion of the cultivation, a sample is taken from the fermentor and smears are made and microscopic examination is done to determine the purity. When found to be free from contamination the cells and the culture liquid have to be separated from each other by means of centrifugation.

Centrifugation

The crude vaccine is dispensed in sterile 500 ml bottles. All bottles are filled to the same total weight, 940 gm, the content of each bottle being approximately 480 ml. Each bottle is covered with sterile screw caps with rubber inlay. The bottles are centrifuge during 60-(90 minutes at maximal speed 2400 RPM in a refrigerated centrifuge.

After centrifugation the supernatant liquid is syphoned off from centrifuged bacterial suspensions and subsequently from 50-60 ml BSS is aseptically added to the bacterial sediment which suspension is thoroughly mechanically shake through a special vibrator. The suspension from each bottle is collected in a 4,4- liter bottle. Samples are taken from each 4-liter bottle for sterility test, the result of which is determined after 14 days incubation. Samples are also drawn for opacity determination. The suspension from each 4-liter bottle is collected to pool bottle, equipped with an air filter, short pipe, two long pipe and a vibro mixer. The suspension is thoroughly homogenized and samples are taken for sterility test.

Opacity determination.

Opacity is determined by dilating the samples with a measured amount of BSS using a burette (25 ml capacity) until the reading is identical with the Fifth International Reference Preparation of Opacity (Plastic Rod) when compared by eye. The opacity of such a suspension is 10 Opacity Units per ml. From this determination one can calculate the bacterial density of the original suspension.

Formula for bacterial counting is as follows:

$$\frac{(10 \quad (V1 + V)2}{Vn}$$ - Opacity units per milliliter

Where

V1 - volume of Original suspension

V2 - volume of BSS added to dilute (V1) to reading equivalent to 10 Opacity Units per milliliter

Inactivation

The "Detox" is suitable and constructed for the detoxification of materials with a maximum volume of 20 liter.

The pooled bacterial suspension is transferred to a special heating bottle which consist of a B-10 bottle equipped with a heating coil, an air filter, a short pipe, a long pipe, a thermocouple and a Vibromixer-stirrer. The "Detox" is connected to a water circulation system and to drain or sink, for the outlet of water.

Connect the assembled "B-10" container with the coil to the "Detox". Connect the Vibromixer on the "B-10" container to a socket of 220 volt. When the vibromixer is switch on, the force of mixing can be regulated with the black knob on the top of the mixer. Connect the "Detox" to a 220 volt socket. Connect the temperature sensor in the "B-10" container to the socket on the Detox. Open the flow valve and the water flow. Select the required temperature on the controller by pressing the center knob and turning the scale. Select the required time on the timer by turning knob.

Switch on the Mains, temperature control and the Timer. The main power is now on the electric system, the recorder and the temperature controller is operating. The recorder will print every 20 seconds while the paper transport is 60 mm/hr. On the timer, select the desired time by turning knob. The clock is now set, and the electric heating element is activated. The water flow passes the heating element and is heated to a temperature of+ 80°C. The heated H_2O circulated through the coil in the "B-10" container and heats the contents of the container. Inactivation time is 10 minutes at 56°C. The returning water enters again the heating element and is used to preheat the incoming water.

As soon as the "on" controller selected temperature is reached, the clock works starts winding off. In the set time period, the temperature controller will maintain the temperature in the container by switching on and off the heating element. After the time period is passed, the heating is automatically switch off and a solenoid valve is opened allowing an extra amount of cold water to pass through the coil and the container is cooled rapidly.

After completion of the heat-inactivation, the suspension is immediately cooled down and merthiolate solution is added at a concentration of 1:5,000 and the suspension is kept at 5°C for 7 days. The sterility test is performed.

Sterility Test. (a) A loopful of vaccine is placed on a BG plate which is then incubated at 35°C for four days and then examined under the microscope.

For the other three tests the volumes inoculated are as follows:

Concentrated vaccine . . . . . 2 ml

Concentrated, blended vaccine . . . . . 2 ml

Final, bulk vaccine . . . . . 10 ml

(b) The sample is inoculated into 300 ml of fluid thioglycollate medium and the container incubated at 30-32°C for 14 days. After three to five days incubation, 1 ml from this container is inoculated into another 300 ml of fluid thioglycollate medium and this container is incubated for a further 14 days. At the time of subculturing, a loopful of material is taken from the original container, Gram-stained, and examined microscopically.

(c) The sample is inoculated into 300 ml of soybean casein-digest medium and the container incubated at 30-32°C for 14 days. After three to five days incubation 1 ml is inoculated into another 300 ml of soybean-casein-digest medium and this container is incubated for further 14 days.

A loopful of material is examined as under (b).

(d) The sample is inoculated into 300 ml of fluid thioglycollate medium and the container incubated at 20-25°C for 14 days. After three to five days incubation 1 ml is incubated into another 300 ml of fluid thioglycollate medium and this container is incubated for a further 14 days. A loopful of material is examined as under (b)

All containers are examined on the third, fourth or fifth, seventh or eight and on the last day.

For the test to be considered satisfactory, there must be no evidence of microbial growth. If any container of plate shows growth, the test is repeated with double the number of containers being inoculated with the volume indicated above. The microscopic examination should show the characteristic staining properties and morphology of B. pertussis.

Viability test. Viability test is performed one week after inactivation. A loopful of crude vaccine is plated on Bordet-Gengou agar, incubated for seven days at 35°C and then examined visually. The vaccine is considered non-viable when no evidence of growth of B. pertussis or any other organism appears on the test medium used.

Pooling of harvest. A pool consisting of the 2 strains of harvest, is done. The 2 strains of varied volumes and bacterial counts are mixed together in a 4-liter bottle. The opacity of the pool is computed based on that of the single harvest. From the pooled bulk a sample is taken for sterility test. When found to be free from contaminants, the vaccine is ready for toxicity, safety, potency and identity test.

Control Tests:

Toxicity Test. Not less than 10 healty white Swiss mice are used, each weighing 14 to 16 grams. They should have access to food and water for not less than two hours before injection.

Each mouse is injected intraperitoneally with half of a single human dose in a volume of 0.5 ml. A control group of 10 mice is injected with an equal volume of 0.9% saline. The total weight of the group of mice is determined before injection, at the end of 72 hours and at the end of seven days after injection. The final bulk is considered satisfactory if at the end of 72 hours the group weight is not less than that at time of injection, and at the end of seven days, the average weight gain per mouse is no less than 60% of that of the control group of mice, in addition no vaccine related deaths should occur.

If any mouse dies the test may be repeated by the aggregate deaths may not exceed 5%.

After passing the toxicity test the bulk is ready for potency test.

Potency test. A satisfactory method of carrying out the assay is as follows:

(a) Mice. Healthy mice, preferably from an inbred strain capable of giving an adequate immune response, are used. They are preferably of the same sex, but if not both sexes are equally distributed thoughout the test and the sexes segregated. The weight of each mouse is at least 10 grams and in a single test the individual weights of the mice are within a 4 - gram range.

A system of randomization of the mice is employed with regard to their distribution into groups, shelf position, order of immunization and order of challenge. For this purpose a table of random numbers or other suitable means may be used.

At least 48 mice are used for each vaccine under test and at least 48 for the standard vaccine. They are distributed into 3 groups each of at least 16 mice for the vaccine under test, and into 3 groups each of at less t 16 mice for the standard vaccine. At least 40 mice are used for control purposes. These are distributed into four groups each of at least 10

mice, one group for the challenge dose and 3 for titrating the challenge suspension in order to determine the ratio of the challenge dose to the LD_{50}.

(b) Immunization of mice. Three 5-fold serial dilutions in physiological saline are made of the final bulk and also of the standard vaccine. The dilutions are chosen in each so as to include the expected 50% effective immunizing dose (ED_{50} or IMD_{50}). Each mouse in each group for immunization is injected intraperitoneally with 0.5 ml of the appropriate dilutions.

(c) The challenge. The interval between immunization and challenge is 14 to 17 days. At least 94 of mice should survive and remain healty during this interval.

The strain used for challenge should be approved by the National Control Authority. To ensure constancy of virulence from test to test, the challenge suspension is prepared from a freeze-dried culture taken from a single lot.

The bacterial suspension for challenge is prepared from a 20-24 hours culture grown on Bordet-Gengou medium or other suitable medium which has been seeded from a rapidly growing culture not more than 30 hours old. The suspension id diluted with a diluent capable of maintaining the viability of the organism. A satisfactory diluent is an aqueous solution containing 1.0% casein peptone and 0.6% sodium chloride adjusted to a pH of 7.1 ± 0.1 the suspension, free from particles of agar or clumps of bacteria is adjusted in such a way that the challenge dose of 0.03 ml is an adequate multiple of the LD_{50} and has an opacity not greater than 0.0001 of an International Unit of Opacity in that volume. (It has been found that a challenge dose of 0.03 ml, approximately 200 times the LD_{50} is satisfactory for the test) this may conveniently be done by first adjusting the suspension to an opacity equivalent to that of the International

Reference preparation and then diluting it accordingly.

For titrating the challenge suspension dilutions of 1/50, 1/250 and 1/1,250 are made.

The immunized mice are challenged by intracerebral injections of the challenge dose. The control mice are injected intracerebrally with the challenge dose and its dilutions. They are injected last. Syringes fitted with needles of nominal external diameter 0.40 mm (27 gauge) are used for the intracerebral injections.

The interval between the preparation of the challenge suspension and the injection of the last mouse dose not exceed 2.5 hours. As a check on the viability of the challenge suspension, a colony count is made after completing the injections by culturing the dilutions used for the control mice. The colony count is usually about one quarter as great as would be expected on the basis of the opacity determination.

(d) Records of results. The mice are observed for 14 days. Mice dying within 3 days are excluded from the test and only those deaths occurring after 3 days are recorded.

(e) Validity of the test. The test is valid if the ED_{50} of each vaccine is between the largest and the smallest immunizing doses, the limits of one standard error of each ED_{50} falls within a range of 64% to 156% the immune response is graded in relation to the immunising doses, and the slopes of the dose response curve of the vaccine under test and the standard vaccine are not significantly different.

(f) Estimate of potency. The ED_{50} of the vaccine under test and the standard vaccine are calculated by a method that provides an estimate of the standard error. The potency is estimated in terms of International Units in the volume which is recommended as a single human dose. The vaccine passes the requirement for potency if the result of the test shows that the best estimate of potency of the vaccine is equal to or more than 4 International

Units in the volume which is recommended as a single human dose.

Innocuity Test. This test is done on final bulk and final lot of pertussis vaccine.

One single human dose but not more than 1.0 ml is injected intraperitoneally into each of five adult mice weighing 17-22 g. and one single human dose, but not more than 5.0 ml, into each of two guinea pigs, weighing 250-350 g.

Vaccine is acceptable if noe of the animals shows signs of ill health in the seven days following the inoculation. If one of the animals dies or shows signs of ill-health during time specified, the test is repeated. The substance passes the test if none of the animals in the second group dies or shew signs of ill-health in the time interval specified.

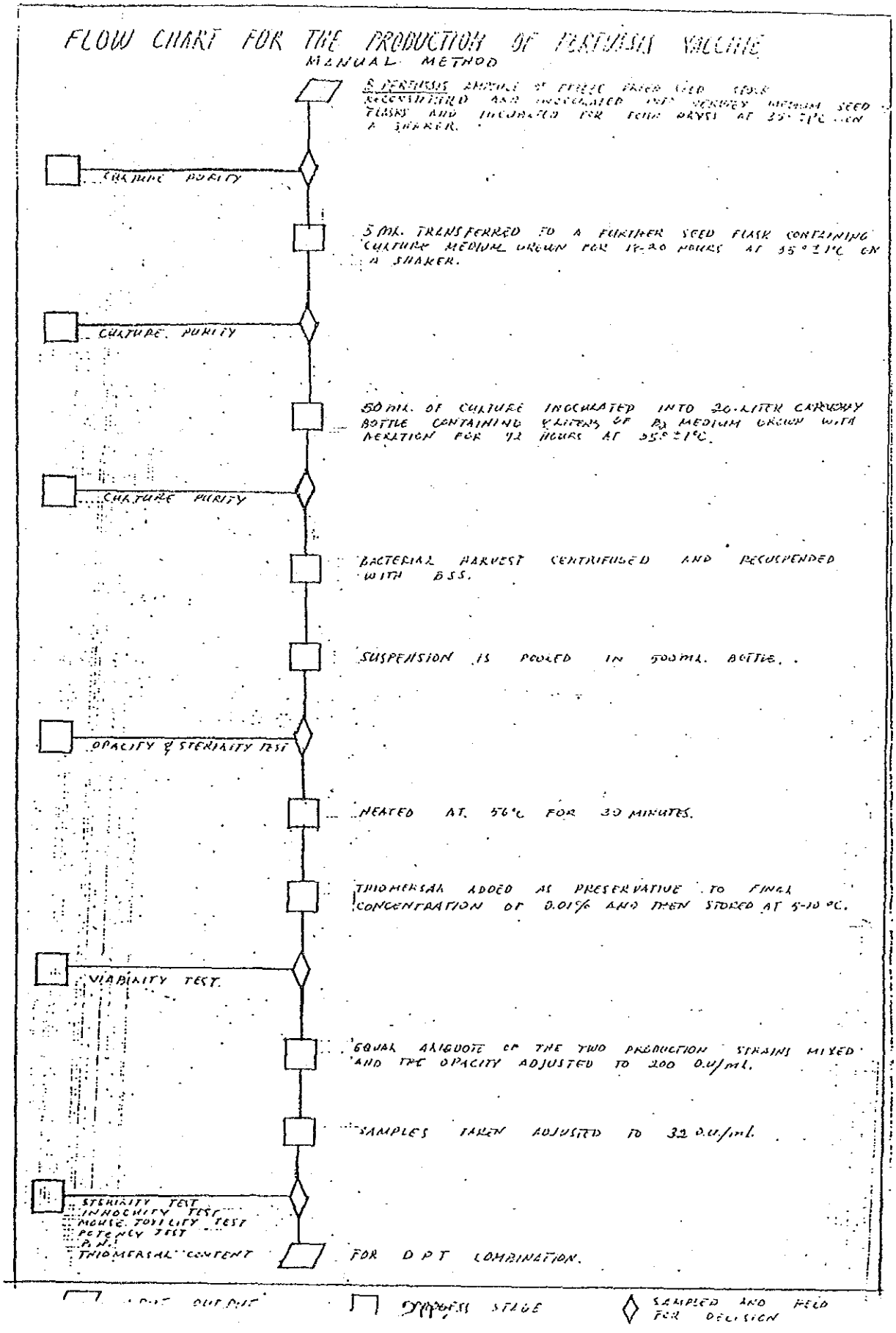
Identity Test. The vaccine is diluted to about 50. O.U. per ml and three loopful are mixed with one loopful of monospecific antiserum on a microscope slide. After mixing, the slide is rocked gently for a few minutes.

