

SCHEDULE F
(See rule 78 and Part X)

¹[***]

²[PART XII B

**REQUIREMENTS FOR THE FUNCTIONING AND OPERATION OF A
BLOOD BANK AND / OR FOR PREPARATION OF BLOOD COMPONENTS.**

I. BLOOD BANKS / BLOOD COMPONENTS

A. GENERAL

1. *Location and Surroundings* : The blood bank shall be located at a place which shall be away from open sewage, drain, public lavatory or similar unhygienic surroundings.
2. *Building* : The building (s) used for operation of a blood bank and/or preparation of blood components shall be constructed in such a manner so as to permit the operation of the blood bank and preparation of blood components under hygienic conditions and shall avoid the entry of insects, rodents and flies. It shall be well lighted, ventilated and screened (mesh), wherever necessary. The walls and floors of the rooms, where collection of blood or preparation of blood components or blood products is carried out shall be smooth, washable and capable of being kept clean. Drains shall be of adequate size and where connected directly to a sewer, shall be equipped with traps to prevent back siphonage.
3. *Health, clothing and sanitation of staff*: The employees shall be free from contagious or infectious diseases. They shall be provided with clean overalls, head-gears, foot-wears and gloves, wherever required. There shall be adequate, clean and convenient hand washing and toilet facilities.

B. ACCOMMODATION FOR A BLOOD BANK.

A blood bank shall have an area of 100 square meters for its operations and an additional area of 50 square meters for preparation of blood components. It shall be consisting of a room each for –

- (1) registration and medical examination with adequate furniture and facilities for registration and selection of donors;
- (2) blood collection (air-conditioned);

1. Part I to Part XIIA omitted by G.S.R. 663 (E), dated 03-07-1992 corrected vide corrigendum G.S.R. 27(E) dated 22-01-1993.

2. Part XIIB and Part XIIC, were sub. By G.S.R. 245 (E) dated 05-04-1999, previously Part XIIB and XIIC, were substituted for Part XIIB by G.S.R. 28(E) dated 22-01-1992 and before that Part XIIB was added by notification number F 1-17/67-D, 24-06-1967.

- (3) blood component preparation. (This shall be air-conditioned to maintain temperature between 20 degree centigrade to 25 degree centigrade);
- (4) laboratory for blood group serology (air-conditioned);
- (5) laboratory for blood transmissible diseases like Hepatitis, Syphilis, Malaria, HIV-antibodies (air-conditioned);
- (6) sterilization-cum-washing;
- (7) refreshment-cum-rest room (air-conditioned);
- (8) store-cum-records.

NOTES :

(1) The above requirements as to accommodation and area may be relaxed, in respect of testing laboratories and sterilization-cum-washing room, for reasons to be recorded in writing by the Licensing Authority and/or the Central Licence Approving Authority, in respect of blood banks operating in hospitals, provided the hospital concerned has a pathological laboratory and a sterilization-cum-washing room common with other departments in the said hospital.

(2) Refreshments to the donor after phlebotomy shall be served so that he is kept under observation in the blood bank.

C PERSONNEL

Every blood bank shall have following categories of whole time competent technical staff:-

- (a) Medical Officer, possessing the qualifications specified in condition (i) of rule 122-G.
- (b) Blood Bank Technician(s) possessing –
 - (i) Degree in Medical Laboratory Technology (M.L.T) with six months' experience in the testing of blood and/or its components; or
 - (ii) Diploma in Medical Laboratory Technology (M.L.T) with one year's experience in the testing of blood and / or its components, the degree or diploma being from a University / Institution recognized by the Central Government or State Government.
- (c) Registered Nurse(s);
- (d) Technical supervisor (where blood components are manufactured), possessing-
 - (i) Degree in Medical Laboratory Technology (M.L.T) with six months' experience in the preparation of blood components; or
 - (ii) Diploma in Medical Laboratory Technology (M.L.T) with one year's experience in the preparation of blood components,

the degree or diploma being from a University / Institution recognized by the Central Government or State Government.

NOTES :

- (1) The requirements of qualification and experience in respect of Technical Supervisor and Blood Bank Technician shall apply in the cases of persons who are approved by the Licensing Authority and/or Central Licence Approving Authority after the commencement of the Drugs and Cosmetics (Amendment) Rules, 1999*.
- (2) As regards, the number of whole time competent technical personnel, the blood bank shall comply with the requirements laid down in the Directorate General of Health Services Manual.
- (3) It shall be the responsibility of the licensee to ensure through maintenance of records and other latest techniques used in blood banking system that the personnel involved in blood banking activities for collection, storage, testing and distribution are adequately trained in the current Good Manufacturing Practices/Standard Operating Procedures for the tasks undertaken by each personnel. The personnel shall be made aware of the principles of Good Manufacturing Practices / Standard Operating Procedures that affect them and receive initial and continuing training relevant to their needs.

D. MAINTENANCE

The premises shall be maintained in a clean and proper manner to ensure adequate cleaning and maintenance of proper operations. The facilities shall include:-

- (1) Privacy and thorough examination of individuals to determine their suitability as donors.
- (2) Collection of blood from donors with minimal risk of contamination or exposure to activities and equipment unrelated to blood collection.
- (3) Storage of blood or blood components pending completion of tests.
- (4) Provision for quarantine, storage of blood and blood components in a designated location, pending repetition of those tests that initially give questionable serological results.
- (5) Provision for quarantine, storage, handling and disposal of products and reagents not suitable for use.
- (6) Storage of finished products prior to distribution or issue.
- (7) Proper collection, processing, compatibility testing, storage and distribution of blood and blood components to prevent contamination.
- (8) Adequate and proper performance of all procedures relating to plasmapheresis, plateletpheresis and leucapheresis.
- (9) Proper conduct of all packaging, labelling and other finishing operations.
- (10) Provision for safe and sanitary disposal of –
 - (i) Blood and/or blood components not suitable for use, distribution or sale.
 - (ii) Trash and items used during the collection, processing and compatibility testing of blood and / or blood components.

*Note : 2nd Amendment Rules, 1999 (w.e.f. 5-4-1999)

E. EQUIPMENT

Equipment used in the collection, processing, testing, storage and sale/distribution of blood and its components shall be maintained in a clean and proper manner and so placed as to facilitate cleaning and maintenance. The equipment shall be observed, standardized and calibrated on a regularly scheduled basis as described in the Standard Operating Procedures Manual and shall operate in the manner for which it was designed so as to ensure compliance with the official requirements (the equipments) as stated below for blood and its components.

Equipment that shall be observed, standardized and calibrated with at least the following frequencies:

EQUIPMENT	PERFORMANCE	FREQUENCY	FREQUENCY OF CALIBRATION
1. Temperature Recorder	Compare against thermometer	Daily	As often as necessary
2. Refrigerated centrifuge	Observe speed and temperature	Each day of use	As often as necessary
3. Hematocrit centrifuge	—	—	Standardise before initial use, after repair or adjustments and annually.
4. General lab. Centrifuge	—	—	Tachometer, every 6 months.
5. Automated Blood typing	Observe controls for correct results	Each day of use	—
6. Haemoglobinometer	Standardize against cyanamethemoglobin standard	—ditto—	—
7. Refractometer or Urinometer	Standardize against distilled water	— ditto —	—
8. Blood container weighing device	Standardize against container of known weight.	— ditto —	As often as necessary
9. Water Bath	Observe temperature	— ditto —	—ditto—
10. Rh view box (wherever necessary)	—ditto—	— ditto —	— ditto—

11	Autoclave	Observe temperature	Each day of use	As often as necessary
12	Serologic rotators	Observe controls for correct results	-- ditto--	Speed as often as necessary.
13	Laboratory thermometers	—	—	Before initial use
14	Electronic thermometers	—	Monthly	—
15	Blood agitator	Observe weight of the first container of blood filled for correct results	Each day of use	Standardize with container of known mass or value before initial use, and after repairs or adjustments.

F. SUPPLIES AND REAGENTS:

All supplies and reagents used in the collection, processing, compatibility, testing, storage and distribution of blood and blood components shall be stored at proper temperature in a safe and hygienic place, in a proper manner and in particular:—

- (a) all supplies coming in contact with blood and blood components intended for transfusion shall be sterile, pyrogen-free, and shall not interact with the product in such a manner as to have an adverse effect upon the safety, purity, potency or effectiveness of the product.
- (b) supplies and reagents that do not bear an expiry date shall be stored in a manner that the oldest is used first.
- (c) supplies and reagents shall be used in a manner consistent with instructions provided by the manufacturer.
- (d) all final containers and closures for blood and blood components not intended for transfusion shall be clean and free of surface solids and other contaminants.
- (e) each blood collecting container and its satellite container(s), if any, shall be examined visually for damage or evidence of contamination prior to its use and immediately after filling. Such examination shall include inspection for breakage of seals, when indicated, and abnormal discoloration. Where any defect is observed, the container shall not be used or, if detected after filling, shall be properly discarded.
- (f) representative samples of each lot of the following reagents and/or solutions shall be tested regularly on a scheduled basis by methods described in the Standard Operating Procedures Manual to determine their capacity to perform as required,

Reagents and solutions	Frequency of testing alongwith controls
Anti-human serum	Each day of use
Blood grouping serums	Each day of use
Lectin	Each day of use
Antibody screening and reverse grouping cells	Each day of use
Hepatitis test reagents	Each run
Syphilis serology reagents	Each run
Enzymes	Each day of use
HIV I and II reagents	Each run
Normal saline (LISS and PBS)	Each day of use
Bovine Albumin	Each day of use.

G. GOOD MANUFACTURING PRACTICES (GMPs) /STANDARD OPERATING PROCEDURES (SOPs):

Written Standard Operating Procedures shall be maintained and shall include all steps to be followed in the collection, processing, compatibility testing, storage and sale or distribution of blood and/or preparation of blood components for homologous transfusion, autologous transfusion and further manufacturing purposes. Such procedures shall be available to the personnel for use in the concerned areas. The Standard Operating Procedures shall *inter alia* include:

1. (a) criteria used to determine donor suitability.
- (b) methods of performing donor qualifying tests and measurements including minimum and maximum values for a test or procedure, when a factor in determining acceptability;
- (c) solutions and methods used to prepare the site of phlebotomy so as to give maximum assurance of a sterile container of blood;
- (d) method of accurately relating the product (s) to the donor;
- (e) blood collection procedure, including in-process precautions taken to measure accurately the quality of blood drawn from the donor;
- (f) methods of component preparation, including any time restrictions for specific steps in processing;
- (g) all tests and repeat tests performed on blood and blood components during processing;
- (h) pre-transfusion testing, wherever applicable, including precautions to be taken to identify accurately the recipient blood components during processing;

- (i) procedures of managing adverse reactions in donor and recipient reactions;
- (j) storage temperatures and methods of controlling storage temperatures for blood and its components and reagents;
- (k) length of expiry dates, if any assigned for all final products;
- (l) criteria for determining whether returned blood is suitable for re-issue;
- (m) procedures used for relating a unit of blood or blood component from the donor to its final disposal;
- (n) quality control procedures for supplies and reagents employed in blood collection, processing and re-transfusion testing;
- (o) schedules and procedures for equipment maintenance and calibration;
- (p) labelling procedures to safeguard its mix-ups, receipt, issue, rejected and in-hand;
- (q) procedures of plasmapheresis, plateletpheresis and leucapheresis if performed, including precautions to be taken to ensure re-infusion of donor's own cells;
- (r) procedures for preparing recovered (salvaged) plasma if performed, including details of separation, pooling, labelling, storage and distribution;
- (s) all records pertinent to the lot or unit maintained pursuant to these regulations shall be reviewed before the release or distribution of a lot or unit of final product. The review or portions of the review may be performed at appropriate periods during or after blood collection, processing, testing and storage. A thorough investigation, including the conclusions and follow-up, of any unexplained discrepancy or the failure of a lot or unit to meet any of its specification shall be made and recorded.
- (2) A licensee may utilise current Standard Operating Procedures, such as the Manuals of the following organizations, so long as such specific procedures are consistent with, and at least as stringent as, the requirements contained in this Part, namely :-
 - (i) Directorate General of Health Services Manual.
 - (ii) Other Organisations or individual blood bank's manuals, subject to the approval of State Licensing Authority and Central Licence Approving Authority.

H. CRITERIA FOR BLOOD DONATION:

Conditions for donation of blood:

- (1) *General* – No person shall donate blood and no blood bank shall draw blood from a person, more than once in three months. The donor shall be in good health, mentally alert and physically fit and shall not be inmates of jail, persons having multiple sex partners and drug-addicts. The donors shall fulfil the following requirements, namely: -

- ¹[(a) the donor shall be in the age group of 18 to 65 years;]
- (b) the donor shall not be less than 45 kilograms;
- (c) temperature and pulse of the donor shall be normal;
- (d) the systolic and diastolic blood pressure are within normal limits without medication;
- (e) haemoglobin which shall not be less than 12.5 grams;
- (f) the donor shall be free from acute respiratory diseases;
- (g) the donor shall be free from any skin diseases at the site of phlebotomy;
- (h) the donor shall be free from any disease transmissible by blood transfusion, insofar as can be determined by history and examination indicated above;
- (i) the arms and forearms of the donor shall be free from skin punctures or scars indicative of professional blood donors or addiction of self injected narcotics.

(2) *Additional qualifications of donor* – No person shall donate blood, and no blood bank shall draw blood from a donor, in the conditions mentioned in column (1) of the Table given below before the expiry of the period of deferment mentioned in the column (2) of the said Table.

Table: Deferment of blood donation

CONDITIONS (1)	PERIOD OF DEFERMENT (2)_
(a) Abortions	6 months
(b) History of Blood transfusion	6 months
(c) Surgery	12 months
(d) Typhoid	12 months after recovery
(e) History of Malaria and duly treated	2 months (endemic) 3 years (non endemic area)
(f) Tattoo	6 months
(h) Breast feeding	12 months after deliver
(i) Immunization (Cholera, Typhoid, Diphtheria, Tetanus, Plague, Gammaglobulin)	15 days
(j) Rabies vaccination	1 year after vaccination
(k) History of Hepatitis in family or close contact	12 months
(l) Immunoglobulin	12 months

(3) No person shall donate blood and no blood bank shall draw blood from a person, suffering from any of the diseases mentioned below, namely:-

- (a) Cancer
- (b) Heart disease

1. Subs. By G.S.R 101 (E), dt:18-2-2011

- (c) Abnormal bleeding tendencies
 - (d) Unexplained weight loss
 - (e) Diabetes-controlled on insulin
 - ¹(f) Hepatitis infection]
 - (g) Chronic nephritis
 - (h) Signs and symptoms, suggestive of AIDS
 - (i) Liver diseases
 - (j) Tuberculosis
 - (k) Polycythemia Vera.
 - (l) Asthma
 - (m) Epilepsy
 - (n) Leprosy
 - (o) Schizophrenia
 - (p) Endocrine disorders
-

I. GENERAL EQUIPMENTS AND INSTRUMENTS.

1. For blood collection room:

- (i) Donor beds, chairs and tables: These shall be suitably and comfortably cushioned and shall be of appropriate size.
- (ii) Bedside table
- (iii) Sphygmomanometer and stethoscope
- (iv) Recovery beds for donors.
- (v) Refrigerators, for storing separately tested and untested blood, maintaining temperature between 2 to 6 degree centigrade with digital dial thermometer, recording thermograph and alarm device, with provision for continuous power supply.
- (vi) Weighing devices for donor and blood containers.

2. For haemoglobin determination:

- (i) Copper sulphate solution (specific gravity 1.053)
- (ii) Sterile lancet and impregnated alcohol swabs.
- (iii) Capillary tube (1.3 x 1.4 x 96 mm for Pasteur pipettes)
- (iv) Rubber bulbs for capillary tubings.
- (v) Sahli's haemoglobinometer / Colorimetric method.

3. For temperature and pulse determination.

- (i) Clinical thermometers
- (ii) Watch (fitted with a second-hand) and a stop-watch.

4. For blood containers:

- (a) Only disposable PVC blood bags shall be used (closed system) as per specifications of IP/USP/BP.
- (b) Anti-coagulants: The anti-coagulant solution shall be sterile, pyrogen-free and of the following composition that will ensure satisfactory safety and efficacy of the whole blood and/or for all the separate blood components.
 - (i) Citrate Phosphate Dextrose Adenine solution (CPDA) or Citrate Phosphate Dextrose Adenine – 1 (CPDA-1) – 14 ml solution shall be required for

1. Subs. by G.S.R. No. 40(E), dated 29-01-2001.

100 ml of blood.

- Note 1.-** (i) In case of single/double/triple/quadruple blood collection bags used for blood component preparations, CPDA blood collection bags may be used.
(ii) Acid Citrate Dextrose solution (A.C.D. with Formula-A) I.P. – 15 ml solution shall be required for 100 ml of blood,
(iii) Additive solutions such as SAGM, ADSOL, NUTRICEL may be used for storing and retaining Red Blood Corpuscles up to 42 days.

Note 2.- The licensee shall ensure that the anti-coagulant solutions are of a licensed manufacturer and the blood bags in which the said solutions are contained have a certificate of analysis of the said manufacturer.

5. Emergency equipments/items:

- (i) Oxygen cylinder with mask, gauge and pressure regulator.
- (ii) 5 per cent Glucose or Normal Saline.
- (iii) Disposable sterile syringes and needles of various sizes.

- (iv) Disposable sterile I.V. infusion sets.
- (v) Ampoules of Adrenaline, Noradrenaline, Mephentin, Betamethasone or Dexamethasone, Metoclorpropamide injections.
- (vi) Aspirin.

6. Accessories:

- (i) Such as blankets, emesis basins, haemostats, set clamps, sponge forceps, gauze, dressing jars, solution jars, waste cans.
- (ii) Medium cotton balls, 1.25 cm adhesive tapes.
- (iii) Denatured spirit, Tincture Iodine, green soap or liquid soap.
- (iv) Paper napkins or towels.
- (v) Autoclave with temperature and pressure indicator.
- (vi) Incinerator
- (vii) Stand-by generator

7. Laboratory equipment:

- (i) Refrigerators, for storing diagnostic kits and reagents, maintaining a temperature between 4 to 6 degree centigrade (plus/minus 2 degrees centigrade) with digital dial thermometer having provision for continuous power supply.
- (ii) Compound Microscope with low and high power objectives.
- (iii) Centrifuge Table Model.
- (iv) Water bath: having range between 37 degree centigrade to 56 degree centigrade.
- (v) Rh viewing box in case of slide technique.
- (vi) Incubator with thermostatic control.
- (vii) Mechanical shakers for serological tests for Syphilis.
- (viii) Hand-lens for observing tests conducted in tubes.
- (ix) Serological graduated pipettes of various sizes.
- (x) Pipettes (Pasteur).
- (xi) Glass slides.

- (xii) Test tubes of various sizes / micrometer plates (U or V type).
- (xiii) Precipitating tubes 6mm x 50mm of different sizes and glass beakers of different sizes.
- (xiv) Test tube racks of different specifications.
- (xv) Interval timer electric or spring wound.
- (xvi) Equipment and materials for cleaning glass wares adequately.
- (xvii) Insulated containers for transporting blood, between 2 degree centigrade to 10 degree centigrade temperatures, to wards and hospitals.
- (xviii) Wash bottles.
- (xix) Filter papers.
- (xx) Dielectric tube sealer.
- (xxi) Plain and EDTA vials.
- (xxii) Chemical balance (wherever necessary).
- (xxiii) ELISA reader with printer, washer and micropipettes.

J. SPECIAL REAGENTS :

- (1) Standard blood grouping sera Anti A, Anti B and Anti D with known controls. Rh typing sera shall be in double quantity and each of different brand or if from the same supplier each supply shall be of different lot numbers.
- (2) Reagents for serological tests for syphilis and positive sera for controls.
- (3) Anti Human Globulin Serum (Coomb's serum).
- (4) Bovine Albumin 22 percent Enzyme reagents for incomplete antibodies.
- ¹(5) ELISA or RPHA test kits for Hepatitis and HIV I & II.
- (6) Detergent and other agents for cleaning laboratory glass wares.

K. TESTING OF WHOLE BLOOD :

- (1) It shall be responsibility of the licensee to ensure that the whole blood collected, processed and supplied conforms to the standards laid down in the Indian Pharmacopoeia and other tests published, if any, by the Government.
- (2) *Freedom from HIV antibodies (AIDS) Tests.* – Every licensee shall get samples of every blood unit tested, before use, for freedom from HIV 1 and HIV II antibodies either from laboratories specified for the purpose by the Central Government or in his own laboratory. The results of such testing shall be recorded on the label of the container.
- (3) Each blood unit shall also be tested for freedom from ²[(Hepatitis B surface antigen and Hepatitis C Virus antibody)] VDRL and malarial parasite and results of such testing shall be recorded on the label of the container.

- NOTES**
- (a) Blood samples of donors in pilot tube and the blood samples of the Recipient shall be preserved for 7 days after issue.
 - (b) The blood intended for transfusion shall not be frozen at any stage.
 - (c) Blood containers shall not come directly in contact with ice at any stage.

1. Subs. By G.S.R. 733(E) dated 21-12-2005
 2. Subs. by G.S.R. 40(E) dated 29-01-2001

L RECORDS :

The records which the licensee is required to maintain shall include *inter alia* the following particulars, namely:

- (1) *Blood donor record*: It shall indicate serial number, date of bleeding, name, address and signature of donor with other particulars of age, weight, haemoglobin, blood grouping, blood pressure, medical examination, bag number and patient's detail for whom donated in case of replacement donation, category of donation (voluntary / replacement) and deferral records and signature of Medical Officer In-charge.
- (2) *Master records for blood and its components*: It shall indicate bag serial number, date of collection, date of expiry, quantity in ml. ABO/Rh Group, results for testing of HIV I and HIV II antibodies, Malaria, V.D.R.L. ¹[(Hepatitis B surface antigen and Hepatitis C Virus antibody)] and irregular antibodies (if any), name and address of the donor with particulars, utilization issue number, components prepared or discarded and signature of the Medical Officer in charge.
- (3) *Issue Register*: It shall indicate serial number, date and time of issue bag serial number, ABO/RH Group, total quantity in ml, name and address of the recipient, group of recipient, unit/institution, details of cross-matching report, indication for transfusion.
- (4) *Records of components supplied*: Quantity supplied; compatibility report, details of recipient and signature of issuing person.
- (5) Records of ACD/CPD/CPD-A/SAGM bags giving details of manufacturer, batch number, date of supply, and results of testing.
- (6) Register for diagnostic kits and reagents used: name of the kits/reagents, details of batch number, date of expiry and date of use.
- (7) Blood bank must issue the cross matching report of the blood to the patient together with the blood unit.
- (8) Transfusion adverse reaction records.
- (9) Records of purchase, use and stock in hand of disposable needles, syringes, blood bags, shall be maintained.

NOTE : The above records shall be kept by the licensee for a period of five years.

M. LABELS :

The labels on every bag containing blood and/or component shall contain the following particulars, namely;

- (1) The proper name of the product in a prominent place and in bold letters on the bag.
- (2) Name and address of the blood bank
- (3) Licence number

¹ Subs. by G.S.R. 40(E), dt. 29.1.2001 (w.e.f. 1.6.2001).

- (4) Serial number
- (5) The date on which the blood is drawn and the date of expiry as prescribed under Schedule P to these rules.
- (6) A colored label shall be put on every bag containing blood. The following color scheme for the said labels shall be used for different groups of blood:

<u>Blood Group</u>	<u>Colour of the label</u>
O	Blue
A	Yellow
B	Pink
AB	White

- (7) The results of the tests for ¹[(Hepatitis B surface antigen and Hepatitis C Virus antibody)] syphilis, freedom from HIV I and HIV II antibodies and malarial parasite.
- (8) The Rh. Group.
- (9) Total volume of blood, the preparation of blood, nature and percentage of anti-coagulant.
- (10) Keep continuously temperature at 2 degree centigrade to 6 degree centigrade for whole human blood and/or components as contained under III of Part XII B.
- (11) Disposable transfusion sets with filter shall be used in administration equipment.
- (12) Appropriate compatible cross-matched blood without atypical antibody in recipient shall be used.
- (13) The contents of the bag shall not be used if there is any visible evidence of deterioration like haemolysis, clotting or discoloration.
- (14) The label shall indicate the appropriate donor classification like “Voluntary Donor” or “Replacement Donor” in no less prominence than the proper name.

NOTES :

- 1. In the case of blood components, particulars of the blood from which such components have been prepared shall be given against item numbers (5), (7), (8), (9) and (14).
- 2. The blood and/or its components shall be distributed on the prescription of a Registered Medical Practitioner.

II. BLOOD DONATION CAMPS.

A blood donation camp may be organized by –

1. Subs. by G.S.R 40(E), dt. 29.1.2001.

- (a) a licensed designated Regional Blood Transfusion Centre ; or
- (b) a licensed Government blood bank; or
- (c) the Indian Red Cross society; or

NOTES:

- (i) “ Designated Regional Blood Transfusion Centre” shall be a center approved and designated by a Blood Transfusion Council constituted by a State Government to collect, process and distribute blood and its components to cater to the needs of the region and that center has also been licensed and approved by the Licensing Authority and Central Licence Approving Authority for the purpose.
- (ii) The designated Regional Blood Transfusion Centre, Government blood bank and Indian Red Cross Society shall intimate within a period of seven days, the venue where the blood camp was held and details of group wise blood units collected in the said camp to the Licensing Authority and Central Licence Approving Authority.

For holding a blood donation camp, the following requirements shall be fulfilled/complied with, namely: -

(A) Premises, personnel etc.

- (a) Premises under the blood donation camp shall have sufficient area and the location shall be hygienic so as to allow proper operation, maintenance and cleaning.
- (b) All information regarding the personnel working, equipment used and facilities available at such a Camp shall be well documented and made available for inspection, if required, and ensuring -
 - (i) Continuous and uninterrupted electrical supply for equipment used in the Camp;
 - (ii) Adequate lighting for all the required activities;
 - (iii) Hand-washing facilities for staff;
 - (iv) Reliable communication system to the central office of the Controller/ Organizer of the Camp;
 - (v) Furniture and equipment arranged within the available space;
 - (vi) Refreshment facilities for donors and staff;
 - (vii) Facilities for medical examination of the donors;
 - (viii) Proper disposal of waste.

(B) Personnel for Out-door Blood Donation Camp:

To collect blood from 50 to 70 donors in about 3 hours or from 100 to 200 donors in 5 hours, the following requirements shall be fulfilled / complied with:

- (i) one Medical Officer and two nurses or phlebotomists for managing 6-8 donor tables;
- (ii) two medico social workers;
- (iii) three blood bank technicians;
- (iv) two attendants;

- (v) vehicle having a capacity to seat 8-10 persons, with provision for carriage of donation goods including facilities to conduct a blood donation camp;

(C) Equipments :

1. BP apparatus.
2. Stethoscope.
3. Blood bags (single, double, triple, quadruple).
4. Donor questionnaire.
5. Weighing device for donors.
6. Weighing device for blood bags.
7. Artery forceps, scissors.
8. Stripper for blood tubing.
9. Bed sheets, blankets/mattress.
10. Lancets, swab stick/tooth picks.
11. Glass slides.
12. Portable Hb meter/copper sulphate.
13. Test tube (big) and 12x100mm (small).
14. Test tube stand.
15. Anti-A, Anti-B and Anti-AB, Antisera and Anti-D.
16. Test tube sealer film.
17. Medicated adhesive tape.
18. Plastic waste basket.
19. Donor cards and refreshment for donors.
20. Emergency medical kit.
21. Insulated blood bag containers with provisions for storing between 2 degree centigrade to 10 degree centigrade.
22. Dielectric sealer or portable tube sealer.
23. Needle destroyer (wherever necessary).

III. PROCESSING OF BLOOD COMPONENTS FROM WHOLE BLOOD BY A BLOOD BANK

The Blood components shall be prepared by blood banks as a part of the Blood Bank services. The conditions for grant or renewal of licence to prepare blood components shall be as follows: -

A. ACCOMMODATION:

- (1) Rooms with adequate area and other specification, for preparing blood components depending on quantum of workload shall be as specified in item B under the heading "1. BLOOD BANKS/BLOOD COMPONENTS" of this Part.
- (2) Preparation of Blood components shall be carried out only under closed system using single double, triple or quadruple plastic bags except for preparation of Red Blood Cells Concentrates, where single bags may be used with transfer bags.

B. EQUIPMENT :

- (i) Air Conditioner;
- (ii) Laminar air flow bench;
- (iii) Suitable refrigerated centrifuge;
- (iv) Plasma expresser;
- (v) Clipper and clips and/or dielectric sealer;
- (vi) Weighing device;
- (vii) Dry rubber balancing material;
- (viii) Artery forceps,
scissors;
- (ix) Refrigerator maintaining a temperature between 2 degree centigrade to 6 degree centigrade, a digital dial thermometer with recording thermograph and alarm device, with provision for continuous power supply;
- (x) Platelet agitator with incubator (wherever necessary);
- (xi) Deep freezers maintaining a temperature between minus 30 degree centigrade to minus 40 degree centigrade and minus 75 degree centigrade to minus 80 degree centigrade;
- (xii) Refrigerated Water bath for Plasma Thawing;
- (xiii) Insulated blood bag containers with provisions for storing at appropriate temperature for transport purposes;

C. PERSONNEL:

The whole time competent technical staff meant for processing of Blood Components (that is Medical Officer, Technical Supervisor, Blood Bank Technicians and Registered Nurse) shall be as specified in item C, under the heading “ 1. BLOOD BANKS/BLOOD COMPONENTS” of this Part.

D. TESTING FACILITIES:

General: Facilities for A, B, AB and O groups and Rh(D) grouping. ¹[(Hepatitis B surface antigen and Hepatitis C Virus antibody)], VDRL, HIV I and HIV II antibodies and malarial parasites shall be mandatory for every blood unit before it is used for the preparation of blood components. The results of such testing shall be indicated on the label.

E. CATEGORIES OF BLOOD COMPONENTS:

(1) **CONCENTRATED HUMAN RED BLOOD CORPUSCLES:**

The product shall be known as “Packed Red Blood Cells” that is Packed Red Blood Cells remaining after separating plasma from human blood.

General Requirements:

- (a) *Storage:* Immediately after processing, the Packed Red Blood Cells shall be kept at a temperature maintained between 2 degree centigrade to 6 degree centigrade.
- (b) *Inspection:* The component shall be inspected immediately after separation of the plasma, during storage and again at the time of issue. The product shall not be issued if there is any abnormality in color or physical appearance or any indication of microbial contamination.
- (c) *Suitability of Donor:* The source of blood for Packed Red Blood Cells shall be obtained from a donor who meets the criteria for Blood Donation as specified in item H under the heading “I. BLOOD BANKS/BLOOD COMPONENTS” of this Part.

1. Subs. by G.S.R. 40(E), dt. 29.1.2001.

- (d) Testing of Whole Blood: Blood from which Packed Red Blood Cells are prepared shall be tested as specified in item K relating to Testing Of Whole Blood under the heading "I. BLOOD BANKS/BLOOD COMPONENTS" of this Part.
- (e) *Pilot samples*: Pilot samples collected in integral tubing or in separate pilot tubes shall meet the following specifications:
 - (i) One or more pilot samples of either the original blood or the Packed Red Blood Cells being processed shall be preserved with each unit of Packed Red Blood Cells which is issued.
 - (ii) Before they are filled, all pilot sample tubes shall be marked or identified so as to relate them to the donor of that unit or Packed Red Blood Cells.
 - (iii) Before the final container is filled or at the time the final product is prepared, the pilot samples tubes accompanying a unit of Packed Red Blood Cells, shall be attached in a tamper-proof manner that shall conspicuously identify removal and re-attachment.
 - (iv) All pilot sample tubes, accompanying a unit of packed red blood cells, shall be filled immediately after the blood is collected or at the time the final product is prepared, in each case, by the person who performs the collection of preparation.

F. Processing:

- (i) *Separation*: Packed Red Blood Cells shall be separated from the whole blood, --
 - (a) if the whole blood is stored in ACD solution within 21 days, and
 - (b) if the whole blood is stored in CPDA-1 solution, within 35 days, from the date of collection. Packed Red Blood Cells may be prepared either by centrifugation done in a manner that shall not tend to increase the temperature of the blood or by normal undisturbed sedimentation method. A portion of the plasma, sufficient to ensure optimal cell preservation, shall be left with the packed Red Blood Cells.
- (ii) *Packed Red Blood Cells Frozen*: Cryophylactic substance may be added to the Packed Red Blood Cells for extended manufacturer's storage not warmer than minus 65 degree centigrade provided the manufacturer submits data to the satisfaction of the Licensing Authority and Central Licence Approving Authority, as adequately demonstrating through in-vivo cells survival and other appropriate tests that the addition of the substance, the material used and the processing methods results in a final product meets the required standards of safety, purity and potency for Packed Red Blood Cells, and that the frozen

product shall maintain those properties for the specified expiry period.

- (iii) *Testing:* Packed Red Blood Cells shall conform to the standards as laid down in the Indian Pharmacopoeia.

(2) PLATELETS CONCENTRATES:

The product shall be known as “Platelets Concentrates” that is platelets collected from one unit of blood and re-suspended in an appropriate volume of original plasma.

General Requirements:

- (i) *Source:* The source material for platelets shall be platelet rich plasma or buffy coat which may be obtained from the whole blood or by plateletpheresis.

(ii) *Processing :*

- (a) Separation of buffy-coat or platelet-rich plasma and platelets and re-suspension of the platelets shall be in a closed system by centrifugal method with appropriate speed, force and time.
- (b) Immediately after collection, the whole blood or plasma shall be held in storage between 20 degree centigrade to 24 degree centigrade. When it is to be transported from the venue of blood collection to the processing laboratory, during such transport action, the temperature as close as possible to a range between 20 degree centigrade to 24 degree centigrade shall be ensured. The platelet concentrates shall be separated within 6 hours after the time of collection of the unit of whole blood or plasma.
- (c) The time and speed of centrifugation shall be demonstrated to produce an unclamped product, without visible haemolysis, that yields a count of not less than 3.5×10^{10} (3.5×10 raised to the power of 10) and 4.5×10^{10} (4.5×10 raised to the power ten) i.e. platelets per unit from a unit of 350 ml and 450 ml blood respectively. One percent of total platelets prepared shall be tested of which 75 per cent of the units shall conform to the above said platelet count.
- (d) The volume of original plasma used for re-suspension of the platelets shall be determined by the maintenance of the pH of not less than 6 during the storage period. The pH shall be measured on a sample of platelets which has been stored for the permissible maximum expiry period at 20 degree centigrade to 24 degree centigrade.
- (e) Final containers used for platelets shall be colorless and transparent to permit visual inspection of the contents. The caps selected shall maintain a hermetic seal to prevent contamination of the contents. The container material shall not interact with the contents, under the normal conditions of the storage and use, in such a manner as to have an adverse effect upon the safety, purity, potency, or efficacy of the product. At the time of filling, the final container shall be marked or identified by number so as to relate it to the donor.
- (iii) *Storage:* Immediately after re-suspension, platelets shall be placed in storage not exceeding for a period of 5 days, between 20 degree centigrade to 24 degree centigrade, with continuous gentle agitation of the platelet concentrates maintained throughout such storage

- (iv) *Testing:* The units prepared from different donors shall be tested at the end of the storage period for –
- (a) Platelet count;
 - (b) pH of not less than 6 measured at the storage temperature of the unit;
 - (c) measurement of actual plasma volume;
 - (d) one percent of the total platelets prepared shall be tested for sterility;
 - (e) the tests of functional viability of the platelets shall be done by swirling movement before issue.
 - (f) if the results of the testing indicate that the product does not meet the specified requirements, immediate corrective action shall be taken and records maintained;
- (v) *Compatibility Test:* Compatible transfusion for the purpose of variable number of Red Blood Cells, A, B, AB and O grouping shall be done if the platelets concentrate is contaminated with red blood cells.

(3) GRANULOCYTE CONCENTRATES:

- (i) *Storage:* It shall be kept between 20 degree centigrade to 24 degree centigrade for a maximum period of 24 hours;
- (ii) Unit of granulocytes shall not be less than 1×10^{10} (i.e. 1×10 raised to the power of 10) when prepared on cell separator;
- (iii) Group specific tests/HLA test wherever required shall be carried out.

(4) FRESH FROZEN PLASMA:

Plasma frozen within 6 hours after blood collection and stored at a temperature not warmer than minus 30 degree centigrade, shall be preserved for a period of not more than one year.

(5) CRYOPRECIPITATE:

Concentrate of anti-hemophiliac factor shall be prepared by thawing of the fresh plasma frozen stored at minus 30 degree centigrade.

(a) *Storage:*

Cryoprecipitate shall be preserved at a temperature not higher than minus 30 degree centigrade and may be preserved for a period of not more than one year from the date of collection.

(b) *Activity:*

Anti-hemophiliac factor activity in the final product shall be not less than 80 units per bag. One percent of the total cryoprecipitate prepared shall be tested of which seventy five percent of the unit shall conform to the said specification.

F PLASMAPHERESIS, PLATELETPHERESIS, LEUCAPHERESIS, USING A CELL SEPARATOR.

An area of 10 square meters shall be provided for apheresis in the blood bank.

The blood banks specifically permitted to undertake the said apheresis on the donor shall observe the criteria as specified in item H relating to Criteria for blood donation “I. Blood Banks/Blood Components” of this Part. The written consent of the donor shall be taken and the donor must be explained, the hazards of apheresis. The Medical Officer shall certify that the donor is fit for apheresis and it shall be carried out by a trained person under supervision of the Medical Officer.

(A) PLASMAPHERESIS, PLATELETPHERESIS AND LEUCAPHERESIS:

The donors subjected to plasmapheresis, plateletpheresis and leucapheresis shall, in addition to the criteria specified in item H relating to the CRITERIA FOR BLOOD DONATION, under the heading "I. BLOOD BANKS/BLOOD COMPONENTS" of this Part being observed, be also subjected to protein estimation on post-pheresis/first sitting whose results shall be taken as reference for subsequent/sitting. It shall also be necessary that the total plasma obtained from such donor and periodicity of Plasmapheresis shall be according to the standards described under validt. Standard Operating Procedures.

NOTE :

- (i) At least 48 hours must elapse between successive apheresis and not more than twice in a week.
- (ii) Extracoporeal blood volume shall not exceed 15% of donor's estimated blood volume.
- (iii) Platelet pheresis shall not be carried out on donors who have taken medication containing Asprin within 3 days prior to donation.
- (iv) If during plateletpheresis or leucapheresis, RBCs cannot be re-transfused then at least 12 weeks shall elapse before a second cytapheresis procedure is conducted.

(B) MONITORING FOR APHERESIS:

Before starting apheresis procedure, hemoglobin or haematocrit shall be done. Platelet count, WBC counts, differential count may be carried out. In repeated plasmapheresis, the serum protein shall be 6 gm./ml.

(C) COLLECTION OF PLASMA:

The quantity of plasma separated from the blood of donor shall not exceed 500 ml. per sitting and once in a fortnight or shall not exceed 1000 ml per month.

PART XII C

I. REQUIREMENTS FOR MANUFACTURE OF BLOOD PRODUCTS

The blood products shall be manufactured in a separate premises other than that meant for blood bank. The requirements that are essential for grant or renewal of licence to manufacture blood products such as Albumin, Plasma Protein Fraction, Immunoglobins and Coagulation Factor Concentrates, shall be as follows, namely: -

A. GENERAL REQUIREMENTS:

1. Location and surroundings, buildings and water supply:

The requirements as regards location and surrounding, buildings and water supply as contained in paragraphs 1.1.1, 1.1.2, 1.1.3 of Part I of Schedule M shall apply *mutatis mutandis* to the manufacture of blood products.

2. Disposal of waste and infectious materials:

- (i) The requirements as regards disposal of waste and infectious materials as contained in paragraph 1.1.4 of Part 1 of Schedule M shall apply *mutatis mutandis* to the manufacture of blood products.
- (ii) Proper facility shall also be provided for potentially infectious materials, particularly HIV I & HIV II ¹[(Hepatitis B surface antigen and Hepatitis C Virus antibody)] through autoclaving, incineration or any other suitable validt. methods.

3. *Health, clothing and sanitation personnel:*

- (i) The requirement as contained in paragraph 3 of Part I of Schedule M shall be complied with.
- (ii) The personnel working in the manufacturing areas shall be vaccinated against Hepatitis B virus and other infectious transmitting diseases.

4. *Requirements for manufacturing area for Blood Products:*

(i) For the manufacture of blood products, separate enclosed areas specifically designated for the purpose shall be provided. These areas be provided with air locks for entry and shall be essentially dust free and ventilated with an air supply. Air supply for manufacturing area shall be filtered through bacteria retaining filters (HEPA Filters) and shall be at a pressure higher than in the adjacent areas.

The filters shall be checked for performance on installation and periodically thereafter, and records thereof shall be maintained.

(ii) Interior surfaces (walls, floors and ceilings) shall be smooth and free from cracks, they shall not shed matter and shall permit easy cleaning and disinfection. Drains shall be excluded from aseptic areas.

1. Subs. by G.S.R. 40(E) dated 29-01-2001

Routine microbial counts of the manufacturing area shall be carried out during manufacturing operations. The results of such counts shall be checked against well documented in-house standards and records maintained.

Access to the manufacturing areas shall be restricted to a minimum number of authorized personnel. Special procedures for entering and leaving the manufacturing areas shall be prominently displayed.

(iii) Sinks shall be excluded from aseptic areas. Any sink installed in other clean areas shall be of suitable material such as stainless steel, without an overflow, and be supplied with water of potable quality. Adequate precautions shall be taken to avoid contamination of the drainage system with dangerous effluents and airborne dissemination of pathogenic micro-organisms.

(iv) Lighting, air-conditioning, ventilation shall be designed to maintain a satisfactory temperature and relative humidity to minimize contamination and to take account of the comfort of personnel working with protective clothing.

(v) Premises used for the manufacture of blood products shall be suitably designed and constructed to facilitate good sanitation.

(vi) Premises shall be carefully maintained and it shall be ensured that repair and maintenance operations do not present any hazard to the quality of products. Premises shall be cleaned and, where applicable, disinfected according to detailed written valid. procedures.

(vii) Adequate facilities and equipments shall be used for the manufacture of blood products derived from blood plasma.

(viii) All containers of blood products, regardless of the stage of manufacture, shall be identified by securely attached labels. Cross contamination shall be prevented by adoption of the following measures, namely: –

(a) processing and filling shall be in segregated areas;

(b) manufacture of different products at the same time shall be avoided;

(c) simultaneous filling of the different products shall be avoided;

(d) ensure transfer, containers/materials by means of airlocks, air extraction, clothing change and careful washing and decontamination of equipment;

(e) protecting containers/materials against the risk of contamination caused by re-circulation of untreated air or by accidental re-entry of extracted air;

(f) using containers that are sterilized or are of documented low “bioburden”,

(ix) Positive pressure area shall be dedicated to the processing area concerned; (x) Air-handling units shall be dedicated to the processing area concerned;

(xi) Pipe work, valves and vent filters shall be properly designed to facilitate cleaning and sterilization. Valves on fractionation/reacting vessels shall be completely steam sterilisable. Air vent filters shall be hydrophobic and shall be validated for their designated use.

5. Ancillary Areas :

(i) Rest and refreshment rooms shall be separated from other areas.

(ii) Facilities for changing and storing clothes and for washing and toilet purposes shall be easily accessible and appropriate for the number of users. Toilets shall not be connected directly with production or storage areas.

(iii) Maintenance workshops shall be separated from production areas. Wherever parts and tools are stored in the production area, they shall be kept in rooms or lockers reserved for that use.

(iv) Animal houses shall be well isolated from other areas with separate entrance.

B. COLLECTION AND STORAGE OF PLASMA FOR FRACTIONATION

(a) Collection:

(1) Plasma shall be collected from the licensed Blood Banks through a cold chain process and stored in frozen condition not warmer than minus twenty degree centigrade.

(2) Individual plasma shall remain in quarantine till it is tested for ¹[(Hepatitis B and Hepatitis C Virus antibody)], HIV I and HIV II.

(3) A sample from pooled – lot plasma of about 10-12 units of different donors shall be tested for ¹[(Hepatitis B and Hepatitis C Virus antibody)] HIV I and HIV II and if the same sample found negative, only then it shall be taken up for fractionation.

(b) Storage Area :

(1) Storage areas shall be of sufficient space and capacity to allow orderly storage of the various categories of materials, intermediates, bulk and finished products, products in quarantine, released, rejected, returned, or recalled products.

(2) Storage areas shall be designed or adopted to ensure good storage conditioning. In particular, they shall be clean, dry and maintained within temperature required for such storage and where special storage conditions are required (e.g. temperature, humidity), these shall be provided, checked and monitored.

(3) Receiving and dispatch bays shall protect materials and products from the weather and shall be designed and equipped to allow containers of incoming materials to be cleaned, if necessary, before storage.

(4) Where quarantine status is ensured by storage in separate areas, these areas shall be clearly marked and their access restricted only to authorized personnel.

(5) There shall be separate sampling area for raw materials. If sampling is performed in the storage area, it shall be conducted in such a way so as to prevent contamination or cross-contamination.

(6) Segregation shall be provided for the storage of rejected, recalled, or returned materials or products.

(7) Adequate facility shall be provided for supply of ancillary material, such as ethanol, water, salts and polyethylene glycol. Separate facilities shall be provided for the recovery of organic solvents used in fractionation.

1. Subs. by G.S.R 40(E), dt. 29.1.2001 (w.e.f. 1.6.2001)

C. PERSONNEL :

(1) *Manufacture :*

The manufacture of blood products shall be conducted under the active direction and personal supervision of competent technical staff, consisting of at

least one person who shall be a whole time employee, with one year practical experience in the manufacture of blood products / plasma fractionation and possesses —

- (a) Post-graduate degree in Medicine—M.D. (Microbiology/Pathology/ Bacteriology/Immunology/Biochemistry); or
- or
- (b) Post-graduate degree in Science (Microbiology); or
- (c) Post-graduate degree in Pharmacy (Microbiology), from a recognized University or Institution.

2. Testing :

The head of the testing unit shall be independent of the manufacturing unit and testing shall be conducted under the active direction and personal supervision of competent technical staff consisting at least one person who shall be a whole time employee. The Head of the testing unit shall have eighteen months practical experience in the testing of drugs, especially the blood products and possesses —

- (a) Post-graduate degree in Pharmacy or Science— (Chemistry/ Microbiology/ Bio-chemistry); or
- (b) Post-graduate degree in Medicine— M.D(Microbiology/Pathology/ Biochemistry), from a recognized University or Institution.

D. PRODUCTION CONTROL :

(1) The production area and the viral inactivation room shall be centrally air- conditioned and fitted with HEPA filters having Grade C (Class 10,000) environment as given in the Table below.

(2) The filling and sealing shall be carried out under aseptic conditions in centrally air-conditioned areas fitted with HEPA Filters Grade A or, as the case may be, Grade B (Class 100) environment given in the said Table.

TABLE AIR CLASSIFICATION SYSTEM FOR MANUFACTURE OF STERILE PRODUCTS.		
<i>Grade</i>	<i>Maximum number of particles permitted per m³</i>	<i>Maximum number of Viable Micro-organism permitted per m³</i>
	<i>0.5 – 5 micron Less than 5 micron</i>	

Drugs and Cosmetics Rules 1945

A (Class 100) (Laminar - Airflow workstation)	3500	None	Less than 1
B (Class 100)	3500	None	Less than 5
C (Class 10,000)	3,50,000	2000	Less than 100

(3) The physical and chemical operations used for the manufacture of plasma fractionation shall maintain high yield of safe and effective protein.

(4) The fractionation procedure used shall give a good yield of products meeting the in-house quality requirements as approved by the Licensing Authority and Central Licence Approving Authority reducing the risk of microbiological contamination and protein denaturation to the minimum.

(5) The procedure adopted shall not affect the antibody activity and biological half- life or biological characteristics of the products.

E. VIRAL INACTIVATION PROCESS :

The procedure used by the licensee to inactivate the pathogenic organisms such as enveloped and non-enveloped virus, especially infectivity from HIV I & HIV II, ¹[(Hepatitis B surface antigens and Hepatitis C Virus antibody)], the viral inactivation and validation methods adopted by the licensee, shall be submitted for approval to the Licensing Authority and Central Licence Approving Authority.

NOTES:

- (1) No preservative (except stabilizer to prevent – protein denaturation such as glycine, sodium chloride or sodium caprylate) shall be added to Albumin, Plasma Protein Fraction, Intravenous Immunoglobulins or Coagulation Factor Concentrates without the prior approval of Licensing Authority and Central Licence Approving Authority.
- (2) The licensee shall ensure that the said stabilizers do not have deleterial effect on the final product in the quantity present so as not to cause any untoward or adverse reaction in human beings.

F. QUALITY CONTROL:

Separate facilities shall be provided for Quality Control such as Hematological, Bio-chemical, Physico-chemical, Microbiological, Pyrogens, Instrumental and Safety testing. The Quality Control Department shall have *inter alia* the following principal duties, namely :-

- (1) To prepare detailed instructions for carrying out test and analysis.
- (2) To approve or reject raw material, components, containers, closures, in-process materials, packaging material, labelling and finished products.
- (3) To release or reject batch of finished products which are ready for distribution.
- (4) To evaluate the adequacy of the conditions under which raw materials, semi-finished products and finished products are stored.

1. Subs. by G.S.R 40(E), dt. 29.1.2001 (w.e.f. 1.6.2001)

- (5) To evaluate the quality and stability of finished products and when necessary of raw materials and semi-finished products.
- (6) To review production records to ensure that no errors have occurred or if errors have occurred that they have been fully investigated.
- (7) To approve or reject all procedures, or specifications impacting on the identity, strength, quality and purity of the product.
- (8) To establish shelf-life and storage requirements on the basis of stability tests related to storage conditions.
- (9) To establish and when necessary revise, control procedures and specifications.
- (10) To review complaints, recalls, returned or salvaged products and investigations conducted there under for each product.
- (11) To review Master Formula Records/Cards periodically.

G. TESTING OF BLOOD PRODUCTS:

The products manufactured shall conform to the standards specified in the Indian Pharmacopoeia and where standards of any product is not specified in the Pharmacopoeia, the standard for such product shall conform to the standard specified in the United States Pharmacopoeia or the British Pharmacopoeia. The final products shall be tested for freedom from HIV I and HIV II antibodies ¹[(Hepatitis B surface antigen and Hepatitis C Virus antibody)].

H. STORAGE OF FINISHED PRODUCT :

(i) The final products shall be stored between two degree centigrade to eight degree centigrade, unless otherwise specified by the Central Licence Approving Authority.

(ii) The shelf-life assigned to the products by the licensee shall be submitted for approval to the Licensing Authority and Central Licence Approving Authority.

I. LABELLING :

The products manufactured shall be labelled as specified in the Indian Pharmacopoeia, the British Pharmacopoeia or the United States Pharmacopoeia which shall be in addition to any other requirement stated under Part IX or Part X of these rules. The labels shall indicate the results of test for ¹[(Hepatitis B surface antigen and Hepatitis C Virus antibody)] freedom from HIV I and HIV II antibodies.

J. RECORDS:

The licensee shall maintain records as per Schedule U and also comply with Batch manufacturing records as specified in Paragraph 9 of Part -I of Schedule M and any other requirement as may be directed by Licensing Authority and Central Licence Approving Authority.

K. MASTER FORMULA RECORDS:

The licensee shall maintain Master Formula Records relating to all manufacturing and quality control procedures for each product, which shall be prepared and endorsed by the Competent Technical Staff, i.e. Head of the manufacturing unit. The Master Formula Records shall contain: –

- (i) the patent or proprietary name of the product along with the generic name, if any, strength and the dosage form;
- (ii) a description or identification of the final containers, packaging materials, labels and closures to be used;

1. Subs. by G.S.R 40(E), dt. 29.1.2001(w.e.f. 1.6.2001)

- (iii) the identity, quantity and quality of each raw material to be used irrespective of whether or not it appears in the finished product. The permissible overage that may be included in a formulated batch shall be indicated;
- (iv) a description of all vessels and equipments and the sizes used in the process;
- (v) manufacturing and control instructions along with parameters for critical steps such as mixing, drying, blending, sieving and sterilizing the product;
- (vi) the theoretical yield to be expected from the formulation at different stages of manufacture and permissible yield limits;
- (vii) detailed instructions on precautions to be taken in the manufacture and storage of drugs and of semi finished products; and
- (viii) the requirements in-process quality control tests and analysis to be carried out during each stage of manufacture including the designation of persons or departments responsible for the execution of such tests and analysis.