Rapid, heavy agglutination should be observed and result is recorded as ± 1,2,3, or 4 plus according to the period after which the serum and vaccine is agglutinated as follows:

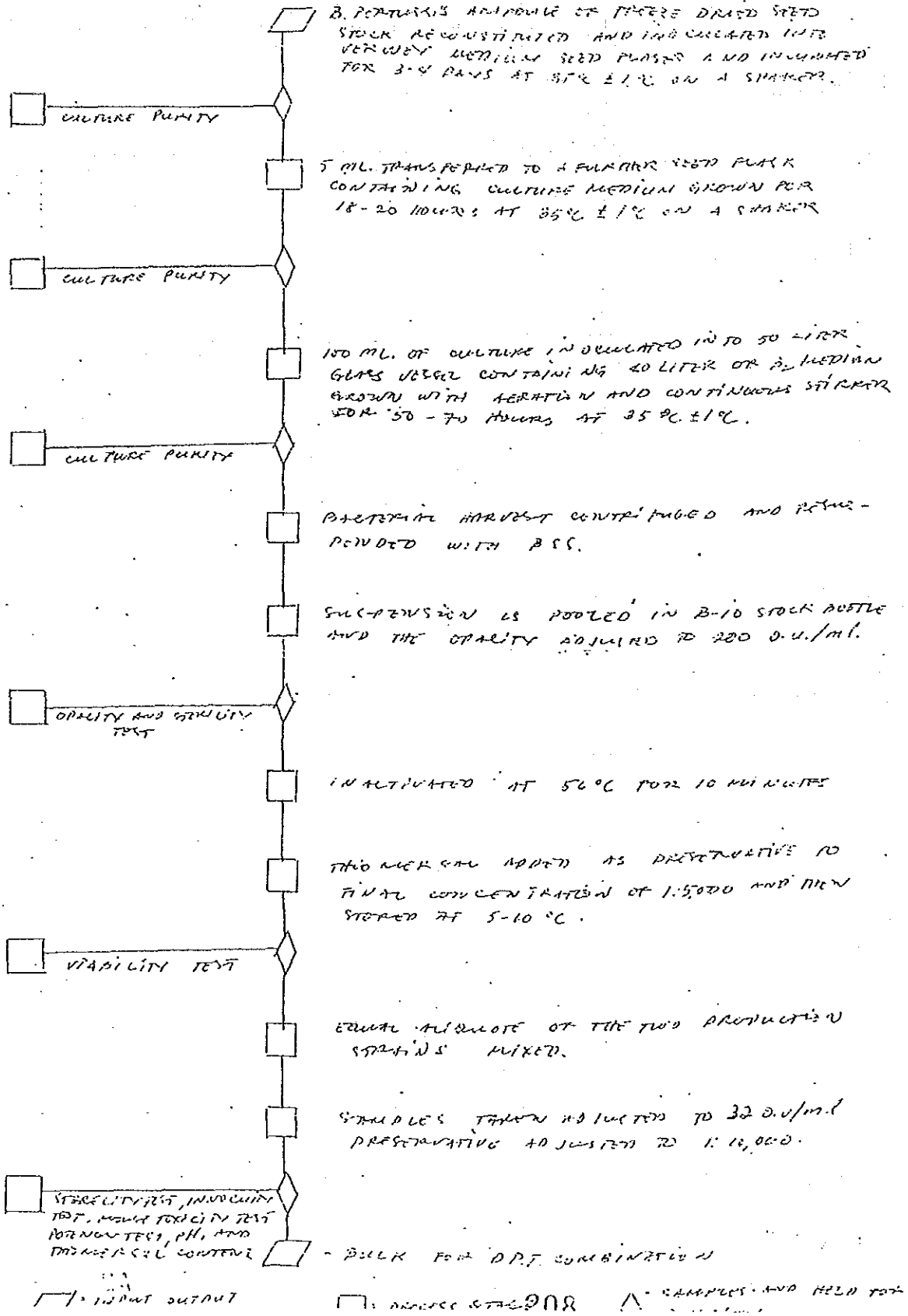
30 seconds - - - - - - - - - - - - 4 ±
60 " - - - - - - - - - - - - 3 ±
90 " - - - - - - - - - - - - 2 ±
120 " - - - - - - - - - - - - 1 ±
120 " - - - - - - - - - - - - Negative

The lowest reading to be considered as end point is ± 4. The control should be negative.

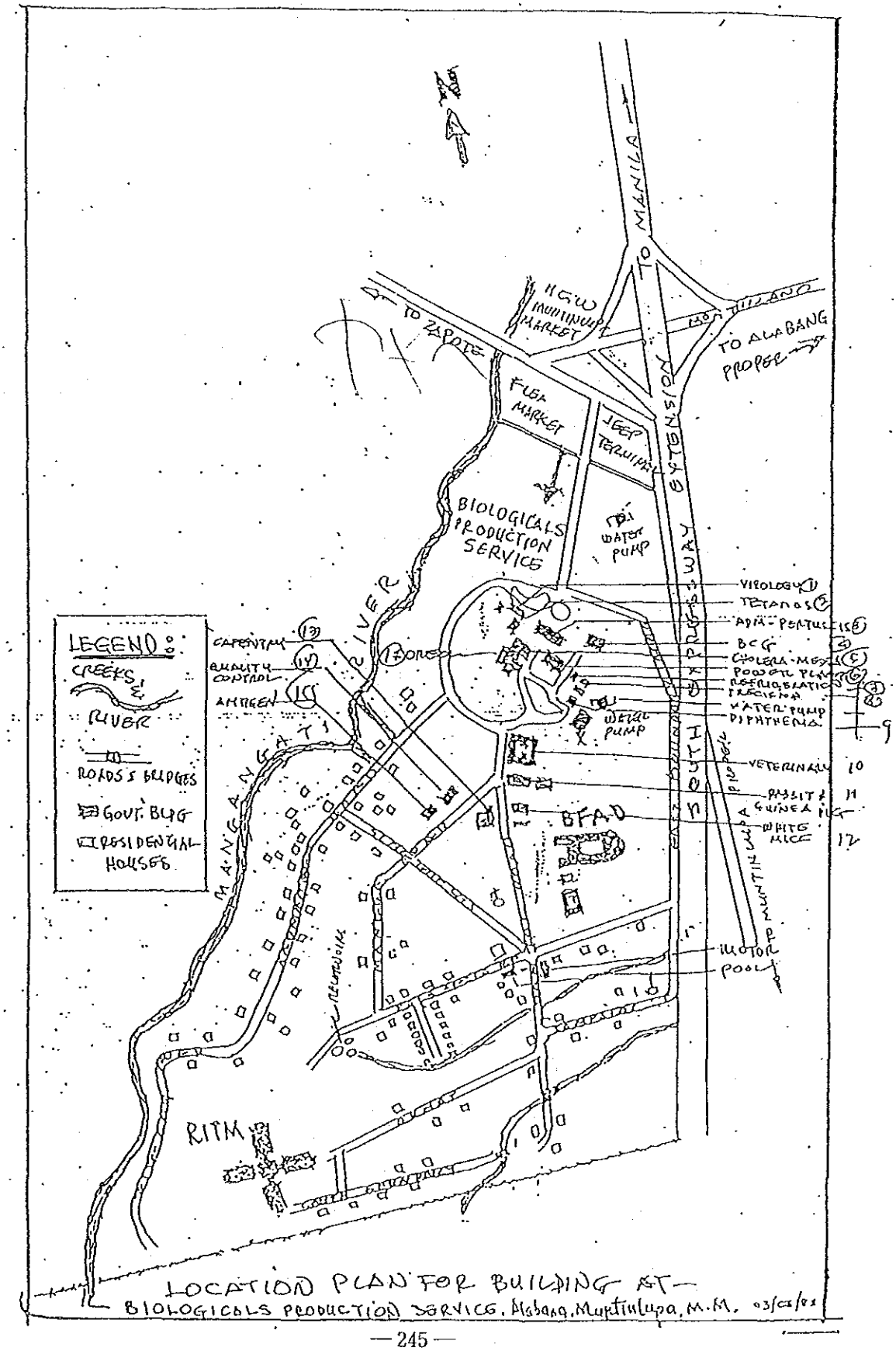
When the results of all tests are satisfactory the bulk is ready for combining with adsorbed diphtheria and tetanus toxoids.


FLOW CHART FOR THE PRODUCTION OF PERTUSSIS VACCINE
MANUAL METHOD
CULTURE PURITY
CULTURE PURITY
CULTURE PURITY
OPACITY & STERILITY TEST
VIABILITY TEST
STERILITY TEST
INNOCUITY TEST
MOUSE TOXICITY TEST
POTENCY TEST
P.H.
THIOMERSAL CONTENT
5 ML. TRANSFERRED TO A FURTHER SEED FLASK CONTAINING CULTURE MEDIUM GROWN FOR 18-20 HOURS AT 35° ± 1°C ON A SHAKER.
50 ML. OF CULTURE INOCULATED INTO 20-LITER CARBOY BOTTLE CONTAINING 9 LITERS OF B2 MEDIUM GROWN WITH AERATION FOR 42 HOURS AT 35° ± 1°C.
BACTERIAL HARVEST CENTRIFUGED AND RECUSPENDED WITH BSS.
SUSPENSION IS POOLED IN 500 ML. BOTTLE.
HEATED AT 56°C FOR 30 MINUTES.
THIOMERSAL ADDED AS PRESERVATIVE TO FINAL CONCENTRATION OF 0.01% AND THEN STORED AT 5-10 °C.
EQUAL ALIQUOTE OF THE TWO PRODUCTION STRAINS MIXED AND THE OPACITY ADJUSTED TO 200 O.U/ML.
SAMPLES TAKEN ADJUSTED TO 32 O.U/ml.
FOR D P T COMBINATION.
SAMPLED AND HELD FOR DECISION

FLOW CHART FOR THE PRODUCTION OF B. PERTUSSIS VACCINE BY FERMENTOR SYSTEM.



20. ワクチン製造所構内図



Republic of the Philippines
Department of Health
BIOLOGICALS PRODUCTION SERVICE
Alabang, Muntinlupa, M.M.

15 June 1989

Mr. Rodolfo B. Villarico
Administrative Officer II
Biologicals Production Service, DOH
Department of Health, Alabang,
Muntinlupa, Metro Manila

S i r :

I am submitting hereunder pertinent information regarding the buildings in the scope of the Biologicals Production Service at Alabang, Muntinlupa, Metro Manila, including the time it was constructed. Those are;(refer to attached schematic as to location of the buildings)

I - VIROLOGY BUILDING

1. One floor construction
2. Floor area 17.06 meters x 37.78 meters
3. Constructed in 1962

II TETANUS LABORATORY

1. Two floor construction(1st floor basement type)
2. floor area- 12.49 meters x 18.59 meters
3. Constructed in 1977

III - ADMINISTRATION-PERTUSSIS BUILDING

1. Three floors
2. 18.60 meters x 41.74 meters floor area
3. Constructed 1939
4. Renovated 1977

IV - B C G BUILDING

1. Three floors
2. 17.98 meters x 24.38 meters floor area
3. Constructed 1950

V - CHOLERA-MEDIA BUILDING

1. One floor
2. Floor area of 24.38 meters x 31.69 meters
3. Constructed 1937
4. Repaired 1964

VI - POWER PLANT

1. One story construction
2. 21.03 meters x 31.69 meters floor area
3. Constructed in 1937

VII - REFRIGERATION SHOP

1. One floor
2. 8.83 meters x 10.65 meters floor area
3. Constructed before World War II

VIII - PRECISION SHOP

1. One floor construction
2. 8.83 meters x 11.27 meters floor area
3. Constructed 1965

IX - DIPHTHERIA TOXOID BUILDING

1. One floor construction
2. 14.93 meters x 21.03 meters
3. Constructed in 1947

X - VETERINARY ,STABLES AND CLINIC

1. One floor - Lateral construction
2. Total area 51.80 meters x 64.02 meters
3. Constructed before World War II

XIa - RABBIT HOUSE

1. One floor
2. 9.44 meters x 27.43 meters floor area
3. Constructed before World War II

XIb - GUINEA PIG HOUSE

1. One floor construction
2. Floor area of 15.84 meters x 24.38 meters
3. Constructed before World War II

XII - WHITE MICE COLONIES

1. One floor construction
2. 14.32 meters x 30.47 meters floor area
3. Constructed 1982

XIII - CARPENTRY SHOP

1. One floor
2. 9.14 meters x 21.33 meters
3. Constructed 1947

XIV - QUALITY CONTROL BUILDING

1. One and one half floor construction
2. 13.10 meters x 30.17 meters total floor area
3. Constructed 19 47 for Small Pox
4. Renovated for Quality control in 1978
5. Mezzanine for animal hose constructed in 1983

XV - ANTIGEN-ANTISERA BUILDING

1. One floor construction
2. 9.14 meters c 43.58 meters floor area
3. Constructed in 1962

XVI - MOTOR POOL AND ANNEXES

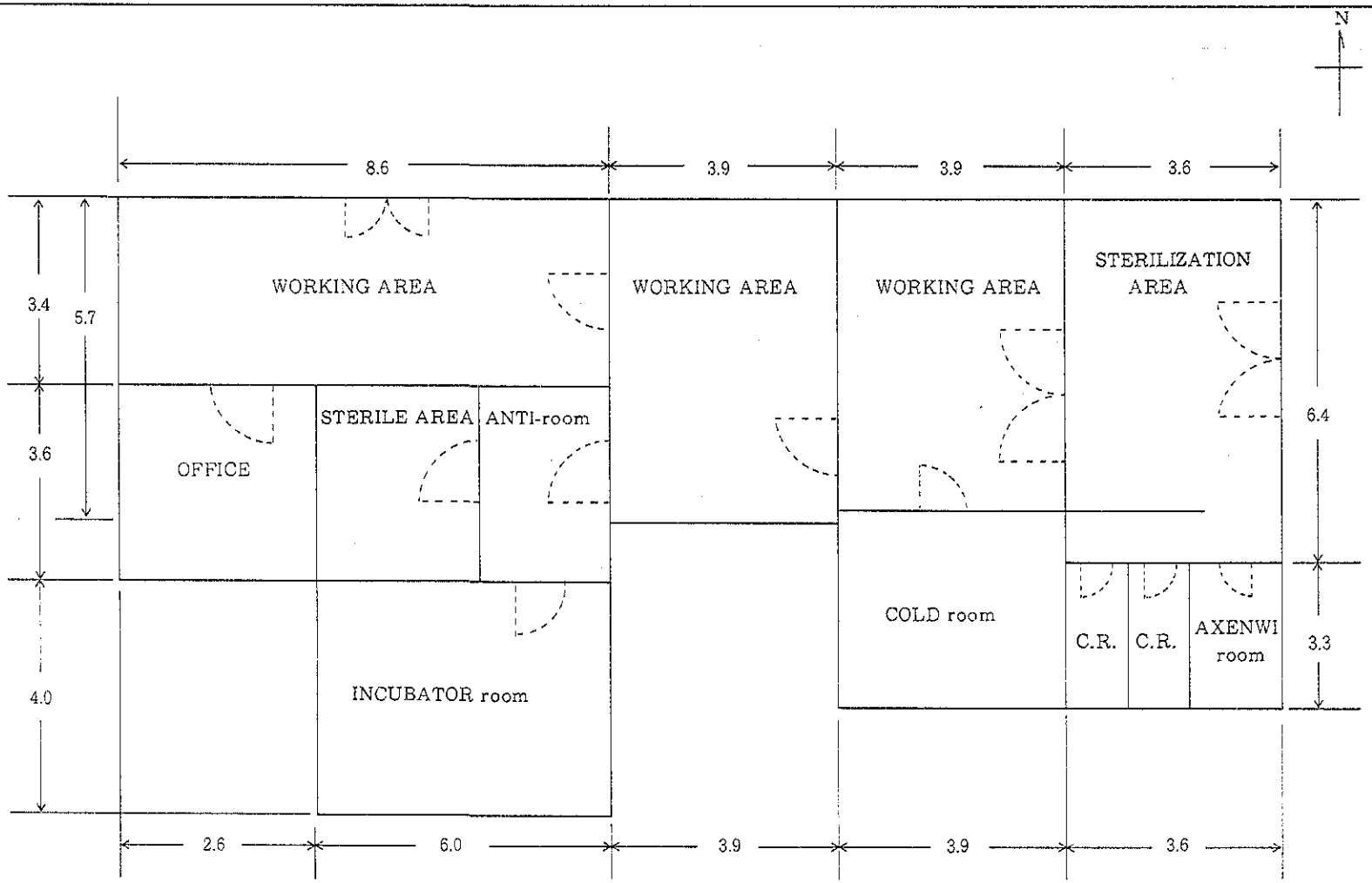
1. One floor(3 sections)
2. a. 8.33 meters x 16.15 meters
   9.44 meters x 15.54 meters
   b. 3.65 meters x 7.31 meters
   c. 19.65 meters x 21.94 meters
3. Constructed as follows;
   a. in 1949
   b. in 1969
   c. in 1971

XVII-ORESOL BUILDING

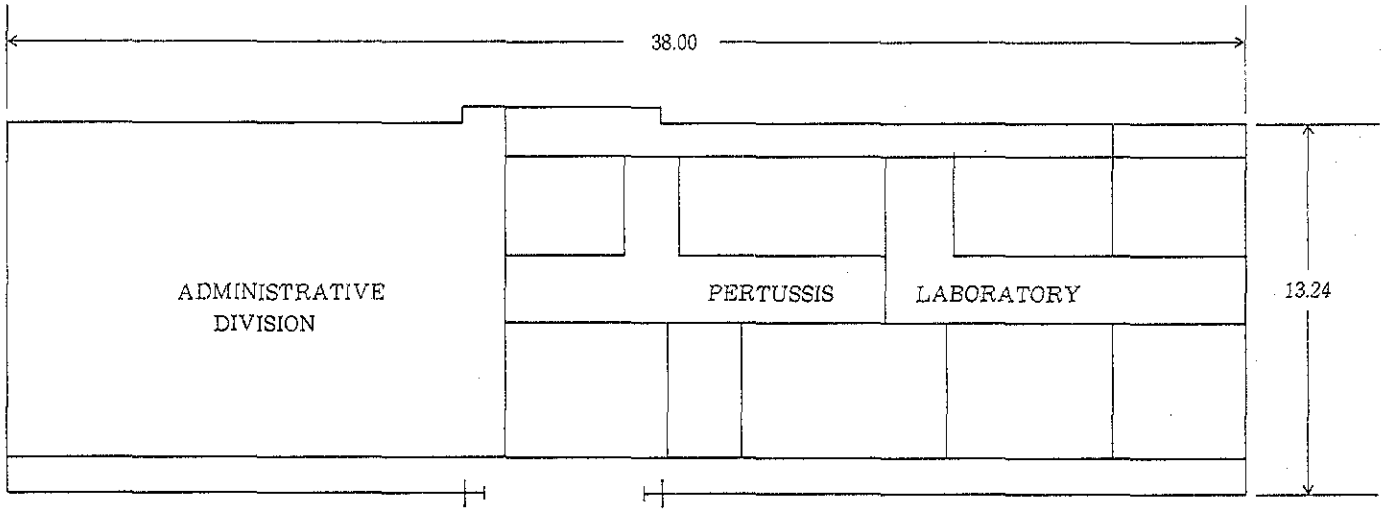
1. One floor constuction
2. 10.00 meters x 13.00 meters floor area
3. Constructed in 1983

SUBMITTED BY:

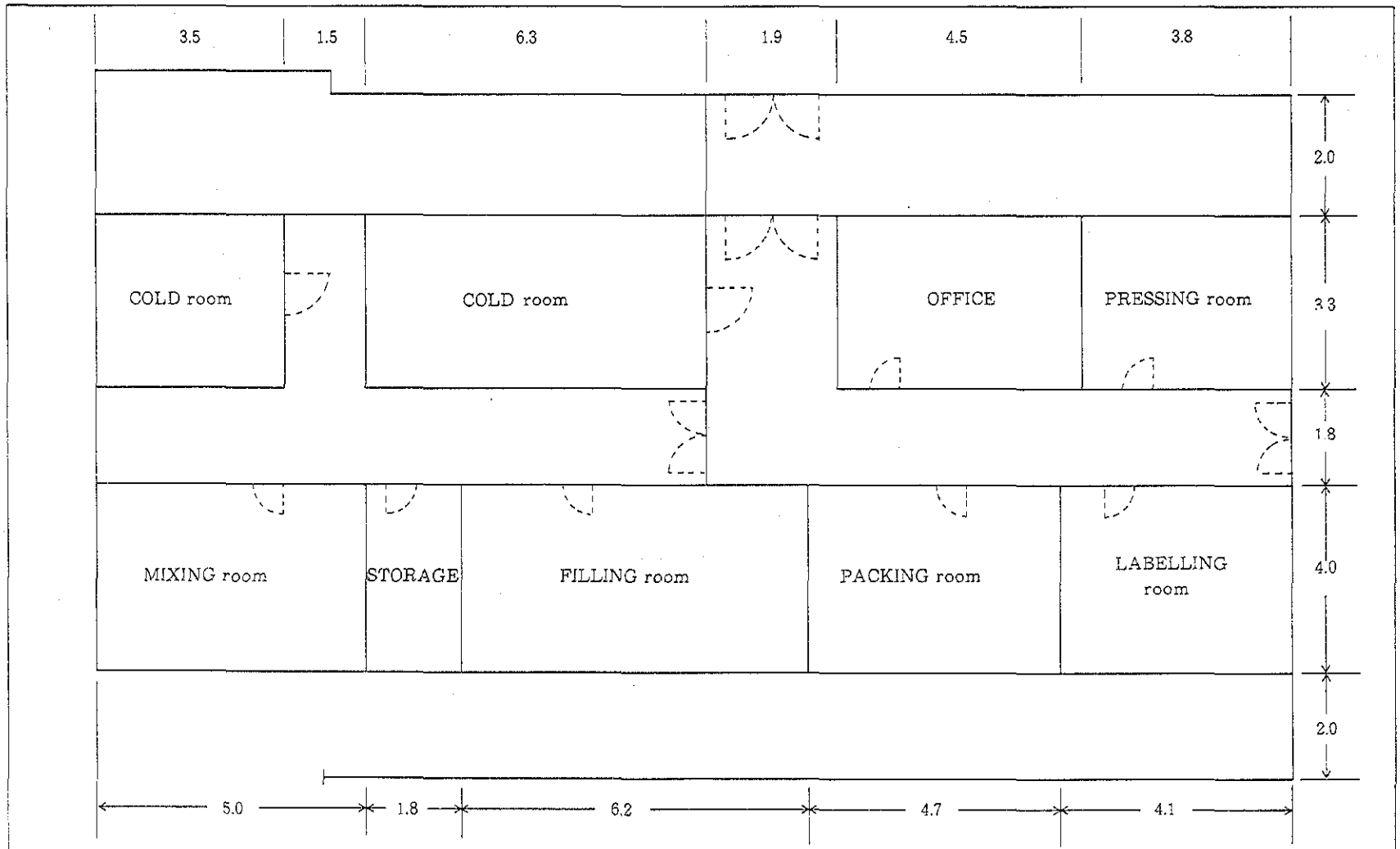
MANUEL A. SAN JUAN
Mechanical Plant Chief