II. REQUIREMENTS FOR MANUFACTURE OF BLOOD PRODUCTS FROM BULK FINISHED PRODUCTS

Where the blood products, such as Albumin, Plasma Protein Fraction, Immunoglobulins and Coagulation Factor Concentrates are manufactured through the manufacturing activities of filling and sealing the blood products from bulk powder or solution or both, the requirements as they apply to the manufacture of blood products from whole blood shall apply *mutatis mutandis* to such manufacture of blood products, unless other requirements have been approved by the Central Licence Approving Authority.]

¹[PART XIID

REQUIREMENTS FOR COLLECTION, PROCESSING, TESTING, STORAGE, BANKING AND RELEASE OF UMBILICAL CORD BLOOD DERIVED STEM CELLS

(A) GENERAL REQUIREMENTS

1. Location, Surroundings and Building: The building (s) for storage of Umbilical cord blood shall be so situated and shall have such measures as to avoid risk of contamination from external environment including open sewage, drain, public lavatory or any factory which produces disagreeable or obnoxious odour or fumes, excessive soot, smoke, chemical or biological emissions.
2. Buildings and premises: (1) The premises used for processing and storage shall be designed, constructed and adapted and maintained to ensure that the above operations and other ancillary functions are performed smoothly under hygienic conditions and in sterile areas wherever required. They shall also conform to the conditions laid down in the Factories Act, 1948 (63 of 1948).

The premises shall be:

- (a) Adequately provided with working space to allow orderly and logical placement of equipment, material and movement of personnel so as to maintain safe operations and prevent contamination;
- (b) Designed / constructed / maintained to prevent entry of insects, pests, birds, vermins and rodent. interior surfaces (walls, floors, ceilings and doors) shall be smooth and free from cracks, and permit easy cleaning, painting and disinfection, and in aseptic areas the surfaces shall be impervious, non-shedding, non-flaking and non-cracking;
- (c) Flooring shall be unbroken and provided with a cove both at the junction between the wall and the floor as well as the wall and the ceiling.

1. Inserted by G.S.R. 899(E) dated 27-12-2011

- (d) Provided with light fitting and grills which shall flush with the walls and not hanging from the ceiling to prevent contamination;
 - (e) If provided with fire escapes, these shall be suitably installed in the walls without any gaps;
 - (f) Provided with the furniture in aseptic areas which is smooth, washable and made of stainless steel or any other appropriate non shedding material other than wood;
 - (g) Provided with separate areas for processing and storage of products to prevent mix- ups, product contaminations and cross contamination;
 - (h) Provided with defined environmental conditions for temperature, humidity, ventilation and air filtration. Classifications shall be defined and, if appropriate, monitored.
- (2) A periodical record of cleaning and renovating of the premises shall be maintained.
3. Disposal of waste and infectious materials:
- (a) Waste materials awaiting disposal shall be stored safely;
 - (b) The disposal of sewage and effluents from the facility shall be in conformity with the requirements of the Pollution Control Board;
 - (c) All bio-medical waste shall be dealt with in accordance with the provisions of the Bio-medical Waste Management and Handling Rules, 1996.
4. Health, clothing and Sanitation of personnel:
- (a) All personnel shall undergo medical examination prior to employment and shall be free from infectious and contagious diseases and thereafter they should be medically examined periodically at least once a year and for this purpose records shall be maintained thereof;
 - (b) All personnel, prior to and during employment, shall be trained in practices which ensure personal hygiene and a high level of personal hygiene shall be observed by all those engaged in the collection, processing, banking of umbilical cord blood;
 - (c) All persons shall wear clean body coverings appropriate for their duties before entering the Processing Zone and the Change Rooms with adequate facilities shall be provided prior to entry into any specific zone;
 - (d) Smoking, eating, drinking is prohibited inside the Laboratory;
 - (e) All personnel working in the Laboratory shall be protected against virus infections.
5. Requirements for Processing, Testing and Storage Areas for Umbilical cord blood stem cells:
- (a) Separate dedicated areas specifically designed for the purpose and the workload shall be provided;
 - (b) There shall be separate areas for designated work purposes namely:-
 - (i) Cord blood Reception: cord blood reception area with space for transient storage of units and physical examination shall have adequate facilities for registration, date entry and generation of bar-coded labels. Air condition area of at least 10.00 Sq. meters shall be provided;
 - (ii) Cord blood processing area: The room shall be clean and have an air handling System to provide a Class 10,000 environment. Entry to this area shall be through air lock. The room will house Class 100 biological safety cabinets for Umbilical cord blood processing. The temperature of the clean room shall be maintained 20 °C to 25°C and with a positive differential pressure of 10-15 pascals and Relative humidity of 50-60% Minimum area shall be 10.00 Sq. meters for the activity;
 - (iii) Haematology and Serology Laboratory: The laboratory shall be equipped and utilized for the purpose of independently testing of Umbilical Cord Blood for ABO grouping and Rh Typing, Total Nucleated Cell Count, Progenitor cell count and

- viability test. The room shall be air-conditioned and area of at least 10.00 Sq. meters shall be provided.
- (iv) Transfusion Transmissible Disease Screening Laboratory: The Laboratory shall be equipped and utilized for screening tests on maternal blood for infectious diseases viz. HIV I & II; Hepatitis B & C virus, syphilis, malaria, CMV and HTLV. The room shall be air-conditioned and area of at least 10.00 Sq. meters shall be provided.
 - (v) Sterility Testing Laboratory: The laboratory shall be used for performing Sterility tests on Umbilical Cord blood unit. The premises may be classified depending on the testing method used. The room shall be air-conditioned with adequate and ancillary area for media preparation, sterilization, incubation and decontamination. Area of at least 10.00 Sq. meters shall be provided.
 - (vi) HLA Typing Laboratory: The Umbilical Cord blood unit shall have arrangements for HLA typing and genetic disease testing. In-house testing can be done by providing a well demarcated laboratory from the processing area for evaluation of possible genetic disease and HLA typing. The area shall have Class 100,000 environment and air-conditioned and area of at least 10.00 Sq. meters shall be provided.
 - (vii) Sterilization-cum-washing: Appropriate facility shall be provided within the premises for proper washing and sterilization. This facility would be optional for laboratories using entirely disposable items.
 - (viii) Records and Store Rooms: There shall be designed record room(s) and store room(s) of at least 10.00 Sq. meters each. The access to record room shall be permitted only to authorized person. The room will have adequate protective facilities as the documents and records are to be preserved for long years.
 - (ix) Cryogenic Storage room: A minimum space of 20.00 sq. meters shall be provided by the licensee. The cryogenic storage room shall have provision for temperature monitoring of storage vessels, liquid nitrogen level in storage vessels and oxygen meter. The service space between each liquid nitrogen storage vessel, supply cylinders and connecting hose should be minimum 1.00 sq. Meters. Separate storage space for other accessories required shall be provided. The room shall be air-conditioned.
 - (x) General Storage area: General storage area shall be provided to store all the consumables, under conditions deemed optimum for storage by manufacturers.

B. COLLECTION AND STORAGE OF PROCESSED UMBILICAL CORD BLOOD COMPONENT

1. Collection:

- (a) Umbilical Cord blood unit specific for an individual will be collected after signing an agreement with the parents, whose child's Umbilical Cord blood is to be collected and the cord blood bank. Private and Public Umbilical Cord blood banking to have different agreements;
- (b) Umbilical Cord blood shall be collected from hospitals, nursing homes, birthing centers and from any other place where a consenting mother delivers, under the supervision of the qualified Registered Medical Practitioner responsible for the delivery;
- (c) The cord blood shall be collected aseptically in a disposable PVC bag, containing adequate quantity of sterile, pyrogen free anti-coagulant and sealed effectively and such PVC Bags shall be procured from licensed manufacturer;
- (d) The Umbilical Cord blood would be collected from a premises operating in hygienic condition to allow proper operation, maintenance and cleaning.

2. Transportation:

- (a) Umbilical Cord blood shall be transported from the birthing center to the designated laboratory under and as per procedure prescribed by the cord blood bank;
- (b) The transportation procedure shall be validated to ensure optimum survival of the Stem Cells;
- (c) The transportation temperature should be between 18 to 28°C;
- (d) The time period between collection and processing shall not exceed 72 hours.

3. Storage:

- (a) The Umbilical Cord blood shall be stored at room temperature between 20 to 25°C in the reception area prior to processing;
- (b) Samples pending tests for specific transfusion transmittable infectious diseases shall be stored in a segregated manner.

Note:- Temperature range between 4 to 37 degrees Celsius, for the whole time period of transit may be allowed beyond the 18°C to 28°C in exceptional cases. The effects of deviation of transit temperature from the optimum, on the product shall be adequately explained by the licensee in the client education booklet.

C. PERSONNEL

Cord blood bank shall have following categories of whole time competent technical staff, namely:-

1. Medical Director:- The operation of cord blood bank shall be conducted under the active directions and supervision of a Medical Director who is a whole time employee and is possessing a Post Graduate degree in medicine – MD [Pathology/Transfusion Medicine/Microbiology] and has experience / training in cord blood processing and Cryogenic Storage.
2. Laboratory In-charge: The laboratory in-charge shall have Post Graduate qualification in Physiology or Botany or Zoology or Cell Biology or Microbiology or Biochemistry or Life Sciences or Graduate in Pharmacy and one year working experience in pathological laboratory licensed by the local health authority or any microbiology laboratory of a licensed drug manufacturing / testing unit and or experience / training in cord blood processing and cryogenic storage.
3. Technical Supervisor (cord blood processing):- The technical supervisor shall have a:
 - (a) Degree in Physiology or Botany or Zoology, Pharmacy or Cell Biology or Bio Sciences or Microbiology or Biochemistry or Medical Laboratory Technology (M.L.T.) with minimum of three years of experience in the preparation of blood components and / or experience or training in cord blood processing and Cryogenic Storage; or
 - (b) Diploma in Medical Laboratory Technology (M.L.T.) with five years experience in the preparation of blood components and experience or training in cord blood processing and Cryogenic Storage shall be essential.
4. Cord Blood Bank Technician(s):- The technicians employed shall have a:
 - (a) A degree in Physiology or Botany or Zoology or Pharmacy or Cell Biology or Bio Science or Microbiology or Biochemistry or Medical Laboratory Technology (M.L.T.) with six months experience and or training in cord blood processing and cryogenic storage; or
 - (b) Diploma in Medical Laboratory Technology (MLT) with one year experience in the testing of blood and / or its components and / or experience or training in cord blood processing and Cryogenic Storage.

D. AIR HANDLING SYSTEM

1. Air handling for sterile areas shall be different from those for other areas. The filter configuration in the air handling system shall be suitably designed to achieve the grade of

air as given in the Table I. The environmental microbiological monitoring of clean areas shall be in accordance to the recommended limits given in Table II.

2. The Processing area shall have HVAC system and fitted with HEPA Filters having Grade C (Class 10,000) environment as given in Table I.
3. The entire processing shall be done conforming to Grade A (Class 100) Standard of air quality.

TABLE I
AIR BORNE PARTICULATE CLASSIFICATIONS FOR MANUFACTURE OF STERILE PRODUCTS

Grade	Maximum number of permitted particles per cubic meter equal to or above			
	At rest (b)		In Operation (a)	
	0.5µm	5 µm	0.5 µm	5µm
A	3,500	0	3,500	0
B(a)	3,500	0	3,50,000	2000
C(a)	3,50,000	2000	35,00,000	20,000
D(a)	35,00,000	20,000	Not defined	Not defined

Notes:-

- (a) In order to reach the B, C and D air grades, the number of air changes shall be related to the size of the room and the equipment and personnel present in the room. The air system shall be provided with the appropriate filters such as HEPA for grades A, B and C. The maximum permitted number of particles in the “at rest” condition shall approximately be as under:-
[Grade A and B corresponds with class 100 or M 3.5 or class 5]; Grade C with Class 10,000 or M 5.5 or ISO Class 7; Grade D with Class 1,00,000 or M 6.5 or ISO Class 8.
- (b) The requirement and limit for the area shall depend on the nature of the operation carried out.

TABLE II
RECOMMENDED LIMITS FOR MICROBIOLOGICAL MONITORING OF CLEAN AREAS “IN OPERATION”

Grade	Air samples Cfu/m3	Settle Plates (dia 90mm) cfu/2hrs	Contact plates (dia 55 mm) cfu per plate	Glove points (Five fingers) cfu per glove
A	Less than 1	Less than 1	Less than 1	Less than 1
B	10	5	5	5
C	100	50	25	-
D	500	100	50	-

Notes:-

- (a) These are average values.
- (b) Individual settle plates may be exposed for not less than two hours in Grade B, C and D areas and for not less than thirty minutes in Grade A area.

E. QUALITY CONTROL

1. Facilities shall be provided for Quality Control such as Haematological, Microbiological and Instrumental testing.

2. Following duties shall be performed under the function of quality control:
 - (a) To prepare detailed instructions for carrying out such tests and analysis;
 - (b) To approve or reject raw materials and consumables, used in any step, on the basis of approved specifications;
 - (c) Haematological tests like Total Nucleated Cell Counts, Mononuclear Cell Count, Enumeration of the population of Stem Cells, Stem Cell viability shall be performed on samples of processed Umbilical cord blood unit;
 - (d) Microbiological Tests shall be done on Maternal Blood samples for freedom from Hepatitis B Surface Antigen, Hepatitis C Virus antibody, HIV I and II antibodies. Syphilis, Malaria, CMV and HTLV. Bacterial and Fungal Culture shall be done on the umbilical cord blood samples;
 - (e) Instruments which would be used to process test and store the UCB unit would be validated before commissioning and calibrated from time to time to check their conformity to specific standards according to an approved and valid protocol;
 - (f) The environmental monitoring of the clean rooms would be done at periodic intervals according to an accepted and validated protocol;
 - (g) All tests mentioned above shall be done in house except tests under itme numbers (e), (f) and test for enumeration of Stem Cell Population, HLA typing and Genetic Disease Testing which may be outsourced to a competent third party approved by the licensing authority.

F. SCREENING TESTS

1. The maternal blood sample shall be tested for
 - (a) Hepatitis B;
 - (b) Hepatitis C;
 - (c) HIV 1 & 2;
 - (d) Syphilis;
 - (e) Malaria;
 - (f) CMV;
 - (g) HTLV
2. The Umbilical Cord Blood shall be tested for
 - (a) Total Nucleated Cell count;
 - (b) Total Mononuclear Cell Count;
 - (c) Progenitor Cell (CD34+) enumeration;
 - (d) Cell Viability;
 - (e) ABO Group and Rh Type;
 - (f) Sterility as regards Bacterial and Fungal contamination status;
 - (g) HLA Matching (Only for allogenic Cord Blood Units).

G. STORAGE

1. The Umbilical cord blood shall be cryopreserved using a controlled rate freezing or equivalent validated procedures. The frozen storage shall be at minus 196°C and shall not be warmer than minus 150°C.
2. There will be no shelf life for this class of product.

H. REFERENCE SAMPLES

1. At least two reference samples shall be collected from cord blood unit product prior to cryopreservation and stored at minus 196°C and shall not be warmer than minus 150°C.
2. At least one additional reference sample shall be stored at minus 76°C or colder for the purposes other than viability analysis.

I. LABELLING

1. Initial label placed during collection shall specify:

- (a) Human Umbilical Cord Blood;
 - (b) Approximate Volume or weight of contents in the collection bag [UCB+ Anticoagulant];
 - (c) Mother's name;
 - (d) Place of collection;
 - (e) Date and time of collection;
 - (f) Collected by;
 - (g) To be labeled in bold, "ROOM TEMPERATURE ONLY– DO NOT REFRIGERATE, DO NOT IRRADIATE";
 - (h) Manufacturing license number.
2. Label at completion of processing and before issue - Cryogenic Storage Label [Statutory label] shall indicate the following:-
- (a) Name of product:- Human Progenitor Cell [HPC] – Cord Blood;
 - (b) Volume or weight of contents;
 - (c) Percentage of Cryoprotectant [DMSO];
 - (d) Percentage of any other additive / preservative;
 - (e) Date of collection [birth]
 - (f) Date of processing
 - (g) Name of manufacturer.....;
 - (h) Manufacturing license number;
 - (i) Storage temperature – not less than, - 196°C and shall not be warmer than minus 150°C,
 - (j) Unique Traceability Number and / or BAR Code.
3. Issue label at the time of release of Cord Blood Unit shall indicate the following namely:-
- (a) Name of manufacturer;
 - (b) License number;
 - (c) All details of the Cryogenic Storage Label;
 - (d) The results of Total Nucleated Cells, Progenitor Cell percentage {CD34+}, Viability;
 - (e) Results of Transfusion Transmittable diseases testing on maternal blood;
 - (f) ABO Group and Rh Type;
 - (g) Date of processing;
 - (h) Result of HLA typing (allogenic);
 - (i) Statement "properly identify intended Recipient and Product";
 - (j) A statement indicating that leukoreduction filters should not be used;
 - (k) Statement "Do not irradiate"
 - (l) Name and address of receiving hospital.

J. RECORDS OR DOCUMENTATION

1. The licensee shall maintain the following records
 - (a) Client / donor enrolment / agreement record;
 - (b) Collection of unit and transportation record;
 - (c) Master record of stored unit;
 - (d) HLA Matching record;
 - (e) Unit Release Register;
 - (f) Stock Register for Blood Collection Bag Cryoprotectant and Preservative, RBC Sedimentation Enhancer;
 - (g) Stock Register for Diagnostic Kits, Reagents and other consumables;
 - (h) Record on feedback after use of cord blood / Adverse reaction record.
2. The following Standard Operating Procedures shall be maintained by the licensee, namely:-
 - (a) Umbilical Cord Blood collection;
 - (b) Transportation of the collected Umbilical cord Blood unit;
 - (c) Processing of Umbilical cord blood unit;
 - (d) Cryogenic storage of processed umbilical cord blood unit;

- (e) Testing of maternal blood for transfusion transmittable infections;
- (f) Testing of Umbilical cord blood for ABO Grouping and Rh Typing;
- (g) Testing of Umbilical cord blood unit for Total Nucleated Cell Count, Mononuclear Cell Count, Progenitor Cell (CD34+) enumeration, and viability;
- (h) Testing of Umbilical cord blood stem cell unit for sterility;
- (i) Disposal of bio medical waste;
- (j) Dispensation of Umbilical cord blood unit;
- (k) Preventive maintenance Protocol for all Instruments;
- (l) Acceptance / Rejection procedure of consumables;
- (m) Environment monitoring of classified areas;
- (n) Any other standard operative procedure as per requirements.

K. CORD BLOOD RELEASE

1. There shall be designated area with adequate space for procedures and records related to cord blood unit selection and release.
2. The cord blood bank shall obtain written or electronic request from the transplant physician or designee for shipment of the cord blood unit.
3. Accompanying documentation at the time of issue from the cord blood bank shall include indications, contra-indications, caution, instruction for handling and use of the cord blood unit including short-term storage and preparation for transplantation.
4. Procedure for transportation of cryopreserved cord blood unit within the facility shall be designed to protect the integrity of the unit and the health and safety of the personnel.
5. Cryopreserved cord blood unit stored at -150°C or colder shall be transported in a liquid nitrogen cooled dry shipper that contains adequate absorbed liquid nitrogen and has been validated to maintain temperature below -150°C for at least 48 hours beyond the expected time of arrival at the receiving facility.

¹[PART XIII]

GENERAL

1. For the purposes of this Schedule, any test or method of testing described in the ²[Indian Pharmacopoeia] shall be deemed to be a method approved by the Licensing Authority.
2. The Licensing Authority shall publish in the Official Gazette from time to time particulars of any test or method of testing approved by him.

1. Renumbered by Notification No. F-18-1/46, dt. 18-6-48

2. Subs. by G.S.R. 19, dated 15-12-1977

¹[SCHEDULE F(I)

PART 1—VACCINES

(A) PROVISIONS APPLICABLE TO THE PRODUCTION OF BACTERIAL VACCINES:

1. Definition.—(1) This part of the Schedule applies to bacterial vaccines made from any micro-organism pathogenic to man or other animal and to vaccines made from other micro-organisms which have any antigenic value.

(2) For the purposes of this part of the Schedule, a bacterial vaccine means a sterile suspension of a killed culture of the micro-organism from which the vaccine derives its name or a sterile extract or derivative of a micro-organism, or a pure suspension of living micro-organisms which have been previously made avirulent.

2. Staff of Establishment.—A competent expert in bacteriology with sufficient experience in the manufacture and standardisation of biological products shall be in charge of the establishment responsible for the production of bacterial vaccine and he shall be assisted by a staff adequate for carrying out the tests required during the preparation and standardisation of the vaccines.

3. Proper Name.—The proper name of any vaccine shall be the name of the micro-organism from which it is made followed by the word “Vaccine” unless this Schedule otherwise provides or if there is no other special provision in this Schedule, some other name as approved by the Licensing Authority. Provided that in the case of the undermentioned preparations the proper name of the vaccine may be as follows: —

1. Anthrax Spore Vaccine (Living).
2. Blackquarter Vaccine.
3. Enterotoxaemia Vaccine.
4. Fowl Cholera Vaccine.
5. Haemorrhagic Septicaemia Adjuvant Vaccine.
6. Haemorrhagic Septicaemia Vaccine (Broth).
- ²[7. Multi Component Clostridial Vaccine.
8. Hemorrhagic Septicaemia Vaccine – Alum Treated.]

4. Records.—Cultures used in the preparation of vaccine before being manipulated into a vaccine, should be thoroughly tested for identity by the generally accepted tests applicable to the particular micro-organisms.

The permanent records which the licensee is required to keep shall include amongst others, a record of the origin, properties and characteristics of the cultures.

5. Combined Vaccines.—Vaccines may be issued either singly or combined in any proportion in the same container. In the case of combination of vaccines, a name for the combined vaccine may be submitted by the licensee to the Licensing Authority, and if approved, may be used as the proper name of the vaccine.

1. Added by Ministry of Health F.P., W.H and U.D. Notfn. No. F.1-6/62-D (SO. 2889), dt. 2-7-1969

2. Ins. by G.S.R. 659(E), dt. 31.8.1994.

6. Preparation— Bacterial vaccines, simple or polyvalent, are prepared from selected cultures after careful examination for their identity, specificity, purity and antigenicity. They may be prepared in the following manner:—

(a) *Formal Cultures or Bacterins*.— The selected pure culture strain or strain are grown in a suitable fluid medium, at an optimum temperature, for an appropriate period. The pure growth is then exposed to the action of solution of Formaldehyde I.P. in suitable concentration and temperature. The product is finally filled in suitable sterilised containers which are subsequently sealed.

(b) *Vaccine of Bacterial Products or Bacterial Derivatives*.—These vaccines are prepared by growing the organisms on suitable media and then deriving specific antigenic constituents of the bacteria by various special methods.

(c) *Living Bacterial Vaccines*.— They are prepared from non-pathogenic but fully immunogenic strains of micro-organism. Strict aseptic precautions are taken throughout the preparation against the introduction of microbial contaminants.

7. General Standards:

(a) *Description*.— Bacterial vaccines are colourless to yellowish brown liquids containing dead or viable bacteria in homogenous suspension.

(b) *Identification*.—All types of vaccines confer active immunity in the susceptible animals which can be demonstrated by injecting suitable experimental animals with the calculated doses of the product and subsequently determining the presence of the protective antibodies in their serum and/or by challenging the vaccinated animals by injecting virulent strain of the homologous organisms. The protected animals should survive the challenge.

(c) *Test for Sterility*.—All bacterial vaccines shall be tested for sterility in accordance with the provision of Rules 115 to 119 (both inclusive). If the vaccine contains added bactericide or bacteriostatic, a quantity of medium sufficient to render the growth inhibitor ineffective is added to the sample, or a suitable substance is added to the sample, or a suitable substance is added in concentration sufficient to render the growth inhibitor ineffective but not itself to inhibit the growth of micro-organism.

(d) *Purity Tests for Living Bacterial Vaccine*.—Petri dishes containing suitable media are streaked with the final product and incubated at 37° C for 72 hours. The vaccine passes the test if no growth of micro-organisms other than those from which the vaccine was prepared is observed. Other tests include examination for motility of the organisms, fermentation reactions and thermoagglutination test and dye-inhibitor tests in case of bruceliza vaccine.

(e) *Safety Test*.— The safety of the vaccine shall be assessed by injecting it in appropriate doses in suitable susceptible animals. No animal should show any untoward, general or local reaction within seven days after inoculation.

(f) *Potency Test*.—Wherever applicable, susceptible experimental animals are inoculated with the calculated doses of the final product. The animals are challenged after the period of immunisation, with virulent infective dose of the homologous culture along with the controls. The potency of the vaccine is assessed by the survival of the vaccinated animals and the death of the controls.

8. Labelling:

(a) The label on the ampoule or the bottle shall indicate:—

- (i) Proper name.
- (ii) Contents in millilitres or doses.
- (iii) Potency, if any.

- (iv) Batch number.
 - (v) Expiry date.
- (b) The label on the outside container shall indicate:
- (i) Proper name.
 - (ii) Contents in Millilitres or doses.
 - (iii) Batch number.
 - (iv) Date of manufacture.
 - (v) Manufacturing licence No.
 - (vi) Manufacturer's name and address.
 - (vii) "For animal treatment only".
 - (viii) Storage conditions.

9. Storage.—Bacterial vaccines shall be stored, protected from light at temperature between 2°C to 4°C and shall not be frozen.

10. Date of manufacture.—The date of manufacture shall be, unless otherwise specified in the individual monograph in this part, as defined in clause (b) of sub-rule (3) of rule 109.

Anthrax Spore Vaccine (Living)

1. Synonyms.—Avirulent Anthrax Spore Vaccine or Bacillus Anthracis Vaccine (Living).

2. Definition.—The vaccine is a suspension of living spores of an uncapsulated avirulent strain of B anthracis in 50 per cent glycerine saline.