SCALE : 1 : 200
DIPHTHERIA LABORATORY
FLOOR PLAN



PERTUSSIS/ADMINISTRATION BUILDING
GROUND FLOOR PLAN



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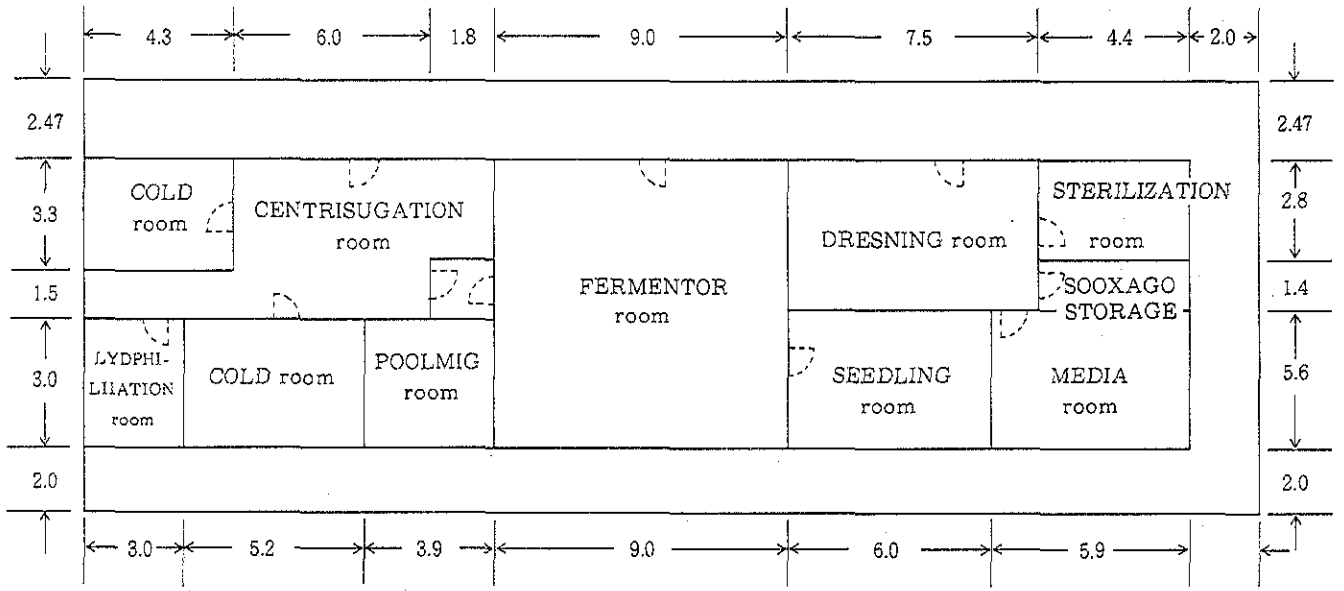
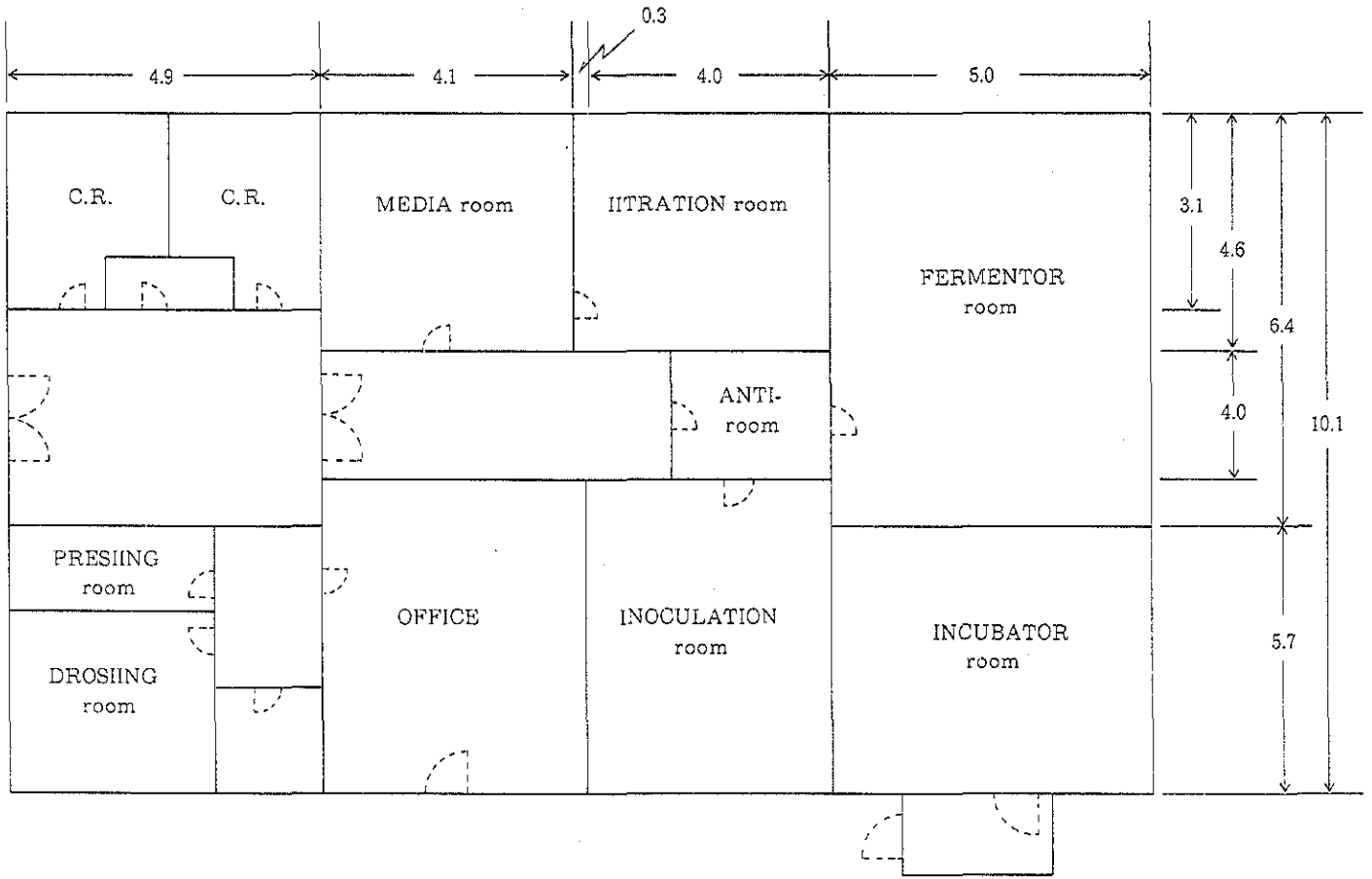
PERTUSSIS LABORATORY
GROUND FLOOR PLAN

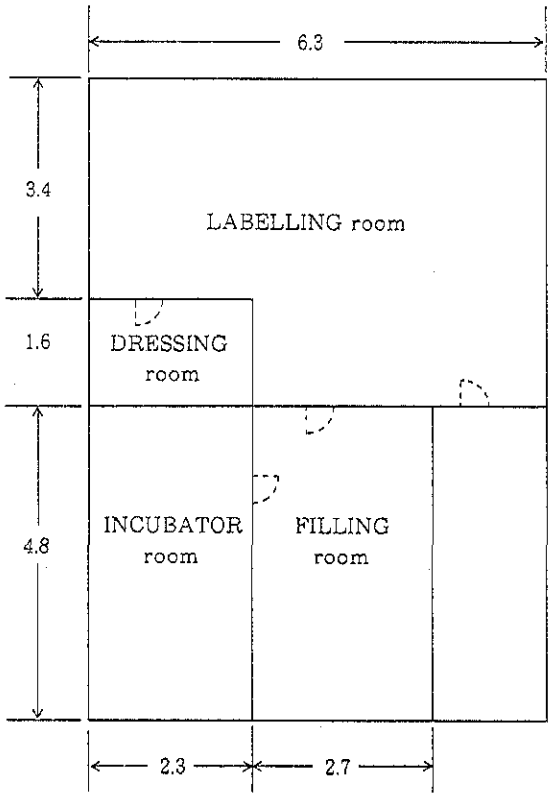


SCALE : 1 : 200

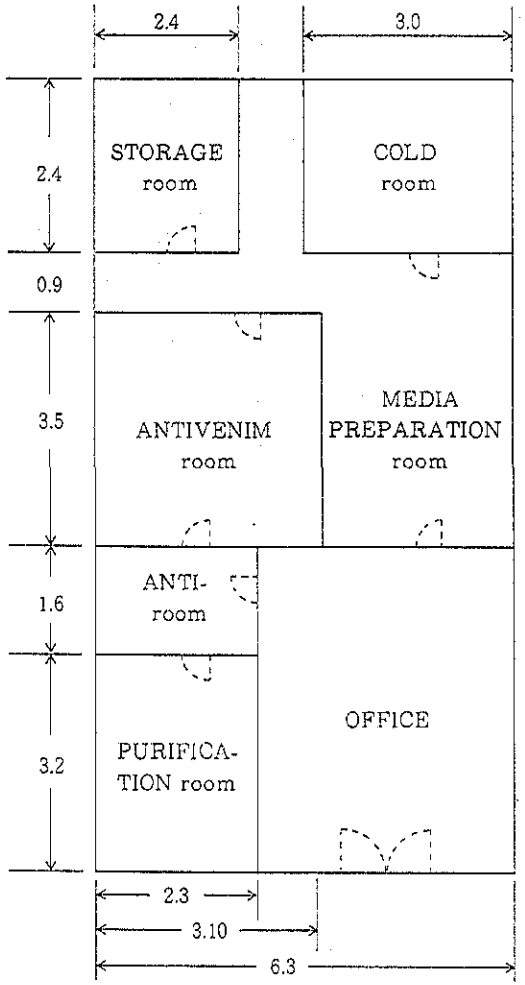
PERTUSSIS LABORATORY
SECOND FLOOR PLAN



TETANUS LABORATORY
FLOOR PLAN



SCALE : 1 : 100
TETANUS LABORATORY
FLOOR PLAN



SCALE : 1 : 100
TETANUS LABORATORY
FLOOR PLAN

WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTE

REGIONAL OFFICE FOR THE WESTERN PACIFIC
BUREAU RÉGIONAL DU PACIFIQUE OCCIDENTAL

(WP)LAB/PHL/DSE/001-E

24 April 1989

ENGLISH ONLY

ASSIGNMENT REPORT

1 February - 3 March 1989

by

Mr Tomo Usmadi[1]
WHO Consultant

Title of project	:	Vaccine quality, safety and efficacy
Location	:	Biological Production Service Alabang, Muntinlupa, Philippines
Participating agencies	:	Biologicals Production Service, Philippines World Health Organization
Subject	:	DPT vaccine production

[1]Head of Department of Assistance Production I, Perum Biofarma, Bandung, Indonesia.

CONTENTS

1. PURPOSE OF VISIT

The terms of reference for the consultant were:

(1) to evaluate the present operational condition of the three fermentation systems for DPT vaccines, installed at the Biologicals Production Service (BPS), Alabang, Muntinlupa, Metro Manila, namely:

2 units - Novo Paljas (diphtheria and pertussis) NW 300, (50) litres capacity;

1 unit - Tetano Paljas (tetanus), (140) litres capacity;

(2) to assess and draw up a list of parts and accessories necessary to ensure continuous operational efficiency of the above systems;

(3) to undertaken a dry-run of the systems with BPS personnel and subsequently trial production by fermentor system;

(4) to assess and make appropriate recommendations on the physical status of support facilities such as electrical, steam, water and compressed air systems, as well as the laboratory system where the fermentors are installed; and

(5) to recommend a suitable training programme for the staff handling the operation, maintenance and repair of the system.

2. OBSERVATIONS AND ACTIVITIES UNDERTAKEN

Observation of the three fermentors

A. Novo Paljas Serial No. 576052/013
For Pertussis

Right fermentor: Temperature sensor and control, pO2 sensor and control were functioning while pH sensor and control should be calibrated.

Left fermentor : Temperature sensor and control, pH sensor and control pO2 sensor and control were all functioning.

Recorder : Right channel for pO2 and pH were out of order and the carbon tape was dry.

B. Novo Paljas Serial No. 379111/041
with Oxytrol for Diphtheria

Right fermentor: Temperature sensor and control and pO2 sensor and control were functioning while pH sensor and control was out of order.

Left fermentor : Temperature sensor and control, pH sensor and control and pO2 sensor and control were all functioning.

Recorder : All channels, right and left, were functioning and carbon tape was dry.

C. Tetano Paljas
Serial no. 578065/033 for Tetanus

Temperature sensor and control and incinerator control and safety were functioning. pH sensor and control and vibro-mixer voltage indicator were out of order.

Recorder: all channels were functioning; carbon tape was dry

Support Facilities:

A. Electric voltage 220V and 3-phases were sufficient

B. 1. Water:
- too much calcium and iron content
- no water treatment was being carried out.

2. Cooling water:
- for Diphtheria and Pertussis the Chiller Hitachi RGU 2003 AYT was in good condition, but the water should be cleaned and changed. The tank was rusty.
- for Tetatnus the Chiller Macartin was still in good condition, but the water should be cleaned and changed. The tank was rusty.

C. Air
- the air compressor is still in good condition; however, they are not using an oil-free compressor. The writer suggests that an oil-free compressor be used for DPT production.

D. Steam:
- the old steam boiler trademark Gabriel was still running, although several flame pipes had been closed due to leakage.
- the steam boiler trademark Oekonom Kessel (Germany) was out of order.

Activities Undertaken

Since many problems were encountered before starting the trial production, it took considerable time to repair the three fermentors and boiler.

1. Pertussis

- by using pH simulator E448, the transmitter of the pH was calibrated.

- the dry carbon tapes were changed for new ones (one set - 6 pieces)

- the burnt-out wire in the recorder for pO2 and pH was substituted with a resistor of 400 okm, 1/2" watt, available in the local market.

- a leak in the right fermentor was sealed by metal epoxy and tested by pressure up to 0.5 bar.

- after all the instruments and controls were tested and restored to good working condition, sterilization of the right and left fermentors was done with distilled water at 110°C, with 0.5 kg/cm pressure.

- trial production with pertussis strain No. 134 produced good results after 52 hours of cultivation, the pH reaching 8.2 and obtaining an extinction of 0.94 (1:5) equivalent to 65 IU/m.l.

2. Tetanus

- one defective transistor in the pH transmitter was substituted with transistor BC 1903 available in the local market and good results were obtained.

- the voltmeter indicator for the vibromizer was repaired.

- during the sterilization, several disturbances had to be overcome, i.e.:

  a. a clogged steam trap under 140 l fermentor

  b. a clogged incinerator caused by sticky-dry medium and rust.

  c. a leak in the rubber diaphragm of the vibromixer shaft seal.

  d. low steam pressure of only 1 kg/cm² caused by too long piping and poor insulation as well as too much load for one steam boiler.

- installation of a new steam pipe and good insulation and reduction of the load to the other departments resulting in sufficient steam pressure (up to 4 kg/cm).

- during the first trial sterilization, the temperature reached 125°C, 1.6 kg cm² could be achieved.

- cooling down from 125°C by recirculating the system through the existing water chiller reached only 46°C and the water tank became hot even when 3 big blocks of ice had been added. The problem was solved by draining the water.

- in the second trial sterilization the pH electrode lost its pressure caused by a too small rubber valve which had to be changed.

- the third trial sterilization was successful.

- trial production was carried out but at 12:00 PM and in the middle of the night the vibromixer and rheostat were short circuited and had to be replaced later with the other vibromixer from the Meta Press and the rheostat from the maintenance department.

- there was also a disturbance in the digital read-out and this had to be changed with a new one.

- trial production then could be continued without disturbing cultivation in the fermentor.

The results of the trial production:

After sterilization of the medium, inoculation was carried out. The cultivation showed a very good growth of Tetanus up to the third day, and the purity was good. But on the fourth day, the culture got contaminated, and therefore had to be discontinued.