3. Preparation.—Avirulent B, anthracis of known antigenicity is grown on suitable medium at pH 7.4 in Roux flasks. After 72 hours incubation at 37° C, the pure sporulated culture growth which shows 70 to 80 per cent sporulation is washed with normal saline and glycerinated to the extent of 50 per cent by weight of the culture washing and the whole suspension is kept at room temperature for twenty-one days to allow for the stabilization of the spores.

4. Standard:

(a) *Description.*— It is slightly opalescent or pale brown semi-viscous liquid.

(b) *Identification.*—Uncapsulated B anthracis which is avirulent can be isolated from the vaccine.

(c) *Sterility test.*— Should comply with the test for sterility described in the general monograph on "Bacterial Vaccine".

(d) *Purity Test.*— Complies with the "Purity Tests for Living Bacterial Vaccine" described under the general monograph on "Bacterial Vaccines".

(e) *Safety Test.*—Four healthy adult guinea-pigs each weighing 300-450 g. not previously treated with any material which will interfere with the test are inoculated subcutaneously, two with 0.2 ml. each and two with 0.5 ml. each of the unglycerinated suspension respectively. Four more guinea-pigs are injected with 1:5 dilution of the glycerinated product in the same manner. No untoward reaction should be observed and none of the animals should die of anthrax during the period of observation for seven days.

(f) *Safety and Potency Test in sheep and goat*—Spore count of the glycerinated suspension is made after twenty-one days from the date of glycerination. Three plates for each of the three dilution 10^5 , 10^6 and 10^7 are made.

Eight sheep and eight goats each weighing not less than 18 kg. are injected subcutaneously in the following manner:—

- two sheep : Each subcutaneously with 10 ml. of the stock suspension (for safety).
- two goats : Each subcutaneously with 5 ml. of the stock suspension (for safety).
- six sheep : Each subcutaneously with one million spores suspended in 50 per cent glycerine saline solution.
- six goats : Each subcutaneously with one million spores suspended in 50 per cent glycerine saline solution.

None of these animals should die of anthrax. Twenty one days after vaccination, the animals are challenged with 100 lethal doses of virulent *B. anthracis* spores along with two healthy sheep and two goats as controls.

All the controls should die of anthrax within 72 hours after challenge and at least 66 per cent of the vaccinated animals should survive. The animals shall be observed for a minimum of ten days from the date of challenge.

¹[(g) *Viable Count*.— The vaccine when plated on suitable media should show 10 million viable spores per cattle dose and 5 million spores per sheep dose.]

5. Labelling and Storage.— Should comply with the requirements for “Labelling” and “Storage” as laid down in the general monograph on “Bacterial Vaccines”.

²[6. *Expiry Date*.— The date of expiry of the potency of the vaccine shall be not more than two years from the date of manufacture if stored in 4° C and six months, if stored at room temperature.]

Blackquarter Vaccine

1. Synonym.—Blackleg vaccine or Quarter Evil Vaccine.

2. Definition.— Blackquarter Vaccine is a culture of *Clostridium chauvoei* grown in a suitable anaerobic fluid medium and rendered sterile and toxic by the addition of Solution of Formaldehyde I.P. in such a manner that it retains its immunising properties.

3. Preparation.—Cultures of *Cl. Chauvoei* are grown in a suitable anaerobic fluid medium and killed by the addition of a suitable concentration of Solution of Formaldehyde I.P. The final product shall be adjusted to pH.7.0.

4. Standards:

(a) *Description.*—It is a yellowish brown liquid containing dead bacteria in suspension.

(b) *Identification.*—It protects susceptible animals against infection with *Cl. Chauvoei*.

(c) *Sterility Test.*—Should comply with the test for sterility described in the general monograph on “Bacterial Vaccine”.

1. Subs. by. G.S.R 659 (E) ,dt.. 31-8-1994.

2. Ins. by. G.S.R 659 (E) ,dt.. 31-8-1994.

(d) *Safety and Potency Tests.*—At least six adult healthy guinea-pigs each weighing 300 g to 450 g are injected subcutaneously each with 3 ml. of the product followed a week later by a second injection with the same dose. They should not show any systemic reaction but may show only a minimum of local reaction. Fourteen days after the second injection six of the vaccinated guinea-pigs are challenged intramuscularly with 25 viable spores of *Cl. Chauvoei* equivalent to 5 c.h.d. along with 0.2 ml. of a 5 per cent solution of calcium chloride. Two controls are used. The controls should die of the specific injection and at least 4 of the six vaccinated animals should survive before the product is passed for issue.

5. *Labelling and Storage.*— Should comply with the requirements of “Labelling” and “Storage” as laid down in the general monograph on “Bacterial Vaccines”.

6. *Expiry Date.*— The date of expiry of the potency of the vaccine shall not be more than twenty-four months from the date of manufacture.

Brucella Abortus (Strain 19 Vaccine) (Living)

1. *Synonym.*—Contagious Abortion Vaccine, (Strain 19) (Living).

2. *Definition.*—*Brucella Abortus* (Strain 19) Vaccine (Living) is a suspension of a pure smooth living culture of *Br. Abortus* of low virulence in normal saline solution.

3. *Preparation.*—Forty eight to seventy-two hour old growth of *Br. Abortus* (Strain 19) on potato agar medium in Roux flasks washed with buffered normal saline solution pH 6.4 and the pure growth from the flasks are pooled together, 0.5 ml. of the pooled product is mixed with 4.5 ml. of normal saline solution at pH 6.4 in graduated centrifuge tube and centrifuge at 3000 r.p.m for one hour. The percentage of cell deposit is assessed by reading the amount of cell deposit obtained.

The concentrated suspension is then diluted with buffer normal saline solution so that the final product contains 0.72 per cent bacterial cell deposit.

4. *Standard:*

(a) *Description.*—It is an almost white turbid liquid containing live bacteria in suspension.

(b) *Identification.*—It consists of Gram-negative bacilli capable of protecting susceptible animals against Brucellosis.

(c) *Sterility Test.*—Should comply with the test for sterility described in the general monograph on “Bacterial Vaccine”.

(d) *Purity Test.*—A smear of the finished products is examined microscopically after staining by Gram’s method for evidence of any contamination. When grown on suitable media, *Br. Abortus* should be obtained in a pure state.

(e) *Safety Test.*—Two healthy guinea-pigs each weighing 300 g. to 450g. are inoculated subcutaneously each with 1.0 ml. of the final product. The guinea-pigs should not show excessive reaction of a toxic nature during the period of observation of ten days.

(f) *Potency Test.*—Each of a group of four healthy guinea-pigs, drawn from a uniform stock and each weighing 300 g. to 450 g. is injected intra-muscularly with 1 ml. of the vaccine, and is challenged nine weeks after vaccination by the intramuscular injection of 1 ml. of a suspension containing 5,000 fully virulent *Br. Abortus* organisms. Each of a group of two unvaccinated guinea-pigs is similarly injected. After a further six weeks, the guinea-pigs are killed and cultures are made from their spleens. More than half of the vaccinated guinea-pigs contain no demonstrable *Br. Abortus* in the spleen; all the controls are infected.

(g) *Viable Count*.—The vaccine when plated on suitable media should show between 14, 000 million and 18,000 million *Br. Abortus* organisms per ml. At least 80 per cent brucella organisms should be in the smooth phase.

4. **Labelling and storage**.— Should comply with the requirements of “Labelling” and “Storage” as laid down in the general monograph on “Bacterial Vaccines”. The liquid vaccine shall be issued fresh as far as possible without allowing any period of storage after manufacture.

5. **Expiry Date**—The date of expiry of the vaccine shall be not more than five weeks from the date of manufacture.

Enterotoxaemia Vaccine

1. **Synonyms**.—Clostridium Welchii, Type D, Formal Culture: Pulpy Kidney Vaccine.

2. **Definition**.—Enterotoxaemia Vaccine is a culture of a highly toxigenic strain of *Clostridium* type D, group is an anaerobic medium rendered sterile and toxic by the addition of Solution of Formaldehyde I.P in such a manner that it retains its immunising properties.

3. **Preparation**.— Selected toxigenic strain of *Cl. Welchii* type D, is grown in a liquid medium under conditions which ensure maximum epsilon toxin production. The culture is checked for purity and toxicity as tested in mice. Solution of Formaldehyde I.P. is added in suitable concentration and the formolised culture is kept at 37° C till the production is sterile and non-toxic.

4. **Standard:**

(a) *Description*- It is a yellowish brown liquid containing dead bacteria in suspension.

(b) *Identification*- When injected into susceptible animals it stimulates the production of epsilon antitoxin of *Cl. Welchii*, type D.

(c) *Sterility Test*- Complies with the test for sterility described in the general monograph on ‘Bacterial Vaccines’.

(d) *Safety and Potency Tests*- At least eight sheep each weighing not less than 18 kg. or twelve rabbits each weighing 1 kg. to 1.5 kg. are used for testing the safety and potency of each brew of the vaccine. Two sheep receive subcutaneously 10 ml. each and the other six sheep receive each 2.5 ml. of the product subcutaneously. The rabbits are given subcutaneously a dose of 5 ml. each. The sheep and rabbits are observed for five days. They should show only a minimum local reaction and no systemic reaction.

The sheep receiving 10 ml. are withdrawn from experiments after five days. Each of the other six sheep is inoculated with a second dose of 2.5 ml. fourteen days after the first injection. The rabbits are inoculated with 5 ml. as a second dose, after one month of the first inoculation. Ten days after the second inoculation the sera of sheep or rabbits are pooled separately. The pooled serum of each group of animal shall contain in each ml. not less than two international units of *Cl. Welchii* epsilon antitoxin which is determined by testing on mice as follows:

One ml. of the pooled serum is mixed with one ml. of the epsilon toxin of *Cl. Welchii* type D, containing 300 mouse-minimum-lethal-doses (mouse m.l.d.) and kept at room temperature for half an hour. At least two mice each weighing not less than 18 g. are each given intravenously 0.2 ml. of the mixture. As control two mice each weighing not less than 18 g. should each receive 0.2 ml. of the toxin containing 300 mouse m.l.d per ml. diluted with equal volume of normal saline. The control mice should die within 1 to 2 hours while the mice receiving the mixture of serum and toxin should survive for at

least two days. Sera containing one International Unit of epsilon antitoxin per ml. will be able to neutralise 150 mouse m.l.d. of epsilon toxin of *Cl. Welchii*, type D.

5. Labelling and Storage.—Should comply with the requirements regarding “Labelling” and “Storage” as laid down in the general monograph on “Bacterial Vaccines”.

6. Expiry Date.—The expiry date of potency of the vaccine shall be not more than twelve months from the date of manufacture.

Fowl Cholera Vaccine (Polyvalent)

1. Synonym.—*Pasteurella Septica* Vaccine (Avian).

2. Definition.—Fowl Cholera Vaccine is a formalised pure broth culture of virulent strains of *Pasteurella Septica* (Avian).

3. Preparation.—The strains are grown separately in nutrient broth for 48 hours at 37° C. The pure growth is killed by the addition of a Solution of Formaldehyde I.P in a suitable concentration. The cultures are then mixed in equal proportions and the final vaccine is bottled in suitable containers.

4. Standard-

(a) *Description.*—It is a light yellow liquid containing dead bacteria in suspension.

(b) *Identification.*—It protects susceptible birds against *P. aviseptica* infection.

(c) *Sterility test.*—Complies with the test for “Sterility” described under the general monograph on “Bacterial Vaccines”.

(d) *Safety Test.*—Two healthy young fowls each weighing not less than 400 g. or twelve healthy mice are inoculated subcutaneously each with 1 ml. of the final product. The birds should not show any untoward reaction during the period of observation for seven days.

5. Labelling and Storage.—Should comply with the requirements of “Labelling” and “Storage” as laid down in the general monograph on “Bacterial Vaccines”.

6. Expiry Date.—The date of expiry of potency of the Vaccine shall be not more than six months from the date of manufacture.

Hemorrhagic Septicaemia Adjuvant Vaccine

1. Synonym.—*Pasteurella Septica* Adjuvant Vaccine.

2. Definition.—The vaccine is a homogenous suspension of formalised agar-washed *Pasteurella septica* with liquid paraffin and lanolin.

3. Preparation.—Pure growth of a highly antigenic strain of *P. Septica* in phase 1 grown on nutrient agar medium containing 0.5 per cent yeast extract is washed with 0.5 per cent formol saline. The pooled suspension is diluted with normal saline to contain approximately 2100 million *P. Septica* organisms per ml. The safety test of this adjusted suspension is conducted on four white mice each weighing not less than 18 g. and observed for three days before it is mixed with liquid paraffin and lanolin in suitable proportion.

The mixture is blended until a homogenous emulsion is obtained which is filled in suitable containers.

4. Standard:

(a) *Description*— It is a white thick oily liquid containing dead bacteria in suspension.

(b) *Identification.*—It protects susceptible animals against infection with *P. Septica*.

(c) *Sterility Test.*—It complies with the test for “Sterility” described in the general Monograph on “Bacterial Vaccines”.

(d) *Safety Test.*—Six white mice each weighing not less than 18 g. are inoculated intraperitoneally each with 0.5 ml. of the vaccine. None of the mice should die of pasteurellosis during the observation period for seven days.

(e) *Potency Test.*—Three susceptible calves in good condition between the ages of nine months to three years are injected intramuscularly, each with 2 ml. of the vaccine, in the case of animals weighing upto 140 kg. and 3 ml. for heavier ones.

Three weeks later these animals along with two healthy animals of the same type and species are challenged subcutaneously with 18 hours old broth culture of *P. Septica* equivalent to at least 50 million mouse minimum infective dose. Both the controls should die of pasteurellosis and at least two out of the three protected animals should survive the challenge dose for a period of seven days.

5. Labelling and storage- Should comply with the requirements for “Labelling” and “Storage” as laid down in the general monograph on “Bacterial Vaccines”.

6. Expiry Date- The date of expiry of potency of the vaccine shall be not more than twelve months from the date of manufacture.

Haemorrhagic Septicaemia Vaccine (Broth)

1. Synonym.—Pasteurella Septica Vaccine (Broth).

2. Definition.—Haemorrhagic Septicaemia Vaccine is formolised culture of a virulent strain of Pasteurella septica in nutrient broth.

3. Preparation.—*Septica* culture is grown in nutrient broth at 37°C. The pure growth is killed by the addition of a solution of Formaldehyde I.P. in a suitable concentration.

4. Standard:

(a) *Description.*—It is a pale yellow liquid containing dead bacteria in suspension.

(b) *Identification.*—It protects susceptible animals against infection with *P.Septica*.

(c) *Sterility Test.*—Complies with the test for “Sterility” described under the general monograph on “Bacterial Vaccines”.

(d) *Safety Test.*—Four healthy rabbits each weighing 1 kg. to 1.5 kg. are inoculated subcutaneously each with 5 ml. of the product. There should be no untoward reaction during the period of observation for seven days. Alternately two rabbits and six mice may be employed. The dose for mice will be 0.5 ml.

5. Labelling and Storage.—Should comply with the requirements of “Labelling” and “Storage” as laid down in the general monograph on “Bacterial Vaccines”.

6. Expiry Date.—The date of expiry of potency of the vaccine shall be not more than six months from the date of manufacture.

Salmonella Abortus Equi Vaccine

1. Synonym.—Equine Abortion Vaccine.

2. Definition.—Equine Abortion Vaccine is a mixture of equal parts of pure formolised cultures of smooth laboratory strains of *Salmonella abortus equi*.

3. Preparation.—The strains are grown separately on plain agar in Roux flasks, for 24-28 hours at 37° C. The pure growth is washed with normal saline solution and the washings are pooled together. The suspension is standardised to contain approximately 600 million *Sal.Abortus equi* organisms per ml. using normal saline solution as diluent. The culture is killed by the addition of sufficient quantity of solution of Formaldehyde I.P in a suitable

concentration and the product is kept at 37°C for seven days. Potassium alum is added to give a final concentration of 1 per cent

4. Standard:

(a) *Description.*—It is an opalescent liquid containing dead bacteria in suspension.

(b) *Identification.*—It protects susceptible animals against infection with *Sal. Abortus equi*.

(c) *Sterility Test.*—Complies with the test for sterility described in the general monograph on “Bacterial Vaccines”.

(d) *Safety Test.*—Six white mice each weighing not less than 18 g. are inoculated intraperitoneally each with 0.5 ml. of the product. None of the mice should die of salmonellosis. The mice are observed for ninety-six hours.

5. Labelling and Storage.—Should comply with the requirements for “Labelling” and “Storage” as laid down in the general monograph on “Bacterial Vaccines”.

6. Expiry Date.—The date of expiry of potency of the vaccine shall be not more than six months from the date of manufacture.

Streptococcus Equi vaccine

1. Synonym.—Strangles Vaccine

2. Definition.—*Streptococcus equi* Vaccine is a phenolised culture of a number of different isolates of *Streptococcus equi* in glucose serum broth.

3. Preparation.—Equal proportions of forty-eight hours old pure cultures of different isolates of *Str. Equi* in serum glucose both are mixed together. The suspension is centrifuged and the deposit is washed with normal saline solution after removing the supernatant. The washed cells are suspended in normal saline and heated in a water bath 65°C for two hours. Phenol and normal saline are added to give a final concentration of 1200 million *Str. Equi* organisms per ml. and 0.5 per cent of phenol in the vaccine.

4. Standard-

(a) *Description.*— It is a slightly opalescent liquid containing dead bacteria in suspension.

(b) *Identification.*—It protects susceptible animals against infection with *Str. Equi*.

(c) *Sterility Test.*—Complies with the test for “Sterility” described in the general monograph on “Bacterial Vaccines”, the nutrient broth being replaced by glucose broth.

(e) *Safety Test.*—Two ponies and two rabbits (each weighing not less than 1 kg.) are inoculated each with 10 ml. and 2 ml. respectively of the final product. The animals should not show any untoward reaction during the period of observation of seven days.

5. Labelling and Storage.—Should comply with the requirements for “Labelling” and “Storage” as laid down in the general monograph on “Bacterial Vaccines”

6. Expiry Date.— The date of expiry of potency of the vaccine shall be not more than six months from the date of manufacture.

Old Adjuvant Vaccine against Pasteurellosis in Sheep and Goats.

1. Synonym- Pasteurella Septica Adjuvant Vaccine for ovines and Caprines.

2. Definition- The vaccine is a homogenous suspension of formolised agarwashed Pasteurella septica of ovine origin with liquid paraffin and lanolin.

3. Preparation— Pure growth of highly antigenic strains (R1, R2, R4) in phase I grown separately on nutrient agar medium containing 0.5 per cent yeast extract is washed with 0.5 per cent Normal saline. Equal quantities of the suspension of three strains diluted with Normal saline to contain approximately 2100 million organisms per ml. is pooled together. The safety test of this adjusted pooled suspension is conducted in for white mice each weighing not less than 18 g. and observed for three days before it is mixed with liquid paraffin and lanolin in suitable proportion.

The mixture is blended until a homogenous emulsion is obtained which is filled in suitable containers.

4. Standards:

(a) *Description*- It is a white thick oily liquid containing dead bacteria in suspension.

(b) *Identification*- It protects susceptible animals against infection with *P. Septica*.

(c) *Sterility Test*- Complies with the test for sterility described in the general monograph on “Bacterial Vaccines”.

(d) *Safety Test*- Six white mice each weighing not less than 18 g. are inoculated intra-peritoneally each with 0.5 ml. of the vaccine. None of the mice should die of Pasteurellosis during the observation period of seven days.

The vaccine is also inoculated into six sheep and six goats in a dose of 3 ml. each intramuscularly and are observed for a period of seven days. During this period none should die of Pasteurellosis.

(e) *Potency Test*- Not being done at present.

5. Labelling and Storage- Should comply with the requirements for “Labelling” and “Storage” as laid down in the general monograph on “Bacterial Vaccines.”

6. Expiry Date- The expiry date of Potency of the Vaccine shall be not more than twelve months from the date of manufacture.

¹[**Multicomponent of Clostridial Vaccine**

1. Synonyms. – Combined anaculture of *Clostridium perfringens* type C and D, *Cl. septicum* and *Cl. oedematiens*.

2. Definition – It consists of four highly antigenic components containing the toxoids of *C. perfringens* type D, *Cl. Perfringens* type C, *Cl. oedematiens* and *Cl. septicum* which are prepared in double strength and then combined in such a proportion that would invoke adequate anti-toxin response in the vaccinated sheep against each antigen incorporated in the vaccine

3. Preparation – The above strains are grown separately in suitable liquid media under conditions which ensure maximum toxin production. The cultures are checked for purity and toxicity in mice. Solution of Formaldehyde I.P. of analytical grade is added to a 0.5 per cent final concentration and formalized cultures are kept at 37°C till the product is sterilized and atoxic. The formalized anacultures are pooled, precipitated by the addition of Aluminium Chloride, 20 per cent solution in distilled water to have a final concentration of the chemical to 10 per cent and pH adjusted to 6.0.the sedimented toxoid is reconstituted to have its original volume in normal saline.

4 Standards :

(a) *Description* – It is whitish liquid when shaken thoroughly to contain killed bacteria and toxoid in suspension.

(b) *Identification* – When injected to susceptible animals it stimulates the production of epsilon and beta antitoxins against *Cl. perfringens* type D and C and also antitoxins against *Cl. septicum* and toxin of *Cl. Oedematiens*.

(c) *Sterility Test* – Complies with the test of sterility described in general monograph on “Bacterial Vaccines.”

(d) *Safety Test* – Four sheeps each are inoculated with 10 ml. S/C of the product and these are observed for 7 days during which period animals shall not show any local or systemic reaction.

(e) *Potency Test* – Eight sheep each are inoculated with 2 doses of vaccines S/C at an interval of 21 days and bled on 10th day after 2nd inoculation for collection of serum for assessing the antitoxin titre against each antigen incorporated in the vaccine. The post-inoculation serum should contain not less than 2 i.u. of epsilon and beta antitoxins of *Cl. perfringens* and 2.5 i.u. of *Cl. septicum* antitoxin and 4 i.u. of *Cl. oedematiens* antitoxin.

5. Labelling and storage - Shall comply with the requirements regarding labelling and storage as laid down in the general monograph on “Bacterial Vaccine”.

6. Expiry date – The expiry date of potency of vaccine shall not be more than 6 months from the date of manufacture.

Haemorrhagic Septicaemia Vaccine – Alum Treated

1. Synonyms – Pasterulla multocida/(Yersinia Multocida) vaccine – Alum treated.

2. Definition -- The vaccine is a formalized culture of a virulent strain of *Pasteurella multocida* in nutrient broth treated with potash alum.

3. Preparation -- A highly potent strain of *Pasteurella multocida* type I in Phase I is grown on nutrient broth at 37°C. The pure growth is killed by the addition of a solution of Formalin I.P in suitable concentration (0.5 per cent). This is treated with Potassium Alum I.P to give a final concentration of 1 per cent.

4. Standard :

(a) *Description* – It is a white suspension containing dead bacteria and alum.

(b) *Identification* – It protects susceptible animals against infection with *P. multocida*.

(c) *Sterility Test* -- It complies with the test for sterility described under general monograph on “Bacterial Vaccines”.

(d) *Safety Test* -- Four healthy rabbits each weighting 1 to 1.5 kg. are inoculated subcutaneously each with 5 ml. of the product. There shall be no untoward reaction during the period of observation for 7 days except slight local swelling. Alternatively two rabbits and six mice may be employed. The dose for mice will be 0.5 ml.

5. Labelling and Storage :-- Shall comply with the requirements of labelling and storage as laid down in the general monograph on “Bacterial Vaccines”.

6. Expiry date – The date of expiry of potency of the vaccine shall be not more than six months from the date of manufacture.]

(B) PROVISIONS APPLICABLE TO THE PRODUCTION OF VIRAL VACCINES

1. Definition- (i) This part of the Schedule applies to viral vaccines live or inactivated made from any virus pathogenic to domestic animals and poultry and made from other modified viruses which have any antigenic value.

(ii) For the purpose of this Part of the Schedule, a virus vaccine means a sterile suspension or a freeze dried powder containing the modified living or inactivated virus particles, which in its original unaltered stage, causes disease from which the vaccine derives its name and which has been prepared from the blood or tissues of a suitable host in which it has been grown *in vivo* or from tissue culture.

2. Staff of Establishment- The establishment in which viral vaccines are prepared must be under the direction and control of an expert in bacteriology with specialized training in virology and sufficient experience in the production of viral vaccines, and he shall be assisted by a staff adequate for carrying out the tests required during the preparation and standardisation of the vaccines.

3. Proper Name- The proper name of any viral vaccine shall be the name of the disease which is caused by the particular virus from which the vaccine is produced followed by the word "vaccine" unless the Schedule otherwise provides, if there is no special provision in the Schedule such other name as is approved by the Licensing Authority. Provided that in the case of the undermentioned preparations the proper name of the vaccine shall be as follows:

- (i) Fowl Pox Vaccines, Chick Embryo Virus (Living).
- (ii) Fowl Pox Vaccine, Pigeon Pox Virus (Living).
- (iii) Horse Sickness Vaccine (Living)
- (iv) Ranikhet Disease Vaccine (Living)
- (v) Ranikhet Disease Vaccine F Strain (Living)
- (vi) Rinderpest Goat Adapted Tissue Vaccine (Living)
- (vii) Rinderpest Lapinised Vaccine (Living)
- (viii) Rinderpest Lapinised Avianised Vaccine (Living)
- (ix) Sheep and Goat Pox Vaccine (Living)
- (x) Swine fever vaccine (crystal violet)
- (xi) Swine fever vaccine lapinised (Living).
- ¹[(xii) Foot and Mouth Diseases Vaccine (Inactivated).
- (xiii) Canine Hepatitis Vaccine (Living).]

²[**4. Records-** The seed virus used in the preparation of vaccine shall, before being used for preparing a batch, be thoroughly tested for purity, safety, sterility and antigenicity by the generally accepted tests applicable to a particular virus. It shall not be more than five passages away from the stock seed virus, unless otherwise prescribed for a particular virus. The stock seed virus shall be maintained by seed-lot system at specified passage level and tested for bacterial, mycoplasmal and extraneous viral contamination. The permanent record which the licensee is required to keep shall include a record of the origin, properties and characteristics of the seed virus from which the vaccines are made.]