The second trial cultivation will be carried out as soon as the materials needed are ready, i.e., seed culture and media.

3. Diphtheria

- One set (6 pieces) of carbon tape was changed with a new one

- A defective pH transmitter was repaired by changing 3 pieces I.C. 741 and 1 piece broken leg transistor BC 1388 available in the local market.

- the pH electrode in the right fermentor showed unstable measurement and cleaning the top of the electrode with NaOH 5N and Hcl 5N did not give good results, so it had to be replaced with a spare one.

- the Novo Paljas fermentor with oxytrol for Diphtheria had been completely calibrated and the sterilization process gave good results. The trial production was carried out at the last due to the medium preparation.

- Rosults of the trial production:

After sterilization of the medium in the fermentor, inoculation was carried out and cultivation is still ongoing.

Other Activities

1. The new steam boiler, Oekonom Kessel, 1500 kg/hr steam capacity had a clogged oil filter which had to be removed; the electrical and electronics circuit had to be repaired, and then calibrated.

This steam boiler was then used in the trial production in the tetano paljas fermentor.

2. A new Amicon Ultra filtration DC 10 LA was installed and a malfunction of the circuit breaker of the power switch was found after changing a resistor for each connection the equipment functioned well.

3. The Bausch and Strobel filling, copping and sealing machine had to be calibrated and the rubber stopper washed in liquid silicon with water which had a good effect on the dispensing process.

4. Two other defective pH transmitters for the Novo Paljas had been repaired for use as spare parts.

5. The defective "Detox" detoxification equipment was repaired since there was a loose wiring connection and it is now in good running condition.

6. Two units of refrigerated centrifuge Heraeus Christ Cauofuge 6-6 were found to have one (1) defective capacitor and two (2) defective overloads and have been repaired.

7. The vibromixer of the Metapress had been adjusted to maximum amplitude but the shaft is too short reaching only up to the middle of the container tank.

A delay of at least three weeks in the start up of vaccine production was encountered due to defects of the fermentor equipment and support facilities. After most of these problems had been resolved, the equipment proved to be operational and essential functions were performed.

During this assignment, for Pertussis, one complete run of the following steps were performed:

- media preparation
- inoculum preparation for seeding and media
- cultivation, i.e. multiplication of the bacteria
- harvesting, i.e. separation of the bacterial mass and cultivation broth
- inactivation of the crude vaccine

Media and inoculum preparation was based on the RIVM (Institute of Public Health and Environmental Hygiene, The Netherlands) method.

Other activities or processes after inactivation could not be carried out due to an already overloaded programme and lack of time.

3. RECOMMENDATIONS

The writer recommends that the following steps be taken:

1. The lack of spare parts and other facilities should be overcome as soon as possible (see Annexes 1, 2 and 3 for lists). Obsolete spare parts from the factory could be a serious problem in maintainng the equipment as it is not sure that all components are available in the local market.

2. Since the pressure drop of steam is mainly due to overloading in other departments of BPS, it is necessary to instal new piping facilities direct from the existing new steam boiler straight to the three fermentors, i.e. 2 Novo Paljas NW 300 and Tetano Paljas.

3. To minimize the percentage of contamination of vaccines produced, the air-conditioning system should be Hepa filtered and a ducting system for aircondition should be installed. Other facilities of sterile rooms should also be provided, such as a Laminaire Flow cabinet.

4. A streamlined transport system should be provided by making the existing lift operational.

5. An autoclave in the DP building should be provided for the sterilization of glassware, media and other materials.

6. To supply the needs of both Diphtheria and Pertussis for distilled water for media and other uses, a distilling apparatus must be installed in the DP building.

7. It is suggested that one small standby generator of about 50KVA should be connected to switch on automatically when electrical failure occurs in the Tetanus, Diphtheria and Pertussis fermentors.

8. As there is a lot of steam vapour during sterilization, there is a need to install an exhaust fan in the fermentor room of DPT.

9. Since the existing and expected problems are both of a technical and technological nature, training of staff abroad in both disciplines should be equally divided, i.e. one each from Diphtheria, Pertussis and Tetanus and two from the maintenance section.

4. ACKNOWLEDGEMENTS

The writer wishes to thank the officials of the Biologicals Production Service, Alabang, especially Dr Bernarto T. Mora, for their courteous and helpful cooperation even during unusual working hours.

ANNEX 1

SPARE PARTS AND FACILITIES NEEDED FOR PERTUSSIS PRODUCTION

A. There is no stock of the following:

1. Air filter for Domnick Hunter
2. Carbon tape for recorder
3. Goose neck for pressure gauge
4. Original silicon tubing (the local silicon tubing is easily broken by heating)
5. O-ring for the fermentor head plate
6. O-ring, nylon tubing and lead anode for PO2 electrode
7. Oxygen, PO2 and temperature transmitter
8. Rubber joint for stirrer shaft (Morse chain)
9. Tube for oxygen probe

B. The following items/spare parts are needed:

1. Breathing filters (big and small)
2. Glass fuses 100 ml
3. Glass fuses 0.8A and 0.6A
4. Membrane for PO2 electrode
5. Nylon and metal clamps #9 and 10
6. pH, PO2 and temperature recorder spare parts
7. Plates for Heraeus Christ centrifuge cryofuge 6-6
8. Rubber lining for centrifuge bottles
9. Sampling glass tube #8 and 9
10. Saturnus ring, close and open size 3/8 and 5/8
11. Seitz filter pads, Steriflo and Clariflo
12. Silicon Paste
13. Silicon Trenn Mittel Spray (for centrifuge)
14. Stainless steel pipe 84001070- 8 x 6
15. Stainless steel pipe 8400189 - 6 x 4
16. Stainless T-pipe, same no. as above
17. St. sila fuse, 110-250V
18. Systeen caps
19. Wash bottle

SPARE PARTS AND FACILITIES NEEDED FOR TETANUS PRODUCTION

1. There is a shortage of silicon tubing, clamps and system caps.
2. A Seitz filter is really essential for filtration.
3. There is a missing part in the Amicon Hollow Fiber Filter, i.e., the connector from the hollow fiber to the container.
4. There are no more spare parts for the following:
   a. Rheostat
   b. Vibromixer
5. There are no more spare parts for the following:
   a. Air filter for Domnick Hunter
   b. Carbon tape for recorder
   c. ph, PO2 and temperature transmitter

SPARE PARTS AND FACILITIES NEEDED FOR DIPHTHERIA PRODUCTION

1. A mixing vessel for medium preparation is essential.
2. A broken rubber connection for stirrer needs immediate replacement.
3. There is a shortage of silicon tubing, clamps and system caps.
4. Peristaltic pumps (2 units) for glucose and NH_4OH are urgently needed for the cultivation process.
5. The shaft of the Vibromixer of the Metapress is too short, reaching only up to the middle of the container tank.
6. No spare parts for the following are available:
   a. Air filter for Domnick Hunter
   b. Carbon tape for the recorder
   c. pH, PO2 and temperature transmitter
   d. Goose neck for pressure gauge
   e. Lead anode and nylon tubing for PO2 electrode
   f. Sampling glass tube
   g. pH electrode and PO2 electrode (spare parts)

DISTRIBUTION

GOVERNMENT

The Secretary of Health
Department of Health
San Lazaro Compound
Sta. Cruz
Manila

The Secretary of Foreign Affairs
Department of Foreign Affairs
PICC Building, CCP Complex
Roxas Boulevard
Manila

The Director-General
National Economic
and Development Authority
Amber Avenue
Pasig
Metro Manila

UNDP

The Resident Representative (2)
of the United Nations
Development Programme
in the Philippines
P.O. Box 1864
Manila

WHO

Regional Director
DPM
DET
DHP
DPC
DPP
CHR
CRP
HIN
RAs
APR (2)
MDO
PIO
PIU
REG
WR, Manila
REG/HQ (6)

OTHER

Mr Tomo Usmadi
Head
Department of Assistance
Production I
Perum Biofarma
Bandung
Indonesia

22. 品質管理に関する調査結果

団員　岩佐三郎

有効性，安全性において共にある水準を下回らず，且つそのばらつきが少ない製品を恒常的に製造することは，生物製剤の生産に課せられた課題である。生物製剤の定義は国によって異なるが，それはさておき，少なくともワクチン等の生物製剤は，その製造原料となるものが恒常性を保ち難い生物由来であり，またその有効成分は多くは不安定な高分子物質から成るのが特徴である。そしてその有効性，安全性の測定には，これまた生物を使って行わねばならない。そこに生物製剤特有の製造技術と品質管理技術とが車の両輪の如く必要となる。以上のような観点から本ワクチン製造プロジェクト事前調査を主として品質管理技術の面から報告する。

1．品質管理部の現状

1)．業務

当部には，通常の品質試験としての力価試験，毒性試験，無菌試験，化学分析試験，BCG菌数試験を担当するセクションに加え，当部のみならず製造所全体が使用する実験動物（マウス，モルモット，ウサギ，ヤギ，ウマ，コブラ）の繁殖飼育を担当するセクションが含まれる。品質管理のための各試験は小分け製品のみならず，一部の試験管内測定を除きバルク段階でのそれらの試験をも行っている。従って試験検体数は多くならざるを得ない。前年度の検体数は 1436 検体で，試験回数にして11,430に及ぶ。勿論この回数には再試験等が含まれており，また必ずしも総て動物試験が課せられた検体とは限らない。また検体には輸入製品が含まれていることは言うまでもない。実験動物の生産頭数は，前年度で，マウス26,360匹，モルモット3,660 匹，ウサギ103 羽と言う。

2)．人員

以上の業務を総員39名で行っているが，現場を日時をかけて細部にわたって検討しなければわからないが，常識的にはかなりの人員不足の感じが免れ得ない。

2．品質管理セクションの問題点

1)　一般試験室

深井報告に指摘されているように，一般品質試験室として化学分析室，無菌試験室があり，加えてBCG 菌数試験室があるが，いずれも狭隘であり空調設備がない。しかし，将来

はともかくとしても，現状の検体数をこなすことは可能かと思われる。ただし行われている試験そのものの質的問題となれば更に現場に立ち入って検討されねば分からない。

2) 動物試験室

モルモット試験室，マウス試験室ともに空調設備は無い。モルモットは見た限りでは良好な印象を受けたが，マウスは痩せている感じを受けた。モルモット試験室には若干のウサギも飼育されていたが，これらはパイロジェン試験に使用されるとのことであった。マウスを使った力価試験（百ワク，破傷風トキソイド，狂犬病ワクチン）の若干の生データを見たが，何となく全体的に用量反応の回帰が小さい印象を受けた。中には用量反応が認め難いものもあった。これが何に起因するのか直ちには分からないが，使用マウスにそれが求められるかもしれない（例えば抗体産生が悪いとか）。とするならば，この問題は，この技協の開始にあたって緊急に解決されねばならないものである（後述）。またこのような成績が出ることに担当責任者が疑問をもたないのは，バイオアッセーについての基本的理解が浅いことによると考えられる。この種のことはマウス体重測定にバネ式2kg 台秤で計っていることにも見受けられる。モルモットを使用する試験としてジフテリアトキソイドの力価試験成績（毒素攻撃法）を見たが，これはまづまづの成績ではないかと考えられる。

3. 動物生産セクションの問題点

1) 小動物繁殖室

繁殖マウスの系統はウエブスターホワイト系であると言う。母子ともに約3000匹が飼育されている。木造建物で開放系であり，感染病の流行が心配されるが，飼育経験 5年の担当者によればそのようなことがなかったと言う。先にも触れたようにこれらのマウスについてはその品質について検討の必要がある。モルモットの毛色は茶，黒，白のブチで土間での青草による群飼育である。モルモット繁殖は比較的難しいと言われているが，自然環境が適しているのか良好のように見えた。しかしこれはマウス，モルモットに通ずることであるが，各個体の生後の週令がどれだけ把握されているかは現場に立ち入って検討されねばならない。ウサギはケージ飼育で一見下痢症状も見当たらず良好のように見えた。ウサギの多くは診断用血清の製造に使われるとの事であった。ウサギ舎の拡張はまだ完成されていなかった。

4. 全体的提言

開発途上国への技術移転に常に付きまとう問題として，いつもそうだとは限らないとは

思うが，その技術を支えているもろもろの科学的基盤と知識をいかに身につけてもらうかが，その技術が定着するかどうかの決め手となると思われる。BPS にも過去WHO その他からの機器供与あるいは研修による製造技術と品質管理技術の訓練があったようだが，必ずしも定着しているとは見受けられない。生物製剤の品質管理の理論と技術は，WHO によれば微生物学，生化学，薬理学，病理学，獣医学等〃の他にバイオアッセーという統計学にわたる一つの体系を形作っていると考えられている。これはどこかの教育機関で教わることの出来るものではないし，また一朝一夕で身につくものではない。以上の観点から以下の提言を述べる。

1) 製造部門と品質管理部門との関係

現在，BPS では製造部門と品質管理部門とは通常考えられているようには仕事上必ずしも明確に区分されてはいない。つまり日本の製造業者では通常常識的には製造担当者が行っているであろうと考えられるものをも含めてバルク段階での諸試験をも後者が行っている。これは将来においては整理されるべきではあろうが，現状のもとでは（人員，試験室の余裕等）止むを得ないのかもしれない。一方その利点としては品質管理担当者が製造担当者と密になり製造の実際について知識を得ることを通じて品質管理の理論と技術を深めるのに役立つであろう。なお品質管理部門の役割は，何もバルク，出来上がり製品の品質試験のみに限定されるものではなく広く製造過程に立ち入って如何に品質にばらつきの少ない製品を製造していくかを品質管理面としてのアドバイスをしていくと言うことが期待されるところである。このような側面があると言う教育も必要であろう。

2) 国の品質管理機関

いわゆる国立品質管理機関を設けるかどうかという問題である。これは将来的には必要になるかと思われるが（例えば国立のBPS をも含めて民間の複数の製造業者が出現するとか），これは現時点では次の理由で賛成しかねる。国立管理機関として具体的には BFAD が考えられるが，この機関のスタッフはワクチン等の生物製剤の品質管理の経験も製造の実際の知識ももちあわせていないと考えられる。一般医薬品の品質管理とは，考え方，手法等の点で全く異なる面が生物製剤の品質管理には多い。結果として，いたづらに権限を与えられた知識と実力の乏しい集団による国家検定あるいは規制は，無用な混乱を製造現場に招く恐れがある。現状では BPSでの出来上がり製品での試験をこれに当てることが現実的であろうと考えられる。フィリッピン国におけるいわゆるnational authorityが誰かを聞き漏らしたが，それにたいするアドバイザーとしての機能をBPS に委託する形で済むこ

とではないかと考える。ただし将来的にはこれらの品質管理にたづさわるBPS 職員の一部をBFADに出向させることで解決されるかもしれない。以上の考えはフィリッピン国の薬事行政上の体系には馴染まない考えかもしれないが。

3) 品質試験

先にも触れたように，今BPS で行われている個々の品質試験の質的問題はもっと立ち入って検討しなければならないが，試験動物，特にマウスの質の問題は早急に解決されねばならない。長期的には別に考えなければならないが，当面この技協で使用するマウスについては,BFAD で軌道にのっているSPF マウスの利用が考えられる。但しこれも試験に使用可能なstrain(株名については，聞き漏らした) であるかどうか検討が必要であろう。現状のマウスについては，取り敢えず定期的な抗体調査によるチェックをアドバイスした。

BPS における各種品質試験は，当然ながらWHO のそれに準じている。日本の品質試験の多くは，長年の試験研究によって前者に比してより優れた方法をとっているものが多い。当然ながら技協においてはこれらの技術をも導入することになるが，この点の理解を十分にしてもらうことが大切である。

4) 一般的事項

今のBPS には製造と品質管理全般にわたってアドバイス出来る専門家はいない。また個々の部門の担当責任者は,50 才前後の女性であり，中堅どころのスタッフが余り見当たらないようである。技術移転の成否は，如何にこれら中堅職員に人を得るかが一つの重要な要素であると考えられる。品質管理の向上には，試験研究活動が欠かせないものであるが，これら中堅職員の専門家としての自立，向上のためにもそれは必要なものである。また研究意欲の向上のためにも必要最低限の専門図書，雑誌が備わることが望ましい。

23. 施設建設計画（深井試案より）

付　録：　　以下に添付した図はＫｉｎｔａｎａｒ同博士の述べた要請の骨子を私なりに理解し、もしそれに従って施設を建設するならばこのような形になるであろうとの具体案の原型であり、参考までに添付したものである。

Ｆｉｇ．１　現在使用中の建物配置図.

Ｆｉｇ．２　比国側資料（資料１．）による将来建物配置図.

Ｆｉｇ．３　改築建物配置図（深井試案）.

Ｆｉｇ．４　Ｂ型肝炎ワクチン製造棟.

Ｆｉｇ．５　百日咳ワクチン・ヂフテリアトキソイド製造棟.

Ｆｉｇ．６　破傷風トキソイド製造棟.

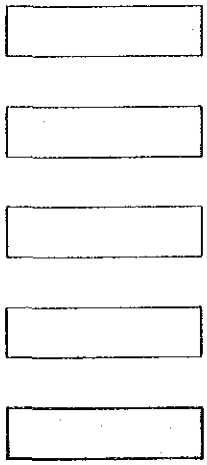
Ｆｉｇ．７　中央製剤棟（分注、凍結乾燥、包装など）.
分注は、バルクの輸入の製品化に備えて
液状製剤のみの分注ラインと
凍結乾燥の可能な分注ラインとの
２系列を考えている。

Ｆｉｇ．８　中央洗浄・滅菌棟.

Ｆｉｇ．９　品質管理棟

（既存建物の内 Administration-Pertussis Bldg., BCG Bldg. は改装して使うことが出来ると見受けられるが、その可否は建築専門家による診断の上判断するべきであろう。）

Legends:



Temperature Controlled

Temperature, Humidity Controlled

NASA Class 100,000 T.H Controlled

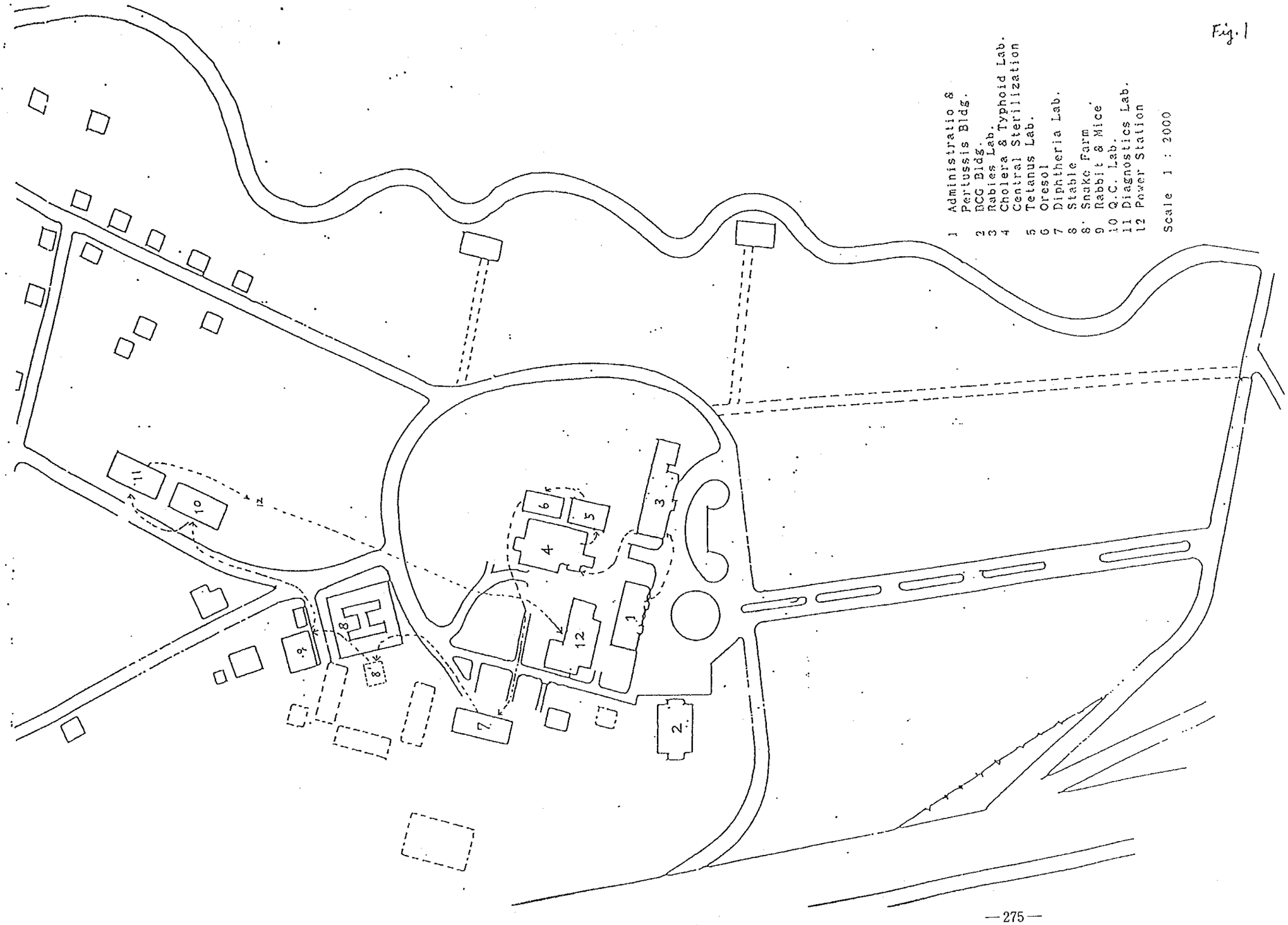
〃 Class 10,000 〃

differential pressure 2 mm Aq.

〃 Class 100

D R	Clothes Changing Booth
A S	Air Shower
D R	Dry Heat Sterilizing Oven
A C	Autoclave (double doored)
P R	Pass Room
Washing	Secondary Washing Room
Preparing	= kitchen; Preparing for sterilization
Seed culture	Preparing Seed culture for fermenter
Detox.	Detoxification (Toxoidization)

Fig. 1


1 Administratio &
Pertussis Bldg.
2 BCG Bldg.
3 Rabies Lab.
4 Cholera & Typhoid Lab.
Central Sterilization
5 Tetanus Lab.
6 Oresol
7 Diphtheria Lab.
8 Stable
8' Snake Farm
9 Rabbit & Mice
10 Q.C. Lab.
11 Diagnostics Lab.
12 Power Station
Scale 1 : 2000