5. Tests- Viral vaccine shall be tested for sterility, safety and potency on suitable test animals and for viability in the case of live vaccines.

(a) *Sterility Test-* All vaccines shall be tested for sterility in accordance with rules 115 to 119. If the vaccine contains added bactericides or bacteriostatic, a quantity of medium sufficient to render the growth inhibitor ineffective is added to the sample or a suitable substance is added in a concentration sufficient to render the growth inhibitor ineffective but not itself to inhibit the growth of micro-organisms.

(b) *Safety Test-* Suitable laboratory animals or large animals or birds may be employed to test the vaccine for safety. Details of the safety test are given in the individual monograph.

1. Ins. by G.S.R. 659(E), dt. 31-8-1994.

2. Subs. by G.S.R. 659(E), dt. 31-8-1994.

(c) *Potency Test-* All virus vaccines for which potency test has been prescribed shall be tested for potency and only those which pass the potency test shall be issued. Details of the potency test are given in the individual monograph.

6. Storage- Live viral vaccines shall be stored, protected from light at sub-zero temperature as required. Other viral vaccines shall be stored at 2 ° C to 4 ° C but shall not be frozen.

7. Condition of housing of animals- (i) The animals used in the production of vaccine must be housed in hygienic conditions in premises satisfactory for this purpose.

(ii) Only healthy animals may be used in the production of vaccine. Each animal intended to be used as a source of vaccine must, before being passed for the production of vaccine be subjected to a period of observation in quarantine for at least seven days. During the period of quarantine the animal must remain free from any sign of disease and must be well kept.

¹[(iii) The poultry birds from which eggs and cell culture for production of vaccines are obtained should be housed in a manner so as to keep them free from extraneous infection and shall be screened at frequent intervals for common bacterial, mycoplasmal and viral infection. The record of the tests and their results shall be maintained by the manufacturers.]

8. Labelling- The provisions of “Labelling” as laid down for Bacterial Vaccines shall also apply to Viral Vaccines. The following additional information shall also be included on the label of the outside container:

(i) The name and percentage of bacteriostatic agent contained in the vaccine.

(ii) If the vaccine as issued for sale contains any substance other than the diluent, the nature and strength of such substances.

9. Date of Manufacture- For the purpose of this part of the Schedule, the date of manufacture shall be what is given unless otherwise stated in the individual monograph, as defined in sub-clause (b) of sub-rule (3) of rule 109.

Fowl Pox Vaccine Chick- Embryos Virus (Living)

1. Synonym- Egg adapted Fowl Pox Vaccine (Living).

2. Definition- Fowl-pox Vaccine, Chick-Embryo Virus (Living) is a suspension of a modified living virus (e.g. Mukteswar Strain) prepared from the chorioallantoic membrane (CAM) of the infected embryo and is either freeze dried or is issued as glycerinated liquid vaccine.

3. Preparation- Active chick-embryos obtained from Salmonella pullorum free flock, are used. ¹[Twelve to thirteen days old embryos are injected with a suitable dilution of the suspension of the infected membrane (seed virus) of chick embryo adopted fowl pox virus.] The suspension of the stock seed virus is dropped on the CAM. After an incubation at 37°C for a suitable period membranes showing discrete or confluent lesions (pocks) are harvested. These are homogenised with adequate quantity of antibiotics (penicillin and streptomycin) ampouled in 0.5 ml. quantities and freeze dried.

4. Standard-

(a) *Description-* Light mauve coloured scales.

(b) *Identification-* When reconstituted vaccine is applied to scarified area of the skin of a fowl it produces characteristic lesions of fowl pox. This product should afford protection against fowl pox.

1. Ins. by. GSR 659(E) , dt. 31-8-1994.

(c) *Moisture Content*- Moisture Content in the finished product should not exceed 1.0 per cent.

(d) *Safety Test*- For testing each batch of fowl pox vaccine twelve healthy cockerels, or other suitable young chicken each weighing not less than 400 g. from the same source are taken. This group of twelve birds is immunized at least twenty-one days previous to the test, with fowl pox vaccine. The vaccine under test is reconstituted in 5 ml. of 50 per cent glycerine saline and administered to fowls as follows:-

Three of the test birds are injected subcutaneously with 0.8 ml. or 10 times the field doses of the vaccine under test. This group serves to indicate whether the product is free from other viruses and bacteria causing septicaemia or not.

Three of the test birds are injected intratracheally with 0.3 ml. or 10 times the field dose of vaccine under test. This group serves to indicate whether the product is free from the virus of infectious laryngotracheitis and similar disease.

[Three of the test birds are injected intranasally with 0.2 ml. of 10 times of the field dose of the vaccine under test. This group serves to indicate whether the product is free from the virus of infectious laryngotracheitis and similar disease.]

The three remaining birds serve as controls. They are isolated and kept under observation for twenty-one days. The birds that succumb during the period of twenty-one days are subjected to a careful postmortem examination. The product is withheld from issue until the vaccine and the test birds are shown to be free from the causative agents of any extraneous disease.

(e) *Sterility test*- Complies with the tests for sterility as described under the general monograph on "Viral Vaccines".

(f) *Potency Test*- For testing of potency three unsusceptible birds each weighing not less than 400 g. are vaccinated using the field dose by the stick method and examined for "takes". Three weeks after vaccination these birds along with two unvaccinated controls are exposed to challenged virus and observed for fourteen days. The vaccinated birds should not manifest any reaction, while the controls should show active "takes".

5. Labelling- Should comply with the requirement for "Labelling" as laid down in the general monograph on "Viral Vaccines".

6. Storage and Expiry date- Freeze dried vaccine shall be expected to retain its potency for periods at temperatures as specified below:-

-15 ° C to - 20 ° C—Twenty-four months.

2° C to 4° C—Twelve months.

Room temperature— upto one month.

The liquid vaccine shall be expected to retain its potency for periods and temperatures as specified below:

2° C to 4 °C— six months.

Room temperature— seven days.

1. Ins. by. GSR 659(E), dt. 31-8-1994.

Fowl- Pox Vaccine, Pigeon Pox Virus (Living)

1. **Synonym-** Fowl Pox Vaccine (Pigeon pox scab).

2. **Definition-** Fowl Pox Vaccine, Pigeon-pox Virus (living) consists of pigeon pox virus in scabs collected from artificially infected pigeons and dried.

3. **Preparation-** Healthy pigeon are scarified on the legs and breast, with a suitable dilution of the suspension of pigeon-pox virus. The pigeons reacting satisfactorily and showing good takes are selected and the superficial skin layer scraped by means of sharp scalpel. The material so collected is freed from feathers, homogenised and dried or freeze dried. The dried pulp is powdered, sieved and ampouled in 0.3 g. quantities and sealed

4. **Standard-**

(a) **Description-** Light cream coloured powder.

(b) **Identification-** When applied to feather follicles by vigorous rubbing, it produces mild reaction in fowls. The product should afford protection to fowls upto six weeks against fowl pox.

(c) **Safety Test-** For testing a batch of vaccine, twelve healthy cockerels, or other suitable young chicken from the same source are made available at the same time. This group of twelve birds is immunised at least twenty-one days previous to the test with fowl pox vaccine. The vaccine under test is reconstituted in 10 ml. of 50 per cent glycerine saline and administered to fowls as follows: -

Three of the test birds are injected subcutaneously with 0.3 ml. or 10 times the field dose of the vaccine to be tested. This group serves to indicate whether the product is free from organisms of septicaemia disease.

Three of the test birds are injected intranasally with 0.2 ml. of the vaccine to be tested. This group serves to indicate whether the product is free from virus of Coryza and similar diseases.

¹[Three of the test birds are injected intratracheally with 0.2 ml or 10 times of the field dose of vaccine under test. This group serves to indicate whether the product is free from the virus of infectious laryngotracheitis and similar diseases.]

The three remaining birds serve as controls. All the birds under test are isolated and held under observation for twenty-one days. All those that succumb are subjected to careful post-mortem examination. The product is withheld from issue until the vaccine and test birds are shown to be free from the causative agents of any extraneous diseases.

(d) **Sterility Test-** Complies with the tests for sterility described under the general monograph on "Viral Vaccines".

(e) **Potency Test-** For testing the potency of a batch of vaccines three susceptible birds each weighing not less than 400 g. are vaccinated using the field dose by the follicular method and examined for 'takes'. Three weeks after vaccination these birds and two healthy susceptible controls are exposed to challenge virus and are observed for fourteen days. The vaccinated birds shall manifest no reaction, while the controls must have active "takes".

5. **Storage and Labelling-** Should comply with the requirements of 'Labelling' as laid down in the general monograph on 'Viral Vaccines'.

6. **Expiry date-** The vaccine shall be expected to retain its potency for periods at temperature as *specified below*:-

-15 ° C to - 20 °C-- two years.

2° C to 4 °C-- twelve months.

Room temperature- Upto one month.

1. Ins. by. GSR 659(E), dt. 31-8-1994.

Fowl Pox Vaccine- Pigeon Pox- Chick Embryos Virus (Living)

1. ***Synonym-*** Chick embryo adapted pigeon pox vaccine (Living).

2. ***Definition-*** Fowl pox vaccine (Pigeon Pox virus) chick embryo adapted virus (living) is a suspension of a modified living virus prepared from the chorioallantoic membranes of the infected embryos and is freeze dried.

3. ***Preparation-*** Active chick embryos obtained from Salmonella Pullorum free stock are used. Twelve to thirteen days old embryos are injected with a suitable dilution of the suspension of the infected membrane (stock seed virus) of chick embryo adapted pigeon pox virus. The suspension of the stock seed virus is dropped on the membrane. The inoculated eggs are incubated at 37 ° C for four days. One of the fourth day embryos that are living, are removed to a refrigerator for chilling for about one hour. Membranes showing discrete lesions (Pocks) are harvested. These are homogenised with adequate quantities of antibiotics, ampouled in 0.5 ml. quantities and freeze dried.

4. ***Standards-***

(a) ***Description-*** Light mauve coloured scales.

(b) ***Identification-*** When reconstituted vaccine is applied to scarified area of the skin of a fowl, it produces characteristics lesions of Fowl Pox. This product should afford protection against pox.

(c) ***Moisture content-*** Moisture content in the finished product should not exceed 1.0 per cent.

(d) ***Safety test-*** For testing each batch of chicks aged four to six weeks from the same source are taken. This groups of twelve birds is immunised at least twenty-one days previous to the test, with fowl-pox vaccine. The vaccine under test is reconstituted in 3 ml. of normal saline solution and administered as under:-

Three of the test chicks are injected subcutaneously with 0.3 ml. or 10 times the field dose of the vaccine under test. This group serves to indicate whether the product is free from other viruses and bacteria causing of septicaemia or not.

Three of the test chicks are injected intratracheally with 0.3 ml. or ten times the field dose. This group serves to indicate whether the product is free from the viruses of infections laryngeotracheitis and similar diseases.

Three of the test chicks are injected with 0.2 ml 1/N of the vaccine under test. This group serves to indicate whether the product is free from the virus of coryza and similar diseases.

The remaining three chicks serve as controls. They are isolated and kept under observation for twenty-one days. The birds that succumb during the period of observation are subjected to careful post-mortem examination. The product is withheld from issue until the vaccine and the test birds are shown to be free from the causative agents of any extraneous disease.

In addition to the above, similar groups of pigeons aged six to nine months old are also injected in a similar way to eliminate psittacosis.

(e) ***Sterility Test-*** Should comply with the tests for sterility described under the general monograph on 'Viral Vaccine'.

(f) ***Potency test-*** For testing potency of a batch of vaccine three susceptible chicks of three to four weeks of age are vaccinated by feather forthicle method (a few forthicles on one leg are injected) and these are examined for 'takes'.

Three weeks after vaccination these chicks along with two unvaccinated chicks are exposed to challenge virus (virulent fowl pox virus) and observed for fourteen

days. The vaccinated chicks should not manifest any reaction while controls should show active 'takes'.

5. **Labelling-** Should comply with the requirements for 'Labelling' as laid down in the general monograph on 'Viral Vaccines'.

6 **Storage-** The freeze dried product is expected to retain its potency for periods at temperatures as specified below:

-15 ° C to 20 ° C—two years.

2° C to 4 ° C—twelve months.

Room temperature- up to one month.

Sheep Pox Vaccine (Living)

1. **Synonym-** Sheep Pox vaccine; Goat pox vaccine.

2. **Definition-** Sheep pox vaccine consists of sheep pox virus collected from sheep artificially infected with sheep pox virus and freeze dried.

3. **Preparation-** Healthy yearling sheep are infected artificially by subcutaneous infection on the undersurface of the previously shaved abdomen with 200- 300 cc. of the freeze dried sheep pox virus (seed material) diluted in 1 : 1 Normal saline solution. On the sixth or seventh day after injection oedematous swelling develops in the injected area with thermal reaction. The sheep which develop good swelling are slaughtered and the gelatinous material present under the skin in the infected area is collected under sterile conditions. This material is mixed with 2 parts by volume of sterile peptone broth of pH 7.2 and homogenised. The homogenised suspension is filtered, ampouled in 0.5 ml. quantities and freeze dried.

4. **Standard:**

(a) **Description-** White scales.

(b) **Identification-** Reconstituted vaccine when applied over the scarified area of the skin of the abdominal region of sheep will produce characteristic local lesion of pox.

(c) **Moisture content-** The moisture content should not exceed 1.0 per cent.

(d) **Safety test-** Two rabbits each weighing not less than 1 kg. are injected subcutaneously each with 1 ml. of 1 : 100 dilution of the vaccine in normal saline solution. These animals are observed for fourteen days. The animals should remain normal.

(e) **Sterility Test-** Complies with the tests for sterility described under the general monograph on 'Viral Vaccines'.

(f) **Potency Test-** Four yearling sheep are vaccinated on the inner surface of the ear by scarification method. The contents of one ampoule of F.D. Sheep Pox vaccine are constituted in 10 cc. of 50 % glycerin saline solution, characteristic 'takes' develop in the scarified area with ulceration and scab formation. Three weeks later these and two more susceptible sheep (Controls) are challenged by scarifying with a suspension of the previous brood of the vaccine on the undersurface of the abdomen. The controls should develop typical lesions of pox and the vaccinated should remain normal.

5.**Labelling-** Should comply with the requirements of 'labelling' as laid down in the general monograph in 'Viral Vaccine'.

6. **Storage and expiry date-** The vaccine is expected to retain potency for period and temperature as specified below:-

-15 ° C to -20 ° C—two years

2 ° C to 4 ° C—three months.

Room temperature- Fifteen days.

Horse Sickness Vaccine (Living)

1. **Synonym-** African Horse Sickness Vaccine, Mouse adapted Polyvalent Horse Sickness Vaccine (Living).

2. **Definition-** Horse sickness vaccine is a suspension of live mouse adapted strains of Horse Sickness Virus (Onderstepoort) prepared from the brains of infected mice and is freeze dried.

3. **Preparation-** Thirty to thirty-five days old white mice are infected intracerebrally with 0.05 ml. of a suitable dilution of the seed virus (6 or 7 types, as the case may be). Groups of large numbers of mice are injected separately with each type of the virus and are housed at 27 ° C to 32 ° C. A majority of these become paralytic on the third and fourth day when they are sacrificed and their brains collected and stored at – 15 ° C to – 20 ° C till the day of processing. For preparing the polyvalent vaccine, equal number of brains collected from mice infected with different types of the virus are homogenised with 5-10 times its volume of sterile lactose buffer medium (pH 7.2) containing antibiotics. The suspension is centrifuged at 1500 r.p.m. for five minutes. The supernatant liquid is distributed in ampoules in suitable quantities and freeze dried.

4. **Standard:**

(a) **Description-** White scaly material.

(b) **Identification-** This product affords protection to horse against horse sickness.

(c) **Safety Test-** Four healthy mice thirty to thirty-five days old are injected intraperitoneally with 0.2 ml. of 10:1 dilution of the vaccine and kept under observation for ten days. All the mice should remain normal throughout the period of observation.

(d) **Sterility Test-** Should comply with the test for sterility described under the general monograph on ‘Viral Vaccines’.

(e) **Viability Test-** Each batch of vaccine is titrated in tenfold dilutions using four mice of thirty to thirty-five days old for each dilution. Each mouse is injected intracerebrally with 0.05 ml and kept under observation for ten days. Mortality and survival ratios are noted and LD₅₀ ml is determined. The minimum acceptable titre is 10–4 LD₅₀ per 0.05 ml.

(5) **Labelling -** Should comply with the requirements of ‘labelling’ as laid down in the general monograph in ‘Viral Vaccines’.

(6) **Storage-** The vaccine may be expected to retain its potency for twelve months if stored – 15 ° C to 20° C and about six months if stored in refrigerator at 2 ° C to 4° C.

Rabies Vaccine (Inactivated)

1. **Synonym-** Antirabic Vaccine (Inactivated)

2. **Definition-** Rabies vaccine is a suspension of the brain tissue of animals, that have been infected with a suitable strain of rabies fixed virus, inactivated with phenol or some other suitable agent.

3. The following particulars relating to this vaccine are the same as those relating to Antirabic vaccine described in Part D of Schedule F to these rules, namely:-

- (i) Strain of fixed Rabies Virus to be used;
- (ii) Staff of Establishment;
- (iii) Condition and housing of animals;
- (iv) Precaution to be observed in preparation;
- (v) Records;
- (vi) Issue.

4. **Preparation-** Healthy sheep or any other suitable species of animal are inoculated subdurally or intracerebrally with an appropriate dose of suspension of a suitable strain of rabbit brain passaged rabies fixed virus. The sheep or animals which get paralysed from the sixth day onwards after the inoculation are sacrificed and their brains collected aseptically. Brain tissue is weighed individually and a suspension of suitable concentration of brain tissue prepared in buffered saline is strained through gauze. The suspension treated with phenol or some other suitable inactivating agent is incubated for an appropriate period.

5. **Standard-**

- (a) *Description-* A grey to pale yellow opalescent suspension.
- (b) *Identification-* Appropriate doses protect mice against subsequent intracerebral inoculation with suitable strain of fixed rabies virus.
- (c) *Safety test-* Not less than five mice, each weighing at least 18 gm., are inoculated intracerebrally with not less than 0.03 ml. of the suitably diluted vaccine. None of the animals should show symptoms of rabies or die of the disease during period of observation of three weeks.
- (d) *Sterility Test-* Should comply with the test for sterility described under the general monograph on 'Viral Vaccine'.

6. **Labelling-** Should comply with the requirements of 'Labelling' as laid down in the general monograph on 'Viral Vaccines'. In addition the label on the container shall indicate the percentage of brain tissue present in the vaccine.

7. **Storage-** The vaccine may be expected to retain its potency for about six months if stored in refrigerator at 2° to 4 ° C.

Rabies Vaccine (Living)

1. **Definition-** Rabies vaccine (living) is a freeze-dried suspension of chick-embryo tissue infected with a suitable attenuated strain of rabies virus.

2. **Preparation-** It may be prepared by the following method. Seed virus consisting of a suspension of the Flury or other suitable strain of chick adapted virus that has been maintained by passage in chick embryos is injected into the yolk-sacs of fertile eggs incubated for a suitable period. After incubation for a further ten days, the embryos are harvested and grind in water for injection to give 33 percent suspension. The suspension is centrifuged to remove coarse particles and the supernatant fluid is distributed into ampoules in 3 millilitre quantities, and freeze dried. The vaccine is reconstituted immediately before use by adding 3 millilitres of water for injection to the contents of an ampoule.

3. **Standard-** It complies with the requirements of general standard of viral vaccines for abnormal toxicity, sterility, and labelling with the following additions.

- (a) *Description-* Dry honey-coloured flakes or powder, readily dispersible in water.
- (b) *Identification-* It protects guinea pig against a subsequent inoculation of rabies street virus. It is distinguished from the inactivated Rabies vaccine by its ability to produce rabies encephalitic on intracerebral injection into mice.
- (c) *Safety-* The guinea pigs used in the test for potency should not show any marked local or systemic reaction during the three weeks following injection with the vaccine.
- (d) *Sterility Test-* Complies with the tests for sterility described under the general monograph on 'Viral Vaccines'.
- (e) *Potency Test-* The contents of an ampoule are dispersed in water for injection to give a 5 per cent suspension and not fewer than twenty guinea pigs, drawn from a uniform stock and each weighing 350 g. to 500 g., are each injected intramuscularly with 0.25 ml. of this suspension. Three weeks later, these guinea pigs and an equal number of similar unvaccinated control guinea pigs are each inoculated with 0.1 ml. of a suitable dilution of canine salivary gland suspension of street virus which is maintained as a 20 per cent suspension at 70 ° C or lower. The guinea pigs are observed for thirty days; not less than 80 per cent of the control guinea-pigs die of rabies and not less than 70 per cent of the vaccinated guinea-pigs are protected.

4. **Storage-** Freeze-dried vaccine should be stored at refrigeration temperatures of 2 ° C to 4 ° C.

5. **Labelling-** The life of the vaccine at room temperature and at refrigeration temperature should be stated on the label.

6. (a) **Action and uses-** Rabies vaccine (living) is used for the prophylactic inoculation of dogs against rabies; one injection should provoke a serviceable immunity lasting for at least a year. The vaccine has been used to a limited extent on cattle.

(b) **Dose-** By intramuscular *injection*: Dogs, the contents of one ampoule reconstituted in 3 ml. of water for injection; cattle five times the dog dose.

Ranikhet Disease Vaccine (Living)

1. **Synonym-** New Castle Disease Vaccine (Living); phemoenteritis Vaccine (Living).

2. **Definition-** Ranikhet Disease vaccine is a suspension of a modified living virus e.g. (Mukteswar strain) prepared from infected embryos and fluids and is freeze dried.

3. **Preparation-** Good fertile eggs obtained from *Salmonella pullorum* free flock are incubated in an egg incubator. Ten days old vigorous embryos are infected with 0.1 ml. of a suitable dilution of a suspension of the virus. Inoculation is done in the allantoic cavity. Embryos are incubated at a suitable temperature. Eggs showing dead embryos twenty-four hours after incubation are discarded. After forty-eight hours incubation the eggs are candled and those showing dead embryos are chilled for a suitable period of time, while embryos alive beyond forty-eight hours are discarded. The fluids and embryos are then collected and spot haemogglutination carried out. The material is homogenised in a blender and ampouled in aliquots of 0.5 ml. quantities and freeze-dried.

4. Standards-

(a) **Description-** Light brown scales.

(b) **Identification-** This product affords protection to fowls against Ranikhet Disease.

(c) **Safety Test-** For testing each batch of freeze dried Ranikhet Disease Vaccine, twelve healthy young chickens, all from the same source each weighing not less than 100 g. are taken and immunised against Ranikhet Disease. Fourteen days later, these birds, are tested as follows with the contents of one ampoule suspended in 100 ml. of normal saline.

Three of the test birds are injected subcutaneously with 0.1 ml. equivalent to ten times the field dose of the vaccine to be tested. This group serves to indicate whether the product is free from viruses or organisms of speticaemia disease.

Three of the test birds are injected intratracheally with 0.1 ml. equivalent to ten times the field dose of he vaccine to be tested. This group serves to indicate whether the product is free from the virus of infectious laryngotracheitis, ¹[***] and similar diseases.

The three remaining birds serve as controls.

²[Three of the test birds are injected intranasally with 0.2 ml of the vaccine to be tested. This group serves to indicate whether the product is free from virus of Coryza and similar diseases.]

All the treated birds and controls are observed daily for fourteen days. All the test birds that succumb are subjected to careful postmortem examination. The product is not issued until the birds under test are shown to be free from the causative agents of any extraneous diseases.

(e) **Sterility Test-** Should comply with the test for sterility described in the general monograph on 'Viral Vaccine'.

1. The word "Coryza" omitted by. GSR 659(E), dt.. 31-8-1994.

2. Ins. by G.S.R. 659(E), dt.. 31-8-1994

(f) *Potency Test*- Four susceptible birds eight to twelve weeks old and each weighing not less than 400 g. are vaccinated by injecting subcutaneously 1 ml. of a 10^{-5} dilution of the product. Two weeks after vaccination these birds and four non-protected birds are challenged by injecting subcutaneously each with 1.0 ml. of a 1: 100 dilution of virulent virus (liver and spleen suspension) or 1.0 ml. of a 1 : 100 dilution of fluid from the embryo infected with virulent Ranikhet Disease virus. The non-protected birds should show symptoms of Ranikhet Disease and die and all the protected birds should remain normal during an observation period of fourteen days.

5. *Labelling*- Should comply with the requirements of 'Labelling as laid down in the general monograph on 'Viral Vaccines'.

6. *Storage*- The vaccine when stored at -15°C to 20°C , may be expected to retain the potency for about one year and about three months if stored in a refrigerator at 2°C to 4°C . The product should not be used if stored for more than ten days outside the refrigerator.

Ranikhet Disease Vaccine F strain (Living)

1. *Synonyms*- New castle disease vaccine F Strain (Living).

2. *Definition*- Ranikhet disease vaccine F. strain is a suspension of a naturally modified living virus (F strain) prepared from the infected embryos, devoid of beaks and eyes and fluids in a frozen state.

3. *Preparation*- Good fertile eggs obtained from Salmonella pullorum free flock are incubated in an egg incubator. Eight days old vigorous embryos are infected with 0.1 ml. of 1 : 100 suspension of Ranikhet disease vaccine F strain virus. Inoculation is done via the allantoic cavity. Embryos are incubated at 37°C . Eggs are candled every day upto four days and the dead ones are discarded. Final candling of the embryos is carried out on the fourth day and only the living ones are chilled in a refrigerator for one hour. The fluids embryos are collected separately. The fluids are tested for spot haemagglutination and sterility test is carried out. The beaks and eye balls of the embryos are removed. The materials are homogenised with adequate quantities of antibiotics in a cool warning blender and ampouled in aliquots of 0.5 ml. quantity and freeze dried.

4. *Standard*-

(a) *Description*- Light brown scales.

(b) *Identification*- This product affords protection to baby chicks against Ranikhet disease.

(c) *Moisture content*- The moisture content should not exceed $1[1.0]$ per cent.

(d) *Potency test*- For testing each batch of the vaccine twelve one-day old chicks are given two drops 1/N of the field dose of the vaccine (5 ampoules selected at random may be reconstituted in 50 ml.) of cold normal saline solution. These are observed for fourteen days and the vaccinated chicks should remain normal throughout the period of observation. This serves the safety test also.

On the fourteenth days the vaccinated chicks are challenged two drops with 1:50 virulent Ranikhet disease virus alongwith 8 control chicks. Four of the controls receive two drops 1/N of the virulent virus while the rest of the four receive 0.5 ml. of the virulent virus. The control chicks should succumb to the challenge virus showing symptoms of Ranikhet Disease while the protected chicks should remain normal throughout the observation period of fourteen days.

(e) *Sterility Test*- Should comply with the tests for sterility described in the general monograph on 'Viral Vaccines'

5. *Labelling*- Should comply with the requirements of "Labelling" as laid down in the general monograph on 'Viral Vaccines'.

6. *Storage*- The vaccine when stored at -15°C to -20°C may be expected to retain the potency for about one year and about three months if stored in a refrigerator at 2°C to 4°C . When removed from the refrigerator, the product should not be used later than ten days.

Rinderpest Goat adapted Tissue Vaccine (Living)

1. **Synonym-** Goat-adapted Cattle Plague Vaccine; Goat Tissue Vaccine (Living).

2. **Definition-** Rinderpest Goat-adapted Tissue Vaccine is the homogenised freeze dried preparation of spleen pulp of goats artificially infected with the suitable strain of rinderpest virus.

3. **Preparation-** Healthy susceptible goats are quarantined for a period of ten days. After this period a batch of selected goats are injected subcutaneously with 2 ml. of a suitable dilution of the suspension of the seed virus. The donor goats are sacrificed after a suitable period when the titre of the virus in the animal body is expected to be maximum, usually four days, and the spleen from animals free from any pathological change or signs are collected under sterile conditions. Smear from each spleen is examined microscopically to exclude spleen which are contaminated from the production batch.

The spleen is freed from fat and fascia and is blended into a smooth pulp in a grinder. The pulp is spread on a shallow dish of glass or stainless steel and is freeze dried.

The freeze dried pulp is then ground into a fine powder and sieved. The powder is ampouled in 0.25 g. or 0.125 g. quantities and freeze dried.

4. **Standard:**

(a) **Description-** Dark brown or chocolate coloured scales or powder.

(b) **Identification-** The product affords protection to susceptible animals against rinderpest.

(c) **Moisture content-** Not more than 1.0 per cent.

(d) **Safety Test-** Each batch of vaccine shall be tested for safety in laboratory animals and cattle or buffalo calves as follows:-

(i) **Small animals-** At least two guinea pigs each weighing 300 g. to 450 g. and two adult rabbits each weighing 1 kg. to 1.5 kg. should be injected each with 1 ml. of 1: 100 suspension of the vaccine subcutaneously and kept under observation for seven days. None of the animals should die. Alternatively, a batch of six white mice each weighing not less than 18 g. may be used, each mouse receiving 0.5 ml. of a dilution 1 : 100 suspension subcutaneously. None of the animals should die.

(ii) **Large animals-** Either cattle of good grade of susceptibility (hill cattle) or buffalo calves may be employed. For each batch of vaccine, three animals should be injected subcutaneously with 1 ml. of 1 : 8000 dilution of the vaccine. These animals should be kept under observation for twelve to fourteen days. None of the animals should show any untowards reactions.

(e) **Sterility Test-** Complies with the tests for sterility described under the general monograph in 'Viral Vaccines'.

(f) **Potency Test-** The animals receiving 1 ml. of 1 : 8000 dilution of vaccine used under safety test mentioned above and kept under observation for fourteen days, should be challenged with 1 ml. of 1 per cent suspension of stock Rinderpest Virulent virus. None of the animals should die of rinderpest within a period of ten days. This test serves as a short potency test for each of the batches.

For conducting a detailed potency test the following procedure may be followed:-

Dilution 1: 8000, 1: 12,000 and 1 : 16,000 shall be tested and for each dilution three susceptible cattle or buffalo calves should be used. Each animal is inoculated subcutaneously with 1 ml. of a dilution of the vaccine, followed twelve to fourteen days later with a standard challenge dose of virulent rinderpest bull virus containing

in 1 ml. of a 1 : 100 suspension of spleen tissue. Two unvaccinated bovines, each receiving the same quantity of the challenge dose acts as controls. These are kept under observation for fourteen days. The end point of protection titre is assessed on the death or survival rate and the dose contained in one gramme of vaccine calculated on the basis of 20 to 40 minimum protective doses being equivalent to one vaccinating dose.

(g) *Virulence and Viability Test*- Two to four goats each weighing not less than 18 kg. are injected with 2 ml. of 1 : 100 suspension of the vaccine and kept under observation for ten days. These animals should show reaction characterised by pyrexia (rise of about 2° C) *anorexia and dullness*.

5. *Labelling*- Should comply with the requirement of 'Labelling' as laid down in the general monograph on 'Viral Vaccines'.

6. *Storage*- The vaccine may be expected to retain its potency for twelve months if stored at - 15° C to -20° C or about three months if stored at 2° C to 4° C.

Rinderpest Lapinised Vaccine (Living)

1. *Synonym*- Rabbit Adapted Cattle Plague Vaccine (Living) Lapinised Vaccine (Living).

2. *Definition*- Rinderpest lapinised vaccine is a suspension of a modified living virus (e.g. Nakamura III Strain) prepared with the blood, spleen and mesenteric lymph glands of infected rabbits and is freeze dried.

3. *Preparation*- Adult rabbits possibly from a known stock, each weighing not less than 1 kg. free from coccidiosis and snuffles, are injected intravenously with 1 ml. of a suitable dilution of a suspension of the stock seed virus. Donor rabbits are sacrificed after a suitable period when the titre of the virus in the animals is expected to be the maximum usually the third day.

Ten millilitres of blood is collected from each rabbit in a defibrinating flask under aseptic condition. Later the animals are sacrificed and the spleen and mesenteric lymph glands collected. Each rabbit is subjected to a thorough post-mortem examination to observe lesions of rinderpest infection.

After harvesting, the blood and the organs (spleen and glands) are homogenised in a suitable proportion if necessary. Adequate quantities of penicillin and streptomycin may be added. The homogenized material is ampouled in suitable quantities and freeze dried.

4. *Standard*-

(a) *Description*- Dark chocolate coloured mass.

(b) *Identification*- This product affords protection to susceptible animals against rinderpest.

(c) *Moisture content*- Not more than 1.0 per cent.

(d) *Safety Test*- For testing a batch 2 guinea pigs each weighing not less than 300 g. are injected subcutaneously with 1 ml. of a 1 : 100 suspension of the vaccine. Alternatively, a group of six white mice each weighing not less than 18 g. is used. Each animal receives subcutaneously 0.5 ml. of 1 : 100 suspension of the vaccine. None of the test animals should die within a period of seven days.

(e) *Sterility Test*- Should comply with the tests for sterility described in the general monograph on 'Viral Vaccines'. If antibiotics have been added the inoculum should be neutralised before doing the test.

(f) *Potency Test*- Dilution 1: 100, 1 : 200, 1 : 400 and 1 : 800 shall be tested and for each dilution 2 susceptible cattle (hill bulls) or buffalo calves should be used. Each animal is inoculated subcutaneously with 1 ml. of a dilution of the vaccine, followed twenty-one days later with a standard challenge dose of a virulent rinderpest virus contained in 1 ml. of a 1 : 100 suspension of spleen tissue. Two unvaccinated bovines each receiving the same quantity of the challenge virus serve as controls. These animals are kept under observation for fourteen days. The end point of the protecting titre is assessed on the death or survival rate and the dose contained in one gramme of vaccine calculated on the basis of twenty minimum protective doses being equivalent to one vaccinating dose.

(g) *Virulence and Viability Test*- Four rabbits each weighing 1 to 1.5 kg. are injected subcutaneously with 1 ml. of 1 : 100 suspension of the vaccine. The animals should react typically showing all the symptoms of rinderpest in rabbits.

5. **Labelling**- Should comply with the requirements of 'Labelling' as laid down in the general monograph on 'Viral Vaccines'.

6. **Storage**- The vaccine may be expected to retain its potency for six months if stored at 15 °C to -20 °C or about a month if stored at 2 °C to 4 °C.

Rinderpest Lapinised Avianised Vaccine (Living)

1. **Synonym**- Lapinised Avianised Vaccine (Living).

2. **Definition**- Rinderpest Lapinised Avianised Vaccine is a suspension of a modified live rinderpest virus of low virulence prepared either with the whole chick embryo or the viscera of the infected chick embryo.

3. **Preparation**- Twelve or thirteen days old active chick embryos from a flock free from Salmonella pullorum infection are injected intravenously with a suitable dilution of the suspension of the stock seed virus in six per cent glucose solution. The embryos are incubated at 38.5° C for five days. At the end of this incubation period, eggs which show living embryos are selected for the preparation of the vaccine. The viscera of the chicks are harvested, care being taken to reject the gizzard and gall bladders. The material is homogenised in a blender with adequate quantities of antibiotics (penicillin and streptomycin added if necessary), and primary freeze dried done. This freeze dried material is ground into a fine powder, ampouled in suitable quantities and finally subjected to secondary freeze drying and sealed under vacuum.

4. **Standard**-

(a) *Description*- Pale cream or yellow coloured sterile powder.

(b) *Identification*- This product affords good grade of immunity to susceptible animals against rinderpest.

(c) *Moisture content*- Not more than 1.0 per cent.

(d) *Safety Test*- For testing each batch, a group of six mice each weighing not less than 18g. is used. Each mouse is injected subcutaneously with 0.5 ml. of a 1 : 100 suspension. Alternatively, two guinea pigs each weighing not less than 300 g. and two rabbits each weighing not less than 1 kg. are injected with 1 ml. of 1 : 100 suspension subcutaneously. These animals should not show any untoward reaction during the period of observation for seven days.

(e) *Sterility Test*- Should comply with the test or sterility as laid down in the general monograph on 'Viral Vaccines'.

(f) *Potency Test*- Healthy highly susceptible cattle (hill bulls) or buffalo calves should be used for testing the potency of each batch of vaccine in suitable dilution. For each dilution two highly susceptible animals should be used. Each animal is inoculated

subcutaneously with 1 ml. of a dilution of the vaccine, followed twenty-one to twenty-eight days after with a standard challenge dose of a virulent rinderpest bull virus contained in 1 ml. of a 1 : 100 suspension of spleen tissue. Two unvaccinated bovines, each receiving the same quantity of the challenge virus serve as controls. All these animals are kept under observation for fourteen days. The end point of protective titre is assessed on the death or survival rate and the dose contained in one gramme of vaccine calculated on the basis of forty minimum protective doses being equivalent to one vaccinating dose.

5. Labelling- Should comply with the requirements of 'Labelling' as laid down in the general monograph on 'Viral Vaccines'.

6. Storage and Expiry date- The vaccine shall be expected to retain its potency for the period at temperatures as specified below:-

-15° C to -20° C .. Six months.

2° C to 4° C . . . One month.

Sheep and Goat Pox Vaccine (Living)

1. Synonym- Sheep Pox Vaccine. Goat Pox Vaccine (Living).

2. Definition- Sheep and Goat Pox Vaccine consists of the virus contained in the scabs collected from sheep artificially infected with the virus.

3. Preparation- Healthy yearling sheep are infected artificially on the shaved portion of the abdomen with a suitable dilution of the suspension of the stock seed virus 50 per cent glycerine saline solution. The material from the semi-dried areas where the pock lesions are evident is collected and dried over calcium chloride or phosphorus pentoxide under vacuum. Dry scabs are powdered, sieved, ampouled in suitable quantities and sealed.

4. Standard:

(a) *Description-* Light cream coloured powder.

(b) *Identification-* This product when applied to scarified area of the skin of the sheep or goats produces characteristic local lesions of pox and should afford protection to sheep and goat against Sheep and Goat Pox.

(c) *Safety Test-* Two rabbits each weighing not less than 1 kg. are injected subcutaneously each with 1 ml. of a 1 : 100 dilution of the vaccine in normal saline solution. These animals are observed for fourteen days. The animals should remain normal.

(d) *Sterility Test-* Complies with the tests for sterility described under the general monograph on 'Viral Vaccines'.

(e) *Potency Test-* Four yearling sheep are inoculated with 1 : 100 suspension of the vaccine in 50 per cent glycerine saline on a scarified area on the abdomen. Fourteen days later, these and two more susceptible sheep are inoculated by the same method with stock virus and observed for a period of fourteen days. The control animals should develop typical lesions of pox and vaccinated animals should remain normal.

5. Labelling- Should comply with requirement of 'Labelling' as laid down in the general monograph on 'Viral Vaccines'.

6. Storage and Expiry date- The vaccine shall be expected to retain its potency for period at temperatures as specified below:-

-15° C to -20° C : Twenty months.

2° C to 4° C : Three months.

Room Temperature : Fifteen days.

Fowl Spirochaetosis Vaccine (Chick Embryo Origin)

1. *Synonym*- Tick Fever Vaccine.

2. *Definition*- The vaccine consists of a merthiolated suspension of chorioallantoic membrane, internal viscera and blood of chick embryos infected with a vaccine strain of spirochaetes and freeze dried.

3. *Preparation*- Eleven days old developing chick embryos are infected with 0.2 ml. of sterile fresh blood containing spirochaetosis via the chorioallantoic membrane. The inoculated embryos are incubated at 37 °C and candled daily and the dead one are discarded. On the seventh day the living embryos are chilled in the refrigerator for two hours. The chilled embryos are harvested separately and necrotic lesions in liver noted. Representative samples of blood should be examined for teaming spirochaetes. The internal viscera, chorio-allantoic membranes and the blood are collected. The material is pooled, weighed and held in deep freeze at – 15 °C to –20 °C for a period of one week. Thereafter the material is blended with equal quantity of Merthiolate (final concentration of merthiolate in the suspension should be 1 : 10, 000) thoroughly for three times, each time the motor running at full speed and the vaccine is ampouled in 2 ml. quantities and freeze dried.

4. *Standard*-

(a) *Description*- Light brownish scales.

(b) *Identification*- The vaccine affords protection when inoculated into the fowls against spirochetosis.

(c) *Moisture content*- The moisture content should not exceed 1.0 per cent.

(d) *Safety and potency test*- Six healthy cockerals ten to twelve weeks old are used for this purpose. Each ampoule of vaccine is reconstituted in 10 ml. of cold distilled water and the six cockerals are injected intramuscularly each with 1 ml. of the reconstituted vaccine and the birds are observed for a period of ten days and the vaccinated birds should remain normal throughout the period of observation. The vaccinated birds are challenged with 0.2 ml. intramuscularly with virulent spirochaete blood along with two susceptible controls. Temperature and blood smear examination of the challenged birds and controls should be carried out daily for a period of ten days. The blood smears of vaccinated birds should remain negative for spirochaetes during the entire period of observation. The controls should react and show spirochaetes in the blood.

(e) *Sterility Test*- Complies with the tests for sterility described in the general monograph on ‘Bacterial Vaccine’.

5. *Labelling*- Should comply with the requirement of ‘Labelling’ as laid down in the general monograph on ‘Viral Vaccines’.

6. *Storage*- The vaccine when stored at –15 °C to – 20 °C may be expected to retain the potency for about one year and about two months if stored in refrigerator at 2 °C to 4 °C.

Swine Fever Vaccine Crystal Violet

1. *Synonym*- Crystal Violet Swine fever vaccine, Hog Cholera Vaccine.

2. *Definition*- Swine fever vaccine, crystal violet is a suspension of blood of swine that have been infected with a suitable virulent antigenic strain of swine fever virus, inactivated with 0.25 per cent crystal violet ethylene glycol at 37 °C for fourteen days.

3. *Preparation*- Susceptible healthy pigs of six to seven months of age belonging to a well established strain or bred are used. Body weight of these animals at this age may vary according to the breed but optimum weight is considered as between 75 to 100 kg. Animals used for production may be procured from well established farms and kept under quarantine

for fourteen days. These are injected intramuscularly with a suitable dilution of the suspension of the virulent blood viruses. Bleeding of the clinically injected animals is carried out on the sixth day. The defibrinated blood from each animal is strained and stored separately in sterile glass containers. To the four parts of defibrinated blood, one part of 0.25 per cent crystal violet- ethylene glycol is added and the suspension after thorough mixing, is stored at 37° C (±0.5) for two weeks. The product is filled in 20 ml. volumes in sterile vials and labelled on the completion of tests.

4. Standard-

(a) *Description-* Very dark violet suspension.

(b) *Identification-* This product affords protection against swine fever but not against African Swine Fever.

(c) *Safety Test-* Two young pigs weighing about 15 to 30 kg. are injected subcutaneously each with 40 ml. of the vaccine batch to be tested. In addition, one unvaccinated susceptible pig is placed in contact.

(d) *Sterility Test-* Should comply with the test for sterility described under general monograph on 'Viral Vaccines'.

(e) *Abnormal toxicity test-* Two guinea pigs are given 1 ml. of vaccine intramuscularly.

Two guinea pigs are given 2 ml. of the vaccine intraperitoneally.

Two mice are given 0.5 ml. of the vaccine subcutaneously.

(f) *Potency Test-* Four susceptible pigs weighing between 20-30 kg. are injected with 5 ml. of the vaccine subcutaneously. After twenty-one days these are challenged with 1 ml. of suitable dilution of the challenge virus subcutaneously. The dose must contain at least 1000 minimum infective doses. At least two control pigs should be used.

5. Labelling- - Should comply with requirement of 'Labelling' as laid down in the general monograph on 'Viral Vaccines'.

6. Storage- The vaccine may be expected to retain its potency for twelve months if stored in refrigerator at 2 °C to 4 °C.

Swine Fever Vaccine Lapinised (Living)

1. Synonym- Lapinised swine fever vaccine, freeze dried lapinised swine fever vaccine.

2. Definition- Swine fever lapinised vaccine consists of the suspension of a modified live swine fever virus prepared from spleens of infected rabbits and is freeze dried.

3. Preparation- Healthy adult rabbits weighing approximately 1000 gms. or over, free from coccidiosis snuffles etc. are injected intravenously with a suitable dose of a dilution of the modified rabbit adapted virus. Rabbits are sacrificed at the height of reaction and spleens are collected with sterile precautions. The collection is later homogenised in a blender using ten per cent yolk phosphate buffer as a diluent. The suspension is ampouled in 0.5 ml. quantities and freeze dried.

4. Standard-

(a) *Description-* Light scales.

(b) *Identification-* This product affords protection against swine fever.

(c) *Moisture content-* The moisture content should not exceed 1.0 per cent.

(d) *Safety Test-* Six mice are injected each with 0.5 ml. of a 1 : 100 suspension of the vaccine. These are kept under observation for seven days. Non should die.

(e) *Viability Test-* Two healthy rabbits are injected intramuscularly with 1 ml. of 1 : 100 suspension of the vaccine. These animals show thermal reaction.

(f) *Sterility Test-* Should comply with the test for sterility described under the general monograph on 'Viral Vaccines'.

(g) *Potency Test-* The vaccine batch under test should be tested on susceptible healthy pigs weighing between 20-30 kg. Two animals for each dilution may be used. The dilutions tested are 1 : 10, 1 : 25, 1 : 50 and 1 : 100. One millilitre of each of these dilutions is injected subcutaneously. One healthy, susceptible, unvaccinated in contact animal should be kept along with the vaccinated animals.

Fourteen to twenty-one days later these animals along with two controls are injected subcutaneously with 1 ml. of the challenge virus containing at least 1000 minimum infective doses.

5. Labelling- Should comply with requirements of 'Labelling' as laid down in the general monograph on 'Viral Vaccines'.

6. Storage- The vaccine may be expected to retain its potency for six months if stored at temperature ranging between - 10° C to - 15° C and for seven days at 2° C to 4° C in the refrigerator.

¹**[Foot and Mouth Disease Vaccine (Inactivated)]**

1. Synonym. – Inactivated Tissue culture mono or polyvalent Foot and Mouth Disease Vaccine.

2. Definition. – Foot and Mouth Disease Vaccine is a liquid product or preparation containing one or more types of foot and mouth disease virus which have been inactivated in such a way that its immunogenic property is maintained. It may also contain an adjuvant. The vaccine is described as monovalent, bivalent, trivalent or polyvalent depending on the number of types of virus used.

3. Preparation. – The virus is propagated in suitable cell culture. The cell culture is infected with an appropriate inoculum of virus and incubated at a suitable temperature for multiplication of virus. The virus is harvested and cellular debris removed by filtration. Inactivation is carried out by a suitable agent such as formaldehyde solution or aziridine compound. The adjuvant may be aluminium hydroxide and/or saponin. In case of inactivated gel vaccine the antigen is concentrated by sedimentation at plus 4 degree C. For preparing a polyvalent vaccine, monovalent antigens are mixed in appropriate quantities to give the final mixture which is the formulated vaccine.

4. Standards :

(a) *Description :-* Aluminium hydroxide gel vaccines settle down to variable degree on storage leaving the supernatant clear.

1. Ins. by G.S.R. No. 659(E), dt. 31-8-1994.

(b) *Identification* – It protects cattle against Foot and Mouth Disease due to homologous type/subtype of virus.

(c) *Sterility test* – It shall comply with the tests for sterility as prescribed under the “general monograph on “Viral Vaccines”.

(d) *Safety test* - The test is carried out on fully susceptible cattle not less than 12 months of age and which have not been sensitized either by vaccination or previous infection. Inoculate 3 susceptible cattles each with 2 ml. of finished product at multiple sites on tongue by intradermal route and observe for 4 days. The same animals are inoculated on 4th day with 3 cattle doses subcutaneously and are observed for a further period of 6 days. The animals should not develop any signs of FMD and remain normal.

(e) *Potency test* – Each batch of the vaccine is to be tested in susceptible cattle of not less than 15 months of age. The potency test in cattle can be done either by :-

(i) *PD₅₀ Method* : The vaccine shall be tested by the determination of PD₅₀ in susceptible cattle by challenging animals vaccinated with appropriate dilution of the vaccine made in adjuvanted or non-adjuvanted diluent as appropriate.

A minimum of 5 animals should be used per dilution and 2 unvaccinated animals to be included as controls to the challenge. All animals are needle challenged with 10,000 ID₅₀ of the homologous strain of virus by inoculation on the tongue on the 21st day of post-vaccination.

The control animals are to be similarly challenged. Animals are observed for 10 days for the development of lesions. Unprotected animals show generalised lesions due to FMD. Control animals must shown generalized lesions. From the number of animals protected in each group the PD₅₀ content of the vaccine is calculated. The vaccine passes the test if an observed PD₅₀ value of 3 or greater is obtained in the test.

(ii) *Percentage protection method* in which groups of ten health susceptible cattle are each injected subcutaneously with the vaccinating dose and 14 – 21 days later the cattle are challenged by intradermal injection into three separate sites on the tongue with 10,000 ID₅₀ of the strain of virus used in the preparation of the vaccine. The vaccine can be passed if atleast seven out of the ten in the group are protected against the development of generalized infection whereas all the controls should react by developing primary and secondary lesions observable in the mouth and feet.

For other reasons if cattle testing is not possible then the potency of the vaccine may be assed in guineapigs either by Lucam ‘C’ index or PD₅₀ method by challenging those which have been previously vaccinated, provided that the correlation has been established between guinea pig challenge test and cattle challenge results.

The estimation of the serum neutralizing antibody titre cattle may be considered as a supportive test to evaluate potency of vaccine.

However, potency testing of vaccines, in cattle, of batches whenever by other accepted methods of testing is in doubt, at atleast one out of every five batches, be undertaken.

5 Labelling :- It is labelled as described under the requirements of ‘labelling’ as laid down in the general monograph, with the additional requirements that the label on the container states the virus types used in the preparation.

6. Storage :- It should be protected from the light and stored between 4° C to 8° C. Under these conditions it may be expected to retain its potency for not less than 12 months. Freezing of aluminium hydroxide vaccine must be avoided. The frozen product will not be fit for use.

Canine Hepatitis Vaccine (Living)

1. **Synonyms** : Infectious Canine Hepatitis Vaccine (Living), Canine Hepatitis Cell Culture Vaccine.

2. **Definition** : Canine Hepatitis Vaccine (Living) is a freeze dried preparation of tissue culture fluid containing the cell culture adapted canine hepatitis virus.

3. **Preparation** : Canine hepatitis vaccine shall be prepared from virus bearing cell culture fluid.

Only stock seed virus which has been established as pure, safe and immunogenic shall be used in the preparation of the vaccine.

Immunogenicity test : Each lot of stock seed virus shall be tested for immunogenicity as follows :

Thirteen canine hepatitis susceptible dogs, 8-14 weeks old shall be used for the test (10 vaccinates and 3 controls). Blood samples may be drawn from these animals and individual serum samples tested for the presence of antibodies, against canine hepatitis virus. Ten dogs shall be injected subcutaneously with predetermined quantity of the virus and remaining 3 dogs are kept as unvaccinated controls. The dose calculation will be based on virus titration in suitable cell culture system. Not less than 14 days post vaccination, the vaccinated and control shall each be challenged intravenously with virulent infectious canine hepatitis virus and observed daily for 14 days. At least 2 out of 3 controls should die and the survivors shall show the clinical signs of canine hepatitis. Nine out of ten vaccinated dogs shall survive and shall not show any signs of infectious canine hepatitis during the observation period.

The stock seed virus shall be tested once in 5 years and maintained under standard conditions as prescribed.

The stock seed virus may be inoculated on a suitable tissue culture system and may be incubated for five to seven days.

The tissue culture fluid is then harvested and titrated in cell culture system for virus content. After appropriate dilution and pooling, the material is stored at minus 20° C until freeze dried. Each vaccine dose shall contain not less than 10^{3.5} TCID₅₀ dose.

4. **Standards :-**

(a) **Description.** – The dried product is a pinkish cream material readily dispersible in water. The reconstituted vaccine is a pinkish liquid.

(b) **Identification.**– It causes characteristic cytopathic effect in dog, pig and ferret kidney monolayers. This can be neutralized by specific antiserum. When inoculated into dogs, the development of specific neutralizing antibodies can be demonstrated by suitable serological tests.

(c) **Moisture content.**– In the finished product moisture content shall not exceed 1.0 per cent.

(d) **Sterility Test.**-- Shall comply with the tests of sterility as described under the general monograph on “Viral Vaccines”.