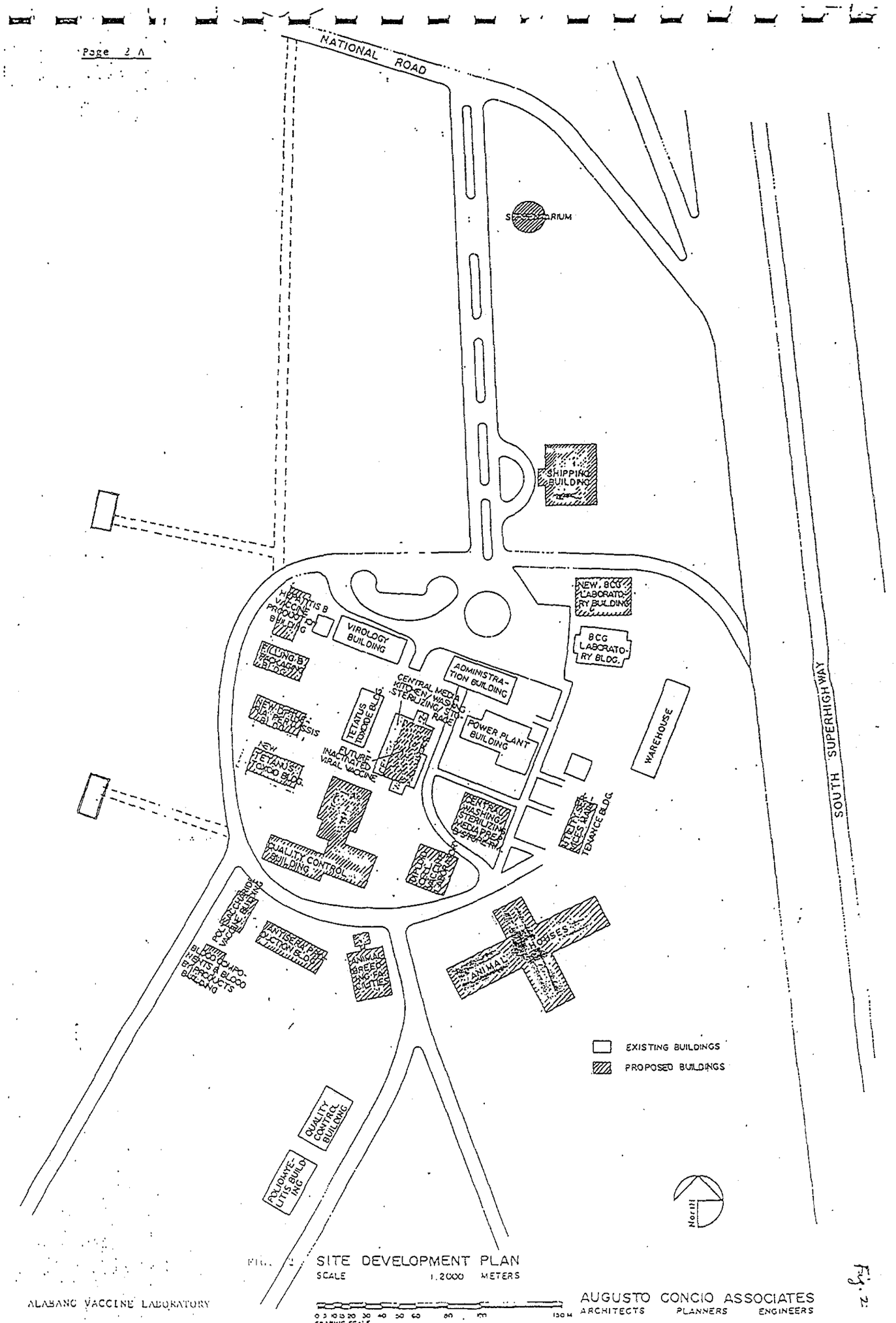


FIG. 2 SITE DEVELOPMENT PLAN

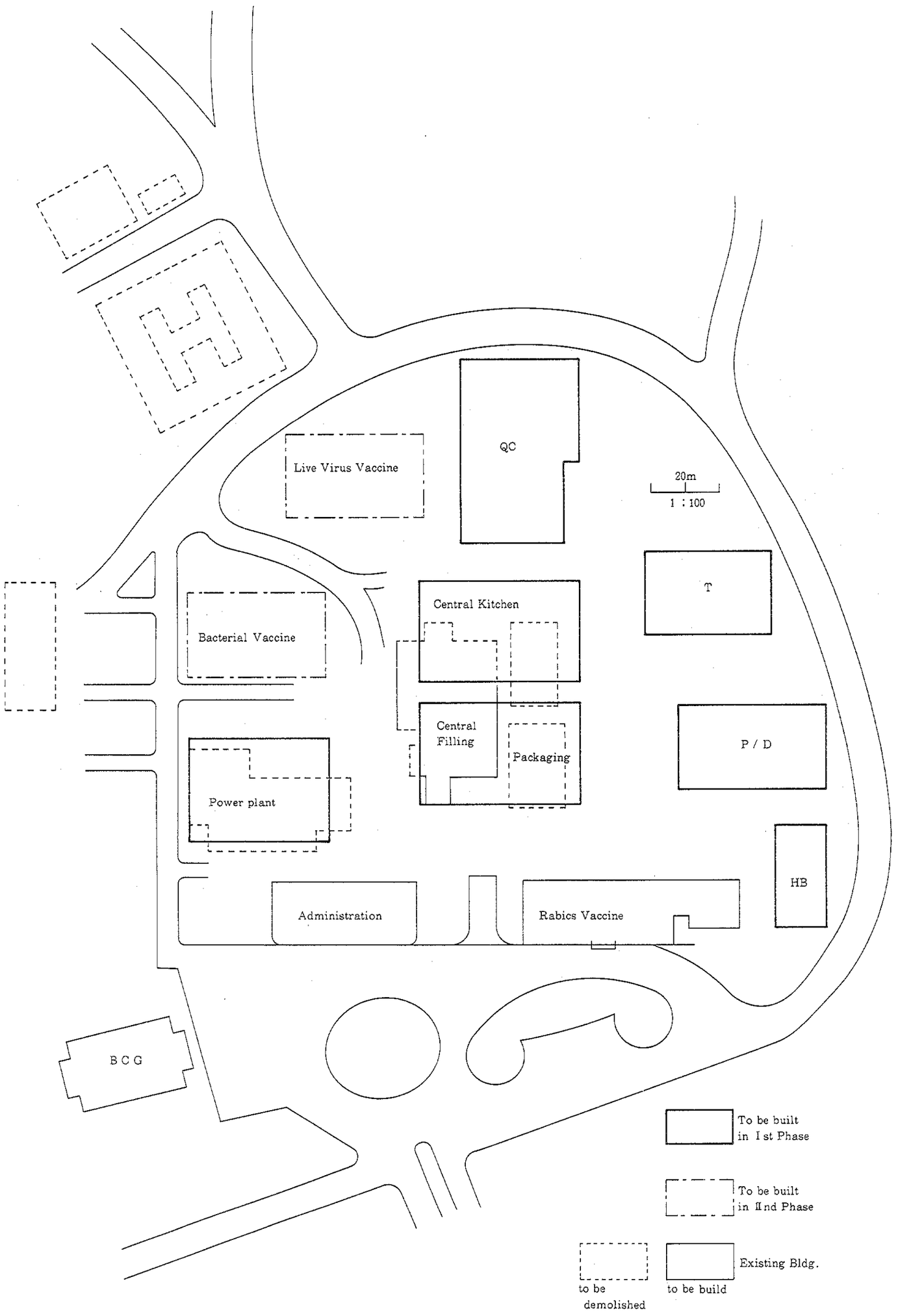
SCALE 1:2000 METERS

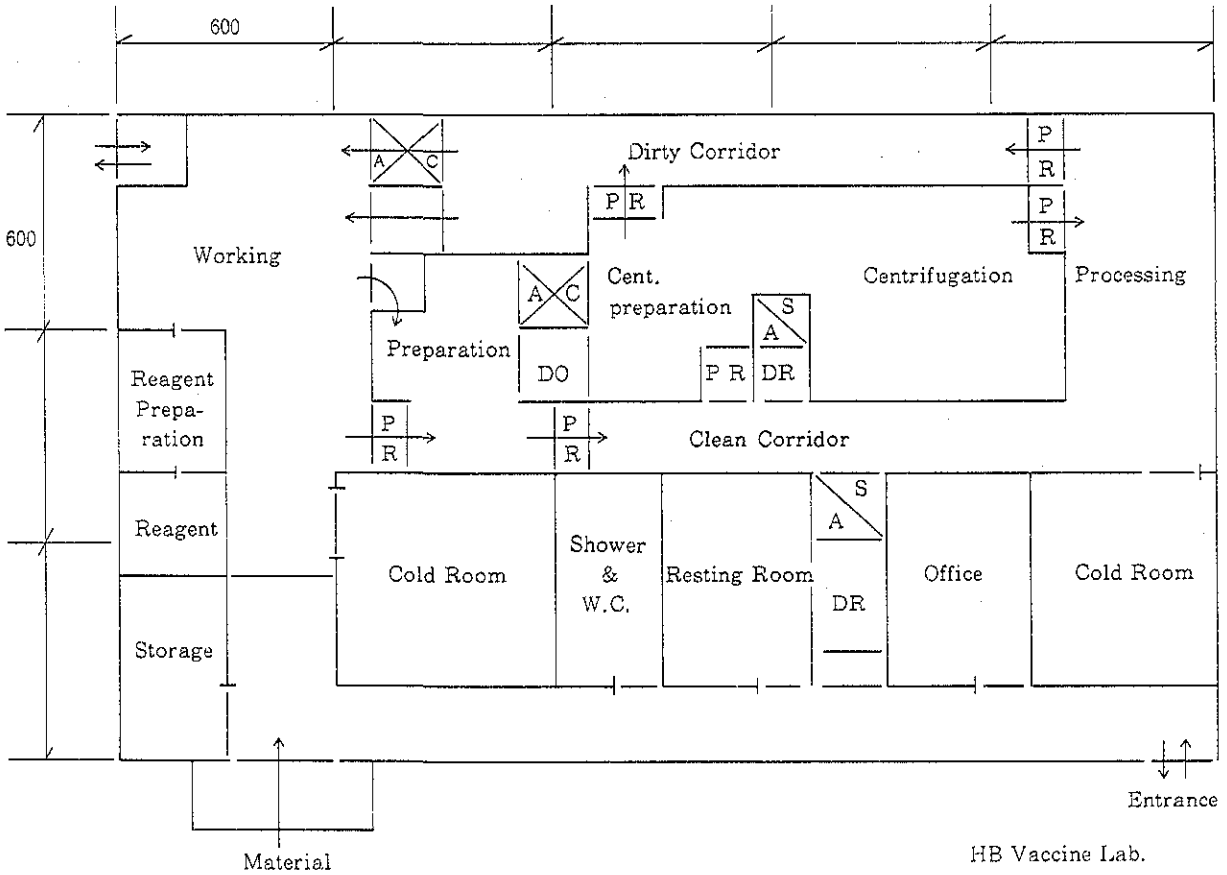
GRAPHIC SCALE 0 5 10 15 20 30 40 50 60 80 100 130 M

ALABANG VACCINE LABORATORY

AUGUSTO CONCIO ASSOCIATES
ARCHITECTS PLANNERS ENGINEERS

Fig. 2


Live Virus Vaccine
QC
20m
1 : 100
T
Central Kitchen
Bacterial Vaccine
Central
Filling
Packaging
P / D
Power plant
HB
Administration
Rabics Vaccine
BCG
To be built
in I st Phase
To be built
in IInd Phase
Existing Bldg.
to be
demolished
to be build

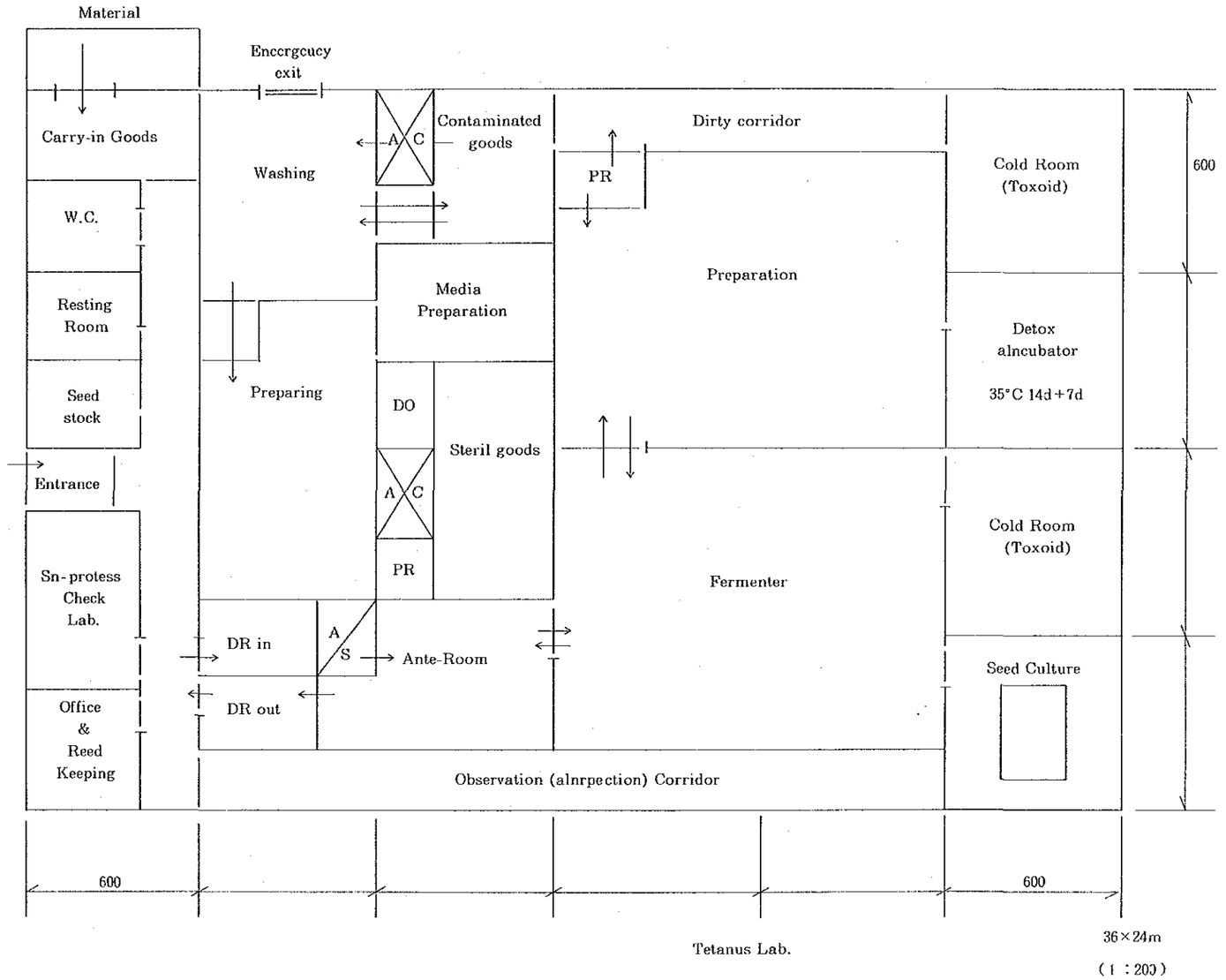


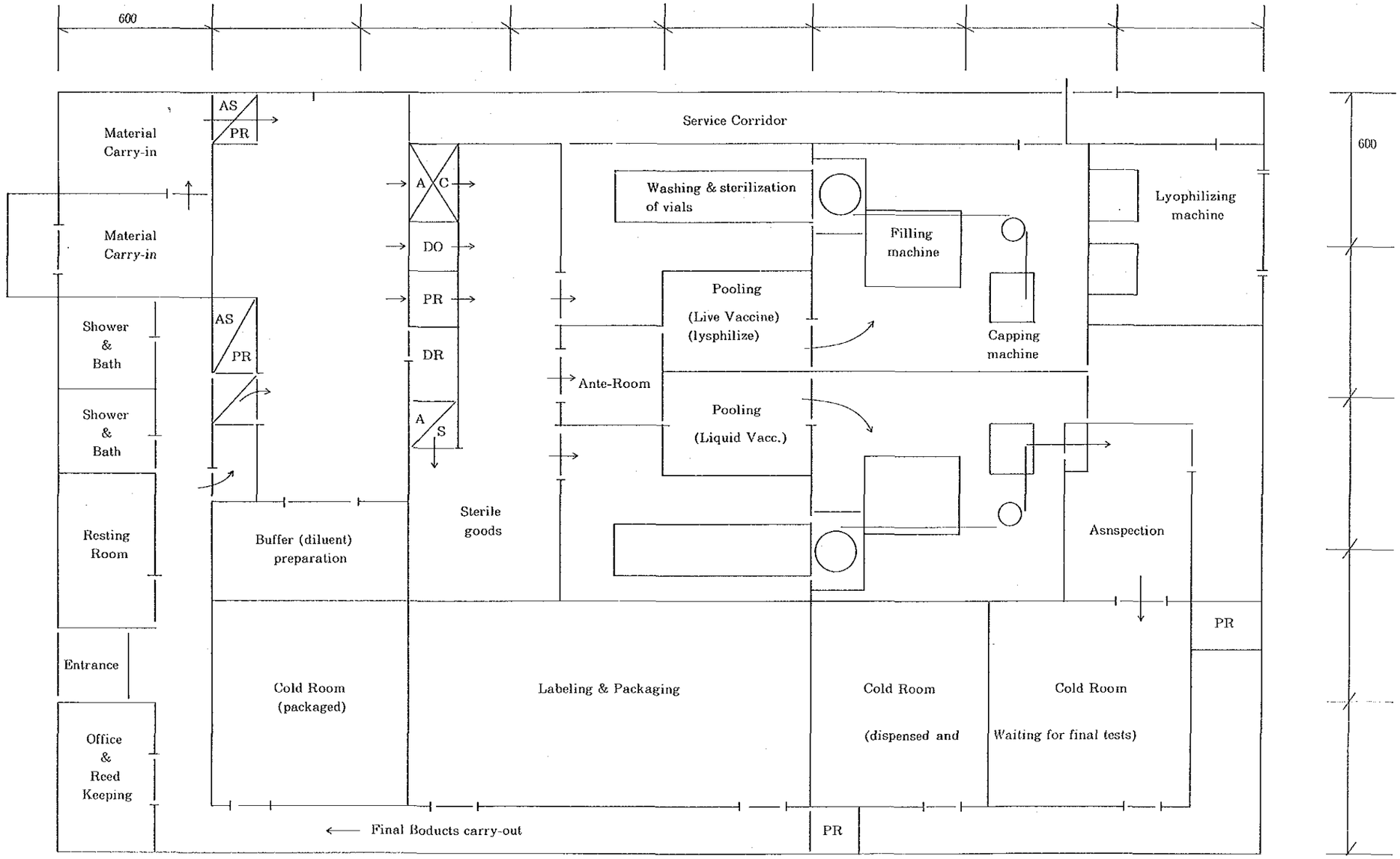
HB Vaccine Lab.