(e) **Safety Test.**-- Mouse safety test – Vaccine prepared for use as recommended on

the label shall be tested. Eight mice shall be inoculated intracerebrally with 0.03 ml and 8 mice shall be inoculated intraperitoneally with 0.5ml. Both the groups shall be observed for seven days. If unfavourable reaction attributable to the product occurs in two or more mice in either group during their observation period, the batch is unsatisfactory.

- (f) *Dog Safety Test.*-- Each of the two susceptible pups aged 8 – 14 weeks shall be injected with vaccine equivalent of 10 vaccinating doses from the batch reconstituted with sterile diluent and administered in the manner recommended on the label and observed for 21 days. None of the pups shall show any unfavourable reaction during the period of observation.
- (g) *Potency test, Virus Titration.* – Samples of finished product shall be tested for virus titre in suitable cell culture system. The batch shall have a virus titre of not less than $10^{3.5}$ TCID₅₀ dose.
- (h) *Potency test in dog.*— Two healthy susceptible dogs of 8-14 weeks of age shall be injected subcutaneously with one Vaccine dose. 14 days after vaccination, specific neutralizing antibodies from both the dogs shall be demonstrable by serological tests.

- 5 Labelling:** Shall comply with the requirement for labelling as laid down in the general monograph on “Viral Vaccines”.
- 6 Storage:** The dry product shall be stored at temperature of minus 20° C or below. The vaccine is expected to retain its potency for about 6 months in the freezing chamber of the refrigerator (temperature) approximately minus 8° C

Duck Plague Vaccine

- 1 Definition.-** Duck plague vaccine is a suspension of modified living virus prepared from infected chick embryos.
- 2 Preparation.-** Fresh fertile hen’s eggs obtained from salmonella free flocks are incubated in an incubator. Nine days old embryos are injected with 0.2 ml of the suitable dilution (1 in 100) of the suspension of the virus on the CAM and incubated at 37° C for 5 days post-inoculation. Dead embryos of the 3rd, 4th and 5th days post-inoculation are harvested. The embryos (devoid of head and legs). Clear fluid and the membranes are collected and homogenized in a Blender, ampoulded in 0.5ml quantities and freeze dried.
- 3 Standards:-**
- (a) *Description.*- Light brown scales.
- (b) *Identification.*- This product affords protection to the ducks against duck plague.

- (c) *Safety Test.*- Four healthy, 8 to 12 weeks old ducks weighting not less than 600 gms are inoculated subcutaneously with 1ml of 10^{-1} dilution of the vaccine and observed for a period of 14 days. During the period of observation, the ducks shall not show any untoward reaction.
- (d) *Sterility test.*- Shall comply with the test for sterility described in the general monograph on “Viral Vaccines”.
- (e) *Potency Test.*- Six susceptible ducks 8 to 12 weeks old each weighing not less than 600 gms are inoculated subcutaneously with 1 ml of 10^{-3} dilution of the vaccine. The minimum virus contents in 1 ml. dose of the vaccine shall be $10^{3.5}$ EID₅₀. 14 days later these ducks are challenged subcutaneously each with 1 ml. of 10^{-2} dilution of the virulent duck plague virus (1000 DEID₅₀) along with 2 unprotected young ducks of about 8-12 weeks age. The unprotected ducks shall show symptoms of duck plague and die within 10 days, while the protected ducks shall remain normal during the observation period of 14 days.
4. **Labelling.**- Should comply with the requirements of labelling as laid down in the general monograph on “Viral Vaccine”.
5. **Storage.**- Vaccine when stored at minus 15° C to minus 20° C may be expected to retain its potency for one year and about three months if stored in the freezing chamber of Refrigerator i.e. minus 5° C.
- Avian Encephalomyelitis Vaccine (Living)***
1. **Synonyms.**- Avin-encephalomyelitis Vaccine Freeze dried.
2. **Definition.**- A virus bearing tissue and fluid suspension from embryonated hen’s legs.
3. **Preparation.**- The stock seed virus which has been established as pure, safe and immunogenic shall be used for preparing the vaccine.
- (i) Each lot of stock seed virus shall be tested for pathogenicity by chicken embryo inoculation test:
- (a) One dose of the seed lot shall be mixed with 9 volume of sterile heat inactivated specific, antiserum to neutralize vaccine virus in the product.
- (b) After neutralization, 0.2 ml of serum vaccine mixture shall be inoculated into each of at least 20 fully susceptible chicken embryos (0.1ml of the inoculum shall be inoculated on CAM of 9-11 days old embryos and 0.1 ml in the allantoic sac).
- (c) Eggs shall be candled for 7 days. Deaths occurring during first 24 hours shall be discarded but at least 18 viable embryos shall survive 24 hours post inoculation for a valid test. All embryos and CAMs from embryos which die after the first day shall be examined.

- (d) If the death or abnormality attributable to inoculums occur, the seed lot is unsatisfactory.
- (ii) **Immunogenicity test.**- Avian encephalomyelitis susceptible chicks, all of same age (8 weeks old) shall be used. 20 chickens shall be inoculated with the field dose of the virus by prescribed route. Ten additional chickens of same age and flock shall be held as unvaccinated controls.

At least 21 days post vaccination, the controls and vaccinates shall be challenged intracerebrally with Virulent avian encephalomyelitis virus and observed each for 21 days. At least 80 percent of controls shall show signs of avian encephalomyelitis or die. At least 19 to 20 vaccinates shall remain free from clinical avian encephalomyelitis during the observation period for the stock seed virus to be satisfactory

4. **Standards:-**

- (a) **Description:-** Greyish white flakes easily dispersible in the diluent.
- (b) **Identification:-** At least 5–6 days old embryonated eggs (from hens with no history of infection with avian encephalomyelitis) shall be inoculated with 0.1 ml of undiluted vaccine into the yolk sac and kept in incubator and then transferred to the brooder where they are allowed to hatch. The hatched chicks shall be raised for 7 days. More than 5 per cent of hatched chicks shall manifest the typical symptoms (weak-leg, leg paralysis tremor etc.) at the end of this period.
- (c) **Moisture Content:** - Shall not exceed 1.0 per cent.
- (d) **Sterility Test:** - Shall comply with the test for sterility described under general monograph on “Viral Vaccines”.
- (e) **Safety Test:** - At least 25 avian encephalomyelitis susceptible birds (6-10 weeks of age) shall be vaccinated with 10 field doses by the recommended route and observed each day for 21 days. If unfavourable reactions attributable to the vaccine occur during the observation period, the batch of vaccine is unsatisfactory.
- (f) **Potency Test:** -
- (i) The vaccine shall be titrated for virus content. To be eligible for release, the batch shall have a virus titre of at least $10^{2.5}$ EID₅₀ per dose.
- (ii) At least 10 susceptible chickens shall be vaccinated with the field dose of the vaccine by prescribed route and 10 chickens from same batch and source shall be kept as unvaccinated controls.

At least 21 days post-vaccination, both the groups shall be challenged intracerebrally with Virulent avian encephalomyelitis virus and observed for 21 days. At least 8 out of 10 controls shall develop recognizable signs or lesions of avian encephalomyelitis and at least 8 out of 10 vaccines should remain normal

- 5. Labelling:** Shall comply with the requirement of labelling as laid down in general monograph on “Viral Vaccines”.

Marek's Disease Vaccine (Living)

1. **Synonyms.-** Herpes virus of Turkey Vaccine HVT vaccine (Living).
2. **Definition:-** Marek's disease vaccine is a suspension of cell free fluid containing live virus.
3. **Preparation.-** The stock seed virus which has been established as pure, safe and immunogenic in avian species shall be used for preparing the seed virus for vaccine production.
 - (i) **Safety Test** – The stock seed virus shall be non-pathogenic for chickens as determined by the following procedure:

The groups of at least 25 chickens each at one day of age shall be used. These chickens shall be of the same source and batch be susceptible to Marek's disease and be kept in isolated group.

Group I: Each chicken shall be injected with 0.2 ml of 10 times as much viable virus as will be contained in one dose of vaccine by intramuscular route.

Group II : Shall serve as controls. At least 20 chickens in each group shall survive for four days postinjection. All chickens that die shall be necropsied and examined for lesions of Marek's disease and cause of death. The test shall be judged according to the following :

At 120 days o age, the remaining chicken in both the groups shall be weighted, killed and necropoised. If at least 15 chicken in each of these two groups have not survived the 120 days period if any of the chicken of Group-I have gross lesions of Marek's disease at necropsy or if the average body weight of the chickens in Group-I is significantly (statistically) different from the average of Group-II at the end of 120 days, the lot of stock seed virus is unsatisfactory.
 - (ii) **Purity test** – Shall conducted in chickens and no lesions other than those typical of Turkey Herpes virus shall be evidenced.
 - (iii) **Immunogenicity test** – Sixty susceptible day old chicks are used. Thirty of them inoculated with the seed virus in a dose corresponding to the field dose of the final vaccine and 14-21 days later challenged by intrabdominal route with virulent Marek's diseas virus, alongwith the other 30 non-vaccinated control chicks. At the end of the observation period when the chicks are 20 weeks old, the surviving chickens are examined for the presence of antibody against Marek's disease by serological tests and postmortem inspection for lesions of Marek's disease.

Any bird dead is thoroughly examined and the cause of death ascertained by necropsy/histopathological examination. All the surviving birds are killed and necropsied. The protection index (P1) is determined by following procedure :

$$1. \text{ Percent MD} = \frac{\text{No. with MD lesions}}{\text{No. with MD lesions} + \text{No. of -ve Surviviours (effective No.)}} \times 100$$

$$2. \quad \text{P.I.} = \frac{\text{Percent MD in controls} - \text{Percent MD in vaccinated}}{\text{Percent MD controls}} \times 100$$

Master seed virus should have P.1 of at least 80 per cent.

Eighty per cent of the chicks in the control group must fall ill specifically. If more than 80 per cent of the vaccinated chickens do not show symptoms or signs of Marek's disease, the seed virus is regarded as sufficiently effective and can be used for production of vaccine.

The seed virus is propagated in duck embryo fibro-blast cell culture, chick embryo fibroblast or any other suitable cell culture (specific pathogen free SPF flock) and when the peak passage level is attained the cell monolayer is suspended in cold diluent of the following composition:

SPGA Stabilizer

0.218% Sucrose.

0.0038% monosodium phosphate.

0.0072% dipotassium phosphate.

L Monosodium glutamate 0.0049 M.

1 per cent bovine albumin fraction (V).

0.25 per cent EDTA (Sterilised by Seitz filtration and stored at minus 10° C). The virus is freed from cell by ultrasonication for 3 minutes interrupted after every 30 seconds) at 100 MA and freeze dried at times 60° C preferably in shelf freeze dried in convenient volumes. The doses per ampoule vial is calculated after titrating the freeze dried product in terms of plaque forming units (PFU) in the corresponding cell monolayers.

4. Standards:

- (a) *Description:* - The cell free freeze dried HVT vaccine look uniformly grayish in colour and easily dispersible in the specified diluent.
- (b) *Identification.* - The vaccine on inoculation in suitable cell culture system shall cause cytopathic effect typical of Herpes virus of Turkey. Specific antiserum of Herpes virus of Turkey shall neutralize the cytopathic effect.
- (c) *Moisture content.* - Moisture content shall not exceed one per cent.
- (d) *Sterility test.* - Shall comply with the test prescribed in general monograph on "Viral Vaccines".
- (e) *Safety Test.*- At least 25 one day old chickens shall be injected with ten times of the field dose of vaccine by intramuscular route. The chickens shall be observed each day for 21 days. Chickens dying during the period shall be examined, cause of death determined and the results recovered as follows :
 - (i) If at least 20 chickens do not survive the observation period, the test is inconclusive.
 - (ii) If lesions of any disease or cause of death are directly attributable to the vaccine the vaccine is unsatisfactory.
- (f) *Potency test.* - The sample shall be titrated in the cell culture system. A satisfactory batch shall contain at least 1500 plaque forming units (PFU) as per dose at the time of release

and maintain at least 1000 PFU till the end of expiry period.

5. **Labelling.** - Shall comply with the requirements of labelling as laid down in general monograph on "Viral Vaccines".
6. **Storage and expiry date.** - The freeze-dried cell free HVT vaccine may be stored at 4°C for 6 months.

Goat Pox Vaccine (Living Cell Culture)

1. **Synonym.** - Goat Pox Vaccine (living), Attenuated Goat Pox Vaccine.
2. **Definition.** - Goat Pox vaccine is freeze-dried preparation, prepared by growing attenuated goat pox virus in kid kidney/testicular cell culture.
3. **Preparation.** - Primary kidney/testicular cell cultures of disease free kid are used. The monolayers infected with the seed virus are incubated at 37° C. The cultures are harvested by three cycles of freezing and thawings 6 to 7 days post infection when more than 80 per cent cells show CPE. The suspension is centrifuged at 1000 rpm for 10 minutes to remove cellular debris being stored at minus 20° C. The suspension is freeze dried after addition of 5 per cent Lactalbumin hydrolysate and 10 per cent sucrose.
4. **Standards:**
 - (a) **Description.** - Light yellow colour.
 - (b) **Identification** - The product affords protection to goat against goat pox.
 - (c) **Moisture content.** - The moisture content shall not exceed 1.0 per cent.
 - (d) **Safety tests.-**
 - (i) **Laboratory animals.** - Six mice, 3 guinea pigs and 3 rabbits are inoculated with 0.2ml intraperitoneally, 0.5 ml and 1.0 ml subcutaneously, respectively with 10 field doses of the vaccine. The inoculated animals during the observation period of 80 days shall remain normal.
 - (ii) **Goat.** - Two susceptible goats of 6 to 8 months of age are inoculated in postaxillary region by subcutaneous route with one-hundred field doses of the vaccine. The inoculated animals shall not develop more than a local reaction of 2 to 3 cms. These animals shall be observed for 10 days.
 - (e) **Sterility test.-** Shall comply with the test for sterility described under the general monograph on "Viral Vaccines".
 - (f) **Titration in cell culture.-** Four randomly selected samples are inoculated in kid kidney cell cultures using 5 tubes for each dilution. The titration shall be repeated thrice. One thousands TCID₅₀ is used as a field dose.
 - (g) **Potency Test.-** The three susceptible goats (8-10 months) are inoculated with 1/10th field dose and 3 susceptible goats (8-10 months) with one field dose, subcutaneously. Three in contract controls are also kept with the inoculated goats. These animals are observed for a period of 14 days and their body temperature recorded daily. The vaccinated

animals shall not show any termal, local or generalized reaction. Twenty one days post infection, the vaccinated and controls and challenged with 10,000 TCID₅₀ of virulent goat pox virus by intradermal route. The temperature of these goats are recorded for a period of 14 days. The vaccinated goats shall not develop localized or generalized reaction while control goats shall develop high fever, localized reaction or even generalized reaction in some cases.

5. **Labelling** .- Shall comply with requirements of labelling as laid down in the general monograph on “Viral Vaccines”.
6. **Storage and expiry date**.- The vaccine is expected to retain its potency for 12 months if stored at minus 15° C to minus 20° C and for three months at 2° C to 4° C.

Sheep Pox Vaccine (Inactivated)

1. **Synonym** .- Formal gel sheep pox vaccine.
2. **Definition** .- Sheep pox vaccine is a formaline inactivated gel treated tissue vaccine.
3. **Preparation**.- Healthy susceptible sheep of 8-12 months of age are inoculated subcutaneously with 500 ml of the 1:100 dilution of the Russian Virulent Sheep Pox Virus. Seven to eight day post inoculation skin of the abdomen alongwith the oedema is collected. The infected tissues are homogenized in 10 percent concentration in phosphate buffer (pH 7.4-7.6) which after the extraction of the virus is mixed with sterile gel and buffer in the following proportion :-

6 percent aluminium hydroxide gel-50 per cent.
Phosphate Buffer (pH 7.6)- 35 per cent.
10 per cent suspension – 15 per cent

This is later formalized and kept at 20-25°C/10°C for varying periods.

4. **Standards**.-
 - (a) **Description** .- It is a greyish white suspension. During storage the gel settles at the bottom, upper layer of the suspension is clear.
 - (b) **Identification**.- This product affords protection to sheep against sheep pox.
 - (c) **Safety test**.- This is carried out by inoculating 2 white mice with 0.2 ml., one guinea pig with 1.0 ml and one rabbit with 3,0 ml of vaccine. The animals should remain clinically healthy for 10 days.
 - (d) **Sterility test**.- This is done by seeding the vaccine on usual bacterial media. The plates and tubes are incubated for 10 days at 37° C. If the pathogenic bacteria are found, the vaccine is rejected while with non-pathogenic bacteria the vaccine is passed for field use.
 - (e) **Potency test**.- This is done by inoculating 4 non immune susceptible sheep preferably exotic breed of 1-2 years with 3 ml of vaccine in the thigh, subcutaneously.

The vaccinated sheep are challenged 15 days after inoculation alongwith 3 controls each with 0.1 ml of virulent virus containing 200 infective doses intradermally under the tail.

The sheep are observed for 10 days and their skin reaction recorded. The vaccine is considered potent if all the vaccinated, sheep do not show thermal or local skin reaction. Vaccine is also potent if 3 vaccinated animals do not develop any reaction and one shows abortive skin reaction, while at least 2 of the 3 controls develop typical sheep pox reaction at the site of inoculation.

5. **Labelling.-** Shall comply with the requirements of labelling as laid down in the general monograph on "Viral Vaccines".
6. **Storage.-** The vaccine shall be stored at 4° C to 5°C. it keeps well at above temperature upto 12 months.

Sheep Pox Vaccine (Living Cell Culture)

1. **Synonym.-** Sheep pox vaccine (Living), attenuated sheep pox vaccine.
2. **Definition.-** Sheep pox vaccine is freeze dried preparation prepared by growing attenuated sheep pox virus in lamb kidney/testicular cell cultures.
3. **Preparation.-** Primary cell cultures prepared from kidney/testicles of disease free lambs are used. The mono layers infected with the seed virus are incubated at 37° C. The cultures are harvested by 3 cycles of freezing and thawing 6 to 7 days post infection when more than 80 per cent Cells show C.P.E. The suspension is centrifuged at 1000 r.p.m. for 10 minutes to remove cellular debris before being stored at minus 20° C. The suspension is freeze dried after addition of 5 per cent Lactalbumin hydrolysate and 10 per cent sucrose.
4. **Standards:-**
 - (a) **Description.-** Light yellow colour.
 - (b) **Identification.-** The product affords protection to sheep against sheep pox.
 - (c) **Moisture contents.-** The moisture contents should not exceed 1.00 per cent.
 - (d) **Safety tests.-**
 - (i) Six mice, 3 guinea pig and 3 rabbits are inoculated with 0.2 ml intraperitoneally 0.5ml and 1.0 ml subcutaneously, respectively containing 10 field doses of the vaccine. The inoculated animals during the observation period of 10 days should remain normal.
 - (ii) One hundred field doses of the vaccine are inoculated subcutaneously into each of 2 susceptibles sheep in postaxillary region. Inoculated animals shall not develop more than a local reaction of 2 to 3 cms.
 - (e) **Sterility test. -** Shall comply with the test for sterility as described under the general monograph on "Viral Vaccines".
 - (f) **Titration in cell culture. -** Four randomly selected samples reconstituted in a maintenance medium are inoculated in lamb kidney cell cultures using 5 tubes for each dilution. The titrations shall be repeated thrice. The TCID₅₀ to be calculated by Read and Muonch method. One thousand TCID₅₀ is calculated as one field dose.
 - (g) **Potency test. -** Three susceptible sheep 8-10 months old are inoculated with 1/10th, field dose and 3 susceptible sheep with one field dose, subcutaneously. Three in contact controls are also kept with the inoculated sheep. These animals are observed for a period

of 14 days and their temperature is recorded daily. The vaccinated animals should not show any thermal, local or generalize reactions. Twenty-one days post infection the vaccinated and controls are challenged with 10,000 ID₅₀ of virulent sheep poxvirus by intradermal route. The temperature of these sheep are recorded for a period of 14 days. The vaccinated sheep should not develop localised or generalized reaction while control sheep should develop high fever, localized reaction or even generalized reaction in some cases.

5. **Labelling.-** Shall comply with requirements of labelling as laid down in the general monograph on "Viral Vaccines".
6. **Storage and expiry date.-** The vaccine is expected to retain its potency for 12 months if stored at minus 15° C to minus 20° C and three months at 2° C to 4° C

Tissue Culture Rinderpest Vaccine

1. **Synonyms.-** Cell Culture Rinderpest Vaccine.
2. **Definition.-** Tissue Culture Rinderpest Vaccine is a freeze dried preparation of live modified rinderpest virus adapted to and propagated in cell culture.
3. **Preparations.-** Primary or secondary monolayer cultures of the kidney cells (Bovine or any other suitable animals) taken from kidney from healthy animals free from any pathological changes shall be used. When secondary cultures are used they shall have retained their original morphology and Karyotype. Kabete 'O' stain of Rinderpest virus developed by East African veterinary Research Organisation (Plowrights strain between the passage levels of 99th and 100th passages) shall be used. The virus harvested from cell monolayer culture prepared from the kidneys of a single calf or serially cultivated bovine kidneys cells (Not more than 10 passages away from the Primary) inoculated with the same seed and harvested together, will be freeze dried with stabilizers in suitable quantities.
4. **Standards.-** It complies with the requirements of general standards of viral vaccines:
 - (a) **Description.-** Dry light yellow coloured flakes readily soluble in chilled and saline or buffered saline.
 - (b) **Identifications.-**
 - (i) Protects cattle against a subsequent challenge with virulent or caprinised rinderpest virus.
 - (ii) It is titrable in tissue culture systems capable of supporting the multiplication of this virus. The test shall be made on at least three separate occasions using a cell culture derived from different animals.
 - (iii) Specificity test shall be performed using an appropriate scrum neutralization test.
 - (c) **Sterility test. -** Each batch shall be tested for bacterial and mycotic sterility as given in general monograph on "Viral Vaccines".
 - (d) **Innocuity test.-** Shall be made on each batch in at least two guinea pigs and six mice. These animals shall be observed for at least two weeks for any untoward reaction.
 - (e) **Safety and efficacy test.-** The test for safety and efficacy shall be performed using the pooled reconstituted contents of not less than 4 ampoules taken at random. The vaccine

shall be injected subcutaneously into each of at least two susceptible cattle free from specific antibodies using the quantity containing not less than 100 fields doses and two further cattle and using 1/10th of the field dose (calculated on the basis of 1000 TCID₅₀ one field dose). The animals shall be housed with at least two unvaccinated animals and observed for a period of three weeks. The vaccine passes the safety test if the cattle show no signs of unusual clinical reactions.

At the end of three weeks all the four animals will be challenged alongwith two in contact cattle with a challenge dose of not less than 10⁴ ACID₅₀ of virulent Rinderpest virus. The vaccine passes the potency/efficacy test if the in contact animals develop rinderpest and all the vaccinated animals remain normal.

5 Labelling.- Shall comply with general monograph on “Viral Vaccines”. Each ampoule or at least 50 percent ampoules in a lot shall contain at least following print :

- (i) TCRP Volume.
- (ii) Batch No. with year.
- (iii) General instructions for use.

6 Storage .- The vaccine when stored at minus 20° C and plus 4 degree C will maintain its titre for 2 years and 6 months respectively.

Canine Distemper Vaccine

1. **Synonyms.-** Canine Distemper Vaccine (Living)- Freeze-dried.
2. **Definition.-** It is freeze dried preparation of either tissues from chick embryo containing eggs adapted strain of canine distemper virus or the cell culture in which modified virus has been cultivated.
3. **Preparation.-** Canine distemper vaccine shall be prepared from virus bearing cell culture, fluid or infected chorioalantoic membrane. Only stock seed virus which has been established as pure, safe and immunogenic shall be used for preparation of vaccine. Stock seed virus propagated in chicken embryo shall be tested for pathogen by chicken embryo test. One volume of the virus shall be mixed with 9 volume of specific sterile heat inactivated serum to neutralize the virus. Mixture shall be inoculated into twenty (9 to 11 days old) chicken embryo (with 0.1 ml on CAM and 0.1 ml in alantoic sac). Embryonated eggs shall be candled for 7 days daily. Death occurring in the first 24 hours shall be discarded. CAMS of embryos which die after 24 hours shall be examined. When necessary embryo sub-culture shall be made to determine the cause of death. The test should be concluded on the 7th day post inoculation.

The surviving embryos and their CAMS are examined. If deaths or abnormality due to the inoculums occur, the seed virus is unsatisfactory.

Immunogenicity test : Thirteen susceptible dogs 8-14 weeks old shall be used for the test (ten vaccinates and 3 controls). Blood samples are drawn from these animals and individual sample is tested for antibodies against canine distemper. Ten dogs shall be injected with a predetermined quantity of the virus and remaining 3 dogs are used as unvaccinated controls. The dose shall be based on the virus titration. At least 21 days post infection the vaccinated and controls shall be challenged intramuscularly with the same dose of virulent canine distemper virus and the animals are observed each day for 21 days. At least 2 out of 3 controls should die and survivor should show the symptoms typical of canine distemper. At least 9 out of 10 vaccinated animals should survive and should not show any clinical signs of canine distemper during the observation period. The stock seed

virus should be tested for immunogenicity at least once in 5 years, if maintained under suitable conditions of storage. Eight days old chicken embryos from a healthy flock are inoculated on their chorioallantoic membrane with bacteriologically sterile virus suspension of egg adapted strain. After incubation for a period of five days, infected membrane and embryos are harvested. The individual embryo is tested for bacterial sterility. Those free from bacterial contamination are made into a 20 percent suspension in a suitable medium. The suspension is distributed in a single dose quantity into the ampoules of vials and freeze-dried.

The ampoules are sealed under vacuum or with pure dry sterile nitrogen before sealing. Alternatively, the virus may be grown on the suitable cell culture. Cells along with the suspending fluid is harvested, distributed in single dose quantity in ampoules and freeze dried.