30×15m

200

890715

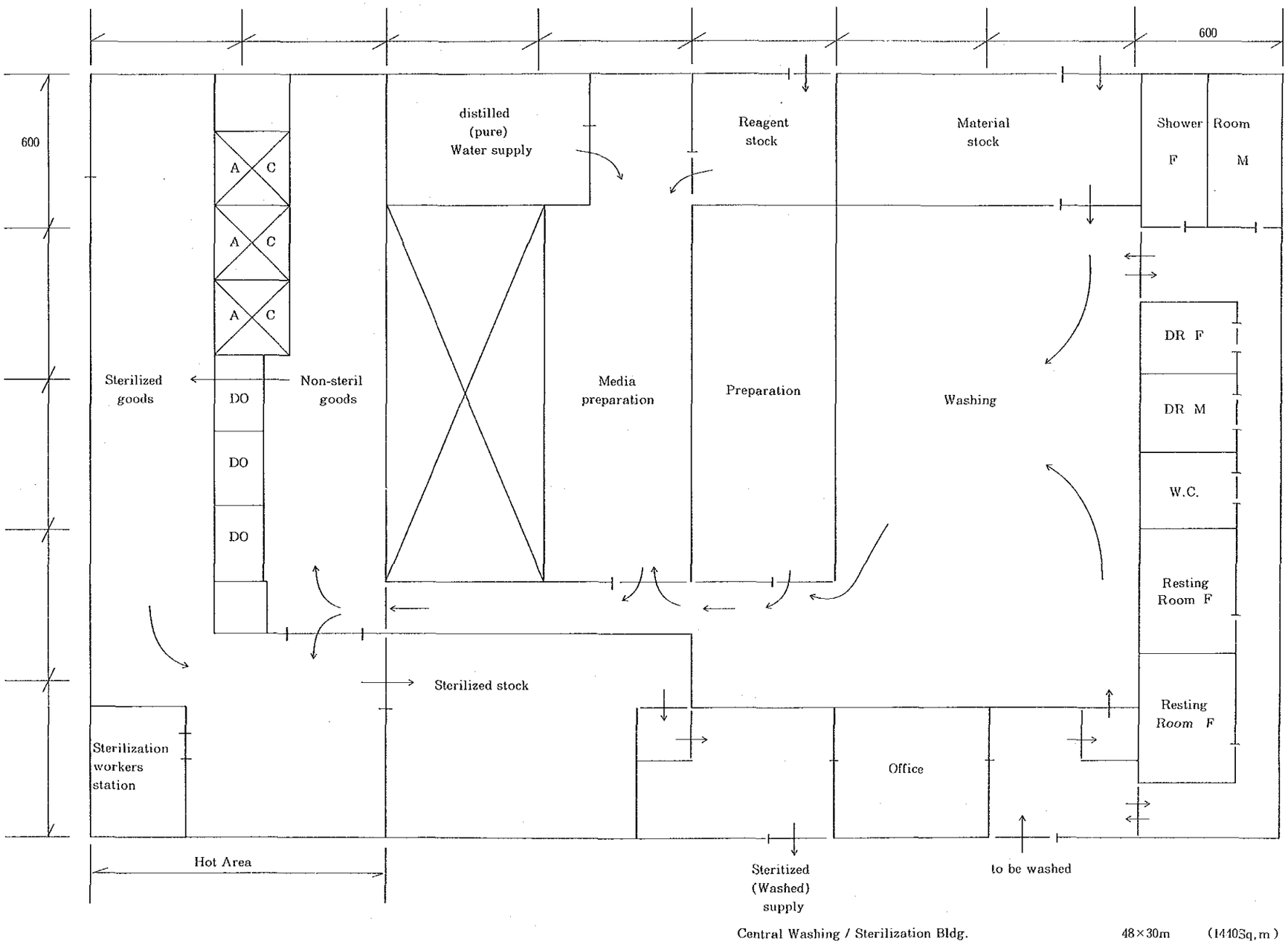

Material
Enccrgcucy exit
Carry-in Goods
Washing
Contaminated goods
A C
Dirty corridor
PR
Cold Room (Toxoid)
600
W.C.
Resting Room
Seed stock
Media Preparation
Preparation
Detox alncubator
35°C 14d+7d
Preparing
DO
Steril goods
Entrance
A C
Cold Room (Toxoid)
Sn-protess Check Lab.
PR
Fermenter
DR in
A S
Ante-Room
Seed Culture
DR out
Office & Reed Keeping
Observation (alnrpection) Corridor
600
600
Tetanus Lab.
36×24m
(1 : 200)



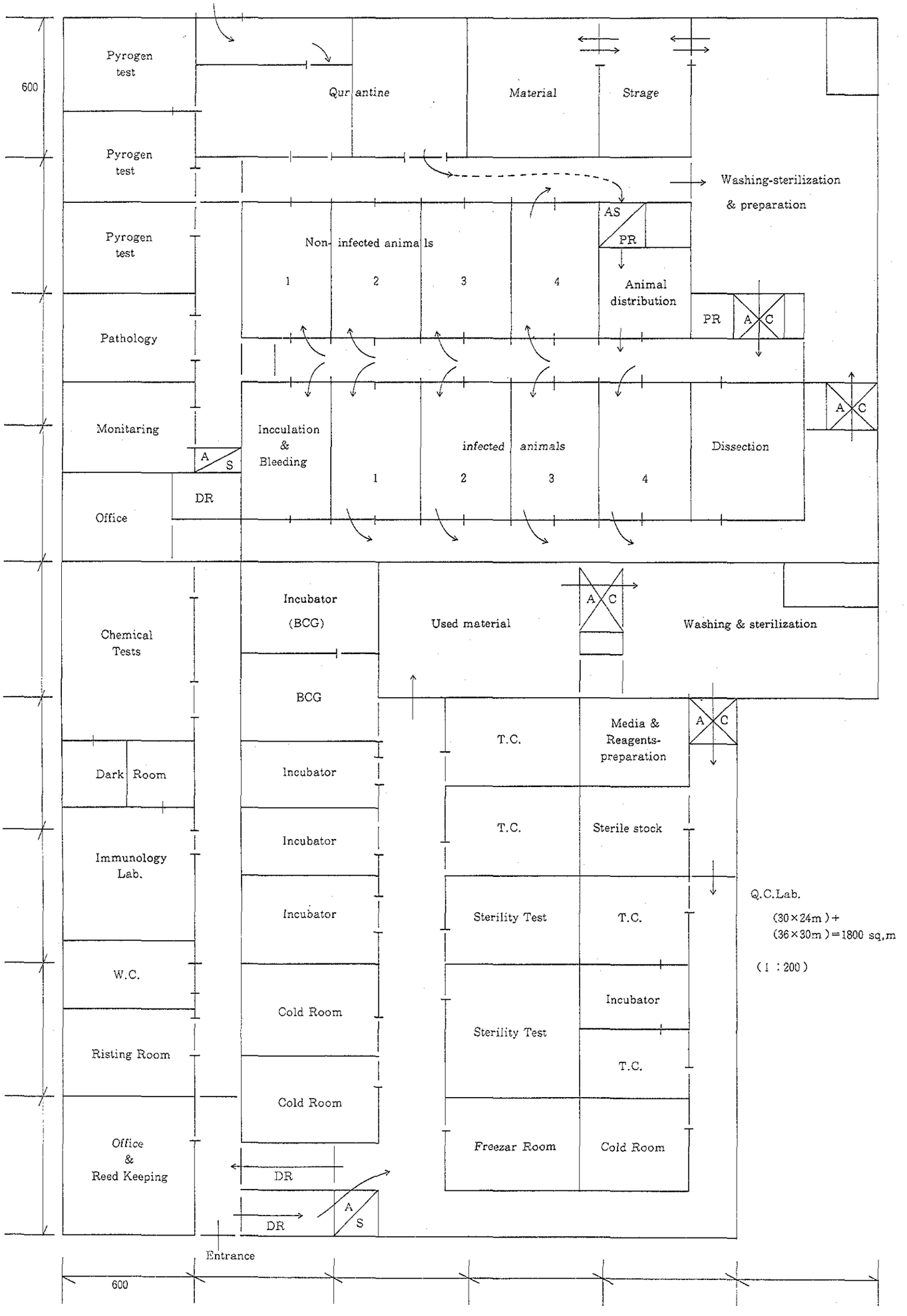
Pooling, Filling, Packaging & Final Products Storage

48×30m

(1 : 200)



Central Washing / Sterilization Bldg. 48×30m (1440Sq,m)


600
Pyrogen test
Pyrogen test
Pyrogen test
Pathology
Monitaring
Office
Qur antine
Material
Strage
Washing-sterilization
& preparation
AS
PR
Non- infected animals
1
2
3
4
Animal distribution
PR
A
C
Inoculation
&
Bleeding
A
S
DR
infected animals
1
2
3
4
Dissection
A
C
Chemical Tests
Dark Room
Immunology Lab.
W.C.
Risting Room
Office
&
Reed Keeping
Incubator
(BCG)
BCG
Incubator
Incubator
Incubator
Cold Room
Cold Room
DR
DR
A
S
Entrance
Used material
A
C
Washing & sterilization
T.C.
Media &
Reagents-
preparation
A
C
T.C.
Sterile stock
Sterility Test
T.C.
Sterility Test
Incubator
T.C.
Freezar Room
Cold Room
Q.C.Lab.
(30×24m)+
(36×30m)=1800 sq.m
(1 : 200)
600

24. フィリピンワクチン製造プロジェクト事前調査団ミニッツ

MINUTES OF DISCUSSIONS
BETWEEN THE JAPANESE PRELIMINARY SURVEY TEAM AND
THE AUTHORITIES CONCERNED OF THE GOVERNMENT OF
THE REPUBLIC OF THE PHILIPPINES ON THE VACCINE PRODUCTION PROJECT

The Japanese Preliminary Survey Team (hereinafter referred to as the "Team") organized by the Japan International Cooperation Agency (hereinafter referred to as "JICA") and headed by Dr. Toshihiko Hasegawa visited the Republic of the Philippines from March 4th to 10th, 1990 for the purpose of making the study on the request of the Technical cooperation for the Vaccine Production and Quality Control Project (hereinafter referred to as "the Project).

During its stay in the Republic of Philippines, the Team exchanged views and had a series of discussions with the Philippines authorities concerned.

As a result of the study and the discussions, the Team and the Philippines authorities concerned came to the tentative understanding of the matters referred to in the document attached hereto.

長谷川敏彦

Dr. Toshihiko Hasegawa
Leader,
Japanese Preliminary Survey Team,
Japan International Cooperation
Agency,
Japan

Dr. Bernardo T. Mora, Jr.
Chief,
Biologicals Production Service
Department of Health
The Republic of the Philippines

ATTACHMENT:

1. The Team confirmed the content, the background and the historical significance of the proposal to Japanese government for the Technical Assistance for the "Upgrading of Vaccine Production and Quality Control activities of the Biologicals Production Service (hereinafter referred to as "BPS") after having discussed with the Philippine Authorities concerned and having visited to survey the activity of BPS.

2. The Team understood that the proposal is aimed to help BPS attained its development plan and is geared toward making BPS as the important strategic focal point being the supplier and distributor of vaccines for the Expanded Program of Immunization. The Team also realized that the Philippine government is committed to the EPI Program of the DOH with the Institution of the National Drug Policy in 1987 in which one of the four pillars is to attain self-sufficiency in Pharmaceutical and Biological Products.

3. Any cooperation that may be agreed upon between Japanese and the Philippine government, the Philippine Authority concerned has expressed the importance of GMP in accordance with the WHO requirements and standard.

4. Both the Team and the Philippine Authority at BPS agreed the urgent need and highest priority in upgrading the vaccine production of DPT and Tetanus Toxoid and Quality Control activity among the items of the development plan of BPS, considering the background of the proposal and situation of Philippines at present time. In addition, the Philippine Authority at BPS has expressed the urgency of strengthening the BCG production due to increasing demands.

5. The Team explained the alternative methods of producing Diphtheria and Tetanus vaccine beside methods using fermentors which can provide enough vaccines of good quality to meet the needs of the entire Philippine population and Philippine side understood the explanation.

6. Both the Team and the Philippine Authority concerned had agreed the importance of the support of good infrastructural functions such as pure water supply, steam supply, etc. and good functioning animal house for the success in upgrading the production of vaccine and quality control activities.

7. Both the Team and the Philippine Authority concerned agreed the importance of commitment and support of Philippine government for the success in upgrading the production and quality control of vaccines at BPS.