4. Standard.-

- (a) *Description.* - It is a dry product, pinkish cream material, readily dispersible in water or a suitable solvent.
- (b) *Identification.* - It infects CAM of fertile eggs. This is neutralized by canine distemper antiserum. It does not cause distemper after injection into susceptible ferrets or dogs but immunizes them against the disease.
- (c) *Moisture content.* - Moisture content in the finished product shall not exceed more than 1.0 per cent.
- (d) *Sterility test.* - Shall comply with the test for sterility as described in the general monograph on "Viral Vaccines."
- (e) *Safety tests.* - (i) *Mice safety test:* Reconstituted vaccine as recommended on the label shall be tested.

Eight mice, 4 weeks old shall be inoculated intracerebrally with 0.03 ml and 8 mice shall be inoculated intraperitoneally with 0.5ml. Both groups shall be observed for 7 days, if unfavourable reaction attributable to the product in either 2 or more mice in either group is observed during observation period, the batch is unsatisfactory.

(ii) *Dog Safety test.*-Inject two healthy dogs eight to fourteen weeks old that have previously been shown to be free from distemper virus-neutralising antibodies by the recommended route with twice the dose stated on the label and observe for 21 days. No significant local or general reaction develops.

- (i) *Potency test.* - (i) *Titration :* Final samples of finished product shall be tested for virus titre, and when tested at any time within the expiry period, it should contain not less than 10^3 ID₅₀ per dose.
- (ii) It shall be carried out in dogs. Two healthy susceptible dogs each of 8-14 weeks of age free from distemper neutralizing antibodies are injected subcutaneously each with one vaccination dose. Serum samples shall be collected from each dog 14 days after vaccination and these shall have specific neutralizing antibodies at a dilution of 1: 100.

6. Labelling.- Shall comply with the requirements of labelling as laid down in the general monograph on "Viral Vaccines".

7. **Storage and expiry date.** - For the freeze-dried product the expiry date is one year when stored at minus 20°C.

Avian Infectious Bronchitis Vaccine (Living)

1. **Synonyms.** - Avian Infectious Bronchitis Vaccine (Living) freeze-dried.
2. **Definition.** - It is a freeze-dried product of low virulent Avian Infectious Bronchitis Virus grown in embryonated hen's eggs of cultivated in cell culture.
3. **Preparation.** - Only stock seed virus which has been established as pure, safe and immunogenic shall be used. Each lot of stock seed virus shall be tested for other pathogens by chicken embryo inoculation tests as follows: -

A lot of seed virus shall be mixed with 9 volumes of sterile heat inactivated specific anti- serum to neutralize and the vaccine virus serum mixture shall be inoculated into each of at least 20 fully susceptible chicken embryos of 9-11 days old (0.1 ml on CAM and 0.1 ml in the allantoic sac). Eggs are candled daily for 7 days. Deaths occurring during first 24 hours shall be discarded but at least 18 viable embryos shall survive 24 hours post inoculation for a valid test. All embryo and CAMS from embryos shall be examined which die after 24 hours. If necessary embryo subcultures shall be made to determine the cause of death. The test shall be concluded on the 7th day post inoculation and surviving embryos including the CAM shall be examined. If death and or abnormality attributable to the stock seed virus occur, the seed lot is unsatisfactory.

Each lot of stock seed virus shall be tested for immunogenicity as below: -

Bronchitis susceptible chickens of the same age and source shall be used. For each method of administration recommended on the label and for each serotype against which protection is claimed, 20 chicks shall be used as vaccinates. Ten additional chickens for each serotypes against which protection is claimed shall be held as unvaccinated controls.

21 to 28 days post vaccination all vaccinates and controls shall be challenged by eye drops with virulent Bronchitis virus. A separate set of vaccinates and controls shall be used for each serotype against which protection is claimed. The challenge virus shall have a titre of at least $10^{4.6}$ EID₅₀ per ml. Trachea swabs shall be taken once 5 days post challenge from each vaccinated and controls. Each swab shall be placed in test tube containing 3 ml of tryptose phosphate broth and antibiotics. The tubes and swabs shall be swirled thoroughly and stored at minus 40° C pending egg inoculation. For each chicken swabs at least 5 chicken embryos, 9-11 days old shall be inoculated in the allantoic cavity with 0.2 ml of broth from each tube. All the embryos surviving 3rd day post inoculation shall be used in evaluation. A tracheal swab shall be positive for virus recovery when any of the embryos show typical infections bronchitis virus lesions such as stunting, curling, kidney urates, clubbed down or death during 4-7 days post inoculation period.

90 percent of the controls should prove positive for virus recovery. If less than 90 per cent of the controls are negative for virus recovery, the stock seed is unsatisfactory. The stock seed virus should be tested for immunogenicity once in 5 years provided it is maintained under standard conditions of the bronchitis virus storage.

4. **Standards.** -

(a) *Descriptio:* It is greyish-white product easily dispersible in the diluent.

(b) *Identification:* (i) The contents of the ampoule are suspended as per the instructions for the field use. The 0.2ml of the suspension shall be inoculated in the allantoic cavity of 9-11 days old chicken embryo and are incubated for 7 days. The lesions typical of infectious bronchitis shall be observed in the embryos at the end of incubation period. The allantoic fluid shall not agglutinate the chicken RBC's.

(ii) Specific antisera against avian infectious bronchitis virus should neutralize the vaccine virus.

(c) *Moisture content.* - Moisture content in the finished product should not exceed 1.0 per cent.

(d) *Sterility test.* - Complies with the test for sterility as described under the general monograph on "Viral Vaccines".

(e) *Safety test.* - Ten healthy susceptible chickens 5-10 days old from the same source batch shall be vaccinated with ten field dose of the vaccine and along with five chicks from same batch as unvaccinated controls by the prescribed route and observed 7 or 21 days post vaccination. Neither severe respiratory symptoms nor death shall occur to more than one experimental chicks, none of the unvaccinated control shall show any clinical symptoms.

(f) *Potency test.* - The minimum virus content of the freeze-dried product shall not be less than $10^{3.5}$ EID₅₀ per bird. The virus content of the vaccine shall be titrated as below:

Serial ten-fold dilution of the freeze-dried material will be made in tryptose phosphate broth. Three to five embryonated eggs (9-11 days old) shall be inoculated with 0.1 ml of each dilution into the allantoic cavity and observed daily for 7 days.

Deaths occurring during the first 24 hours shall be discarded. The surviving embryos are examined for the evidence of infection and EID₅₀ shall be calculated by the Reed and Muench Method/spearman and Karber method.

5. *Labelling* .- Shall comply with the requirement of labelling as laid down in the general monograph on "Viral Vaccine".

6. *Storage and expiry date.*- Shall be stored at 4° C for six months].

PART II –ANTISERA

Provisions applicable to the production of all Sera from Living Animals

1. *Definitions-* (i) This Part of the Schedule applies to antibacterial sera, anti-viral sera and anti-toxic sera which are prepared by injecting bacteria or viruses or their products into buffalo- bulls or other suitable animals so as to produce active immunity which is manifested by the formation of anti-body.

(ii) For the purpose of this Part of the Schedule an anti-serum means sterile liquid anti- serum concentrated and unconcentrated, solutions of globulins or their derivatives or solid forms which can be reconstituted when necessary.

2. *Staff of Establishment-* The establishment shall be under the direction and control of a competent expert in bacteriology and serology with adequate training in immunology and standardisation of biological products and knowledge of animal management. He shall be assisted by a staff adequate for carrying out the tests required during the course of preparation of the sera and standardisation of the finished products.

3. *Proper Name-* The proper name of the antiserum shall be the recognised scientific name of the diseases or its causative organism or some generally recognised abbreviations thereof preceded by the prefix 'anti', and followed by the word 'serum; as for example, 'Anti- Anthrax serum'. The proper name of any antitoxin may be formed from the word 'Anti-toxin' preceded by the name of the organism from which the toxin was prepared, and followed, if desired, by a term indicating the source or the strain of that organism provided where there is no special provision in the Schedule, the name as approved by the Licensing Authority may be adopted.

4. Records-

(1) The permanent records which the licensee is required to keep shall include the following particulars: -

- (a) As to the culture- Evidence of the identity and specificity of the cultures.
- (b) As to the procedure used in immunising the animals;
 - (i) The method of preparing the cultures or antigen used for immunisation.
 - (ii) The dosage and methods employed in administering the culture or antigen.
 - (iii) The period in the course of immunisation at which blood is withdrawn for the preparation of the serum.
- (c) Any test which may have been applied to the serum to determine its content of specific antibodies or its specific therapeutic potency and purity.

(2) If the licensee desired to treat the performance of any tests recorded under sub-paragraph (i) (c) of this paragraph as determining the date of completion of manufacture for the purpose of rule 109 he shall submit full particulars of the proposed test to the Licensing Authority and obtain his approval.

5. Cultures- The cultures used in immunising the animals shall be at all times open to inspection, and specimens shall be furnished for examination at the request of the Licensing Authority.

6. Quantity -

- (a) Any antiserum shall be issued for veterinary use in the form of either.
 - (i) Actual serum, i.e., the liquid product of decantation of the coagulated blood or plasma without any addition, other than antiseptic or subtraction, or
 - (ii) A solution of the purified serum proteins containing the specific antibodies.

(b) At the time of issue, the liquid shall be clear or show at the most a slight opalescence or precipitate. Preparations of the natural serum shall not contain more than 10 per cent of solid matter. A solution of serum protein shall not contain more than 20 per cent of solid matter.

1. Precautions to be observed in preparations-

- (i) Laboratories where sera are exposed to the air in the course of the process of preparation must be separated by a sufficient distance from stables and animals houses to avoid the risk of aerial contamination with bacteria from animal excreta, and must be rendered fly proof to prevent such contamination by insects. Such laboratories must have impervious walls and floors and must be capable of being readily disinfected when necessary.
- (ii) A special room with impervious walls must be provided for the collection of blood from the living animals.
- (iii) An efficient system of manure removal must be used which will prevent its accumulation in the vicinity of any room where blood or serum is collected or handled.
- (iv) An adequate number of sterilizers must be provided for the sterilization of all glassware or other apparatus with which the serum may come into contact in the course of its preparation.
- (v) All processes to which the serum is subjected during and after the collection from the animals, must be designed to preserve its sterility, but in the case of a artificially concentrated sera, it shall suffice that the process of concentration is

conducted with scrupulous cleanliness and in such a manner as to avoid unnecessary dangerous contamination.

(vi) The laboratories in which the testing of sera for potency, sterility and freedom from abnormal toxicity are carried out must be adequate for the purpose. An adequate supply of animals for use in such tests and suitable housing for such animals must be provided.

(vii) Provision must be made for complying with any special conditions which may be laid down in the Schedule relating to the production and issue of the particular serum, in respect of which the licence is granted.

8. *Unhealthy or Infected Animals-* If an animal used in the production of sera is found to be suffering from an infection except one produced by living organisms against which it is being immunized, or shows signs of serious or persistent ill health not reasonably attributable to the process of immunisation, the licensee shall immediately report the matter to the Licensing Authority and shall, if the authority orders an inspection and the Inspectors so directs, cause such animals to be killed and a postmortem examination of it to be made, and take steps to prevent any serum obtained from the animal being sold or offered for sale until permission is given by the Licensing Authority. If the result of the postmortem is such as to bring under suspicion, the health of any of the other animals used for the production of sera, the Licensing Authority may prohibit the use of those animals for the production of sera or may take such other steps as may be necessary to prevent the issue of sera which may be dangerous to animal health.

Provided in the case of emergency, the person in charge of the establishment may order the destruction of an animal used in the production of sera and suspected of infection, and shall in that case given notice forthwith to the Licensing Authority and shall permit an Inspector to be present at the postmortem examination.

9. *Conditions and Housing of animals:*

(i) The animals used in the production of sera should be adequately housed under hygienic environments.

(ii) Only healthy animals free from disease should be used in the preparation of sera.

(iii) Every animal intended to be used as the source of serum must be subjected to a period of observation in quarantine for at least seven days before being admitted to the animal sheds in which the serum yielding animals are housed.

(iv) In case of horses and other equidae, every animal used as source of serum shall either be actively immunized against tetanus or shall be passively immunized against the disease by injection of tetanus antitoxin in such doses as to ensure the constant presence of that antitoxin in the blood during the whole period of the use of the animals as a source of serum.

Anti-Sera and their General Standard

Anti-sera contain the immune substances that have a specific prophylactic or therapeutic action when injected into animals exposed to or suffering from a disease due to a specific microorganism or its toxin. Anti-sera are classified into three groups.

- (i) Antitoxic sera (Antitoxin)
- (ii) Antibacterial sera.
- (iii) Antiviral sera.

Antisera are usually issued in an unconcentrated form for animal use but may be concentrated and also freeze dried. However, for the purpose of the Schedule the word 'antisera' is also used for the unconcentrated liquid sera only. A suitable bacteriostatic agent in a concentration sufficient to prevent the growth of microorganisms is added to the liquid serum.

General Standard

1. **Description-** Liquid native or unconcentrated antisera are yellow or yellowish brown in colour. They are initially transparent but may become turbid with age. They are almost odourless except for the odour of any bacteriostatic agent that may have been added.
2. **Identification-** The test for identity is described in the individual monograph.
3. **Acidity or Alkalinity-** All native antisera have a pH of 7.0 to 8.5.
4. **Abnormal Toxicity-** All anti-sera shall comply with the following tests or freedom from abnormal toxicity.
 - (a) Two healthy mice each weighing not less than 18 g. are injected subcutaneously each with 0.5 ml. of the sample and observed for five days. None of the mice should show any abnormal reaction or die.
 - (b) Two healthy guinea pigs each weighing 300 g. to 450 g. are injected subcutaneously each with 5 ml. of the sample and observed for seven days. None of the guinea-pigs should show any abnormal reaction or die.
5. **Sterility-** All anti-sera shall comply with the tests for sterility described in rules 115 to 119.
6. **Potency-** The potency of each preparation, when the available methods permit, is determined by the appropriate biological assay, and it is described under the individual monograph.
7. **Total Solids-** Native antisera should not contain more than 10 per cent solid matter.
8. **Labelling-** Should comply with the provisions for 'Labelling' as laid down for 'Bacterial Vaccines.'
9. **Storage-** Liquid preparations of antisera shall be stored, protected from light at temperature between 2 °C to 4 °C and shall not be frozen.
10. **Date of Manufacture-** The date of manufacture shall be unless otherwise specified in the individual monograph in this part is as defined in clause (b) of sub-rule (3) of rule 109.
11. **Containers-** All antisera are distributed in sterilised containers of a material which is inert towards the substance and which are sealed to exclude micro-organisms.
12. **Expiry Date-** The expiry date of potency of all sera shall not be more than twenty-four months after the date of a manufacture.

Anti-Anthrax Serum

1. **Synonym-** Bacillus Anthracis Anti-serum.
2. **Definition-** Anti-anthrax serum is the serum of animals that confers a specific protection against *Baccillus anthracis*.
3. **Preparation-** The antiserum may be prepared in buffalo bulls after repeated injections of cultures of *B. anthracis* of a virulent strain.
4. **Standard-** It complies with the requirements in the general provisions for antisera under Description, Acidity or Alkalinity, Abnormal Toxicity, Sterility, Solids, Labelling, Storage and Expiry date.

Identification – It protects animals against infection with *B. Anthracis*

Anti-Blackquarter Serum

1. **Synonym-** Blackleg Antiserum, Clostridium Chauvoei-Anti serum
2. **Definition-** Anti-Blackquarter serum is the serum of suitable animals containing the Substances that have a specific neutralising effect on *Clostridium Chauvoei*.

3. **Preparation-** It is prepared by injecting subcutaneously or intramuscularly increasing doses of formolised cultures of *Cl. Chauvoei* into buffalo bulls.

4. **Standards-** It complies with the requirements described in the general provisions for antisera under Description, Acidity or Alkalinity, Abnormal Toxicity, Sterility, Solids, Labelling, Storage and Expiry date.

Identification- It protects susceptible animals against infection with virulent strains of *Cl. Chauvoei*.

Anti-Fowl-Cholera Serum

1. **Synonym-** Pasteurella Septica Antiserum (Avian).

2. **Definition-** Fowl Cholera Antiserum is the serum of animals containing the substances that confer a specific protection to fowls against virulent strain of Pasteurella Septica (Avian).

3. **Preparation-** Antiserum is prepared from buffalo bulls after they have been subjected to an injection of killed cultures of virulent strain of *Pasteurella Septica* (Avian) followed by injections of living cultures of the same organism.

4. **Standard-** It complies with the requirements described in the general provision for anti-sera under Description, Acidity or Alkalinity, Abnormal Toxicity, Sterility, Solids, Labelling, Storage and Expiry date.

Identification- It protects susceptible fowls against infection with Pasteurella Septica (Avian) and its homologous strains.

Anti- Haemorrhagic Septicaemia Serum

1. **Synonym-** Pasteurella Septica Antiserum.

2. **Definition-** Anti-Haemorrhagic Septicaemia Serum is the serum of animals containing the substances that confer a specific protection to susceptible animals against virulent strains of *Pasteurella Septica*.

3. **Preparation** – The antiserum is prepared from buffalo bulls after they have been subjected to repeated injections of formolised cultures of standard strain *Pasteurella Septica* with adjuvants, followed by suitable doses of virulent culture of the organism.

4. **Standard.**-It complies with the requirements described in the general provisions for antisera under Description, Acidity or Alkalinity, Abnormal Toxicity, Sterility, Solids, Labelling, Storage and Expiry date.

Identification- It protects susceptible animals against infection with homologous strains of *Pasteurella Septica*.

Anti-Rinderpest Serum

1. **Synonym-** Cattle Plague Antiserum

2 **Definition-** Anti-Rinderpest Serum is the serum of buffalo bulls containing the substances that confer a specific immunity to susceptible animals against virulent strains of the virus of *rinderpest*

3. **Preparation** – The antiserum is prepared from buffalos who have reacted to a dose of virulent rinderpest virus, which is injected simultaneously with a predetermined quantity of anti rinderpest serum so as to control the severity of the reaction (serum-simultaneous-method).

4 **Standard-** It complies with the requirements described in the general provisions for antisera under Description, Acidity or Alkalinity, Abnormal Toxicity, Solids, Labelling, Storage and Expiry date.

(i) **Identification-** It protects susceptible animals against rinderpest.

(ii) **Potency**-Five Buffalo-calves of about one year of age in good condition are used for the test. Three are injected subcutaneously with the anti-rinderpest serum under test at the rate of 10 ml. per 46 kg. body weight, subject to a minimum of 20 ml. per animal. These together with the two remaining, are simultaneously injected subcutaneously at a different site with 1 ml. of a 1: 100 dilution of spleen suspension of virulent bull-virus.

The animals should be observed for fourteen days during which time the serum treated animals should exhibit no symptoms of rinderpest other than rise in temperature and slight intestinal disturbances, while the controls develop more severe symptoms or die.

Salmonella Pullorum Anti Serum

1. **Synonym**- Salmonella Pullorum Anti Serum.

2. **Definition**- Salmonella Pullorum anti-serum is the sera from fowls and contains antibodies against Salmonella Pullorum. It is used for standardizing batches of Salmonella Pullorum antigens and also used as a control along with the sera suspected for pullorum disease.

3. **Preparation**- The serum is prepared after intravenous inoculation with smooth culture suspension of Salmonella Pullorum in healthy birds.

4. **Standards**- It complies with the requirements in the general provisions for anti-sera under Description, Acidity, Alkalinity, Sterility, Solids, Labelling, Storage and Expiry date.

5. **Identification**- It should give positive agglutination with Salmonella pullorum antigen.

Standard Anti-Brucella Abortus Serum

1. **Synonym**- National counterpart of standard anti-Brucella Abortus serum.

2. **Definition**- Standard Anti-Brucella abortus serum is the serum which contains 1000 International Units (I.U) per ml. and is used for standardizing batches of brucella antigens and is also used as a control along with the sera suspected for brucellosis.

3. **Preparation**- The serum is prepared after intravenous inoculation of suspension of smooth culture of B. abortus (strain 99) in rabbits or cattle and subsequently diluting it suitably with brucella-free healthy serum so that when tested with standardized Brucella abortus tube test antigen, it gives 50% agglutination at 1/500 final serum dilution.

4. **Standard**- It complies with the requirements in the general provision for anti-sera under Description, Acidity, Alkalinity, Sterility, Solids, Labelling, Storage and Expiry date.

Identification- It should give agglutination with Brucella antigen.

PART III- DIAGNOSTIC ANTIGENS

Provisions Applicable to the Manufacture and Standardisation of Diagnostic Agents (Bacterial Origin)

1 **Definition**- This Part of the Schedule applies to reagents of bacterial origin employed for various tests.

2 **Staff of establishment**- A competent expert in bacteriology with sufficient experience in the manufacture and standardisation of veterinary biological products shall be in charge of the establishment responsible for the production of various diagnostic agents of bacterial origin and he may be assisted by a staff adequate or carrying out the tests required during the preparation and standardisation of various diagnostic agents.

3 **Proper Name**- The proper name of any diagnostic agent is the name of micro-organism from which it is made, followed by the word 'antigen' unless the Schedule

otherwise provides, or, it may be derived from the name of the organism responsible for the causation of the disease or if there is no special provision in the Schedule, the name approved by the Licensing Authority. In the case of the undermentioned preparations the proper name of the diagnostic agent may be as follows:

1. Abortus Bang Ring (A.B.R.) Antigen.
2. Brucella Abortus Coloured Antigen.
3. Brucella Abortus Plain Antigen.
4. Johnin.
5. Mallein.
6. Salmonella Abortus Equi "H" Antigen.
7. Salmonella Pullorum Coloured Antigen.
8. Salmonella Pullorum Plain Antigen.
9. Tuberculin.

4. **Records-** Cultures used in the preparation of diagnostic agents of bacterial origin must, before being manipulated into an agent be thoroughly tested for identity by the generally accepted tests applicable to the particular micro-organism. The permanent record which the licensee is required to keep shall amongst other include a record of the origin, properties and characteristics of the cultures.

5. **Preparation-** Diagnostic agents of bacterial origin are prepared from selected cultures after their careful examination for the identity, specificity, purity and antigenicity. They may be prepared in the following manner:

(a) *Formolised Antigens-* The selected pure culture strain grown in a suitable medium at an optimum temperature for an appropriate period. The pure growth is then exposed to the action of a solution of Formaldehyde I.P. in a suitable concentration and at an appropriate temperature for a suitable period.

(b) In some cases, the diagnostic agents are prepared by growing the organisms on suitable media and then deriving specific protein constituents of the bacteria by various methods.

6. **General Standard:-**

(a) *Description-* Diagnostic agents may be clear opalescent or coloured liquids.

(b) *Identification-* Some exhibit specific agglutination when mixed with the serum of the animals infected with homologous organisms and others when injected into the animal body in appropriate doses cause specific reactions like hypersensitiveness, local and general reaction, if the animal is infected with homologous organisms.

(c) *Sterility Test-* All antigens shall be tested for sterility in accordance with rules 114 to 119.

(d) *Standardisation-* It is carried out either by determining the definite concentration in the product or by observing the general and local reactions in healthy and artificially infected animals with various standard dilutions of the product.

7. **Labelling-** As under general provisions for the bacterial vaccines with the addition that it is meant for diagnostic purposes only.

8. **Storage-** All antigens are stored, protected from light at a temperature between 2 ° C to 4 ° C.

9. **Date of manufacture-** The date of manufacture shall be unless otherwise specified in the individual monograph in this part as defined in clause (b) of sub- rule (3) of rule 109.

Abortus Bang Ring (A.B.R.) Antigen

1. **Synonym-** Milk Ring Test Antigen.

2. **Definition-** The antigen is a suspension of pure growth culture of standard strain of Brucella abortus strain 99 strained supravivally with 2,3,5, triphenyl tetrazolium chloride suspended in 0.85 per cent saline containing 1 per cent glycerol and 1 per cent phenol.

3. **Preparation-** Smooth strain of Brucella abortus strain 99 is grown in potato infusion agar for 48 to 72 hours in Roux flasks, at 37 ° C. Condensation fluid if any is pipetted off before washing. Each flask is washed with about 20 ml. of normal saline. The pooled washing is filtered through a gauze and the filtrate is collected in a measuring cylinder. To every 500 ml. of the filtrate 1 g. of 2, 3, 5, triphenyl tetrazolium chloride is added immediately. The container is shaken for thirty minutes till the tetrazolium salt is dissolved. The product is taken out and kept at 37 ° C for two hours. After incubation the product is heated at 65° C in a water bath for thirty minutes. It is cooled and centrifuged at 3000 r.p.m for one hour. The supernatant is pipetted off and sediment is suspended in normal saline containing 1 per cent glycerol and 1 per cent phenol and filtered through sterile cotton wool. This forms concentrated antigens.

Standardization of the Strained Antigen

An aliquot portion of the microbial suspension stained with phenylte-trazolium is taken, representing the initial undiluted suspension. 1 ml. per tube of this initial undiluted stained suspension is added to six test tubes, followed by increasing quantities of the glycerolphenol diluent as follows:-

Tube	Undiluted Stained Suspension	Diluent
1	1	0.6
2	1	0.8
3	1	1.0
4	1	1.2
5	1	1.4
6	1	1.6

The contents of each tube are then diluted tenfold with the same diluent and serve as antigen for a tube agglutination test with the Standard Serum (or its national counterpart). In this way, six sero-reactions will be carried out. During this procedure, the concentrated strained microbial suspension should be kept in the refrigerator at 4 ° C.

The agglutination reactions are read after forty-eight hours at the agglutination titre of the Standard Serum, previously determined with the usual unstained antigen in the tube test, corresponding to the correct dilution of the standard antigen.

The next step, therefore, is to dilute the concentrated stained suspension to the same extent as the tube whose tenfold dilution has given the correct agglutination titre, i.e. the concentration of antigen in the tube before the tenfold dilution had been made.

4. **Standard:**

(a) **Description-** It is a red colour liquid containing dead bacteria in suspension.

(b) *Identification*- It shows formation of a specific cherry red coloured ring in the cream layer when mixed with pooled samples of milk taken from animals suffering from brucellosis.

(c) *Sterility Test*- Should comply with tests for sterility described in the general monograph on 'Diagnostic Antigen'. The test shall, however, be done before colouring.

5. **Labelling and Storage**- Should comply with the requirements of 'Labelling' and 'Storage' as laid down in the general monograph on 'Diagnostic Antigens'.

6. **Expiry Date**- The date of expiry of potency shall be not more than nine months from the date of manufacture when stored 2 ° C to 4° C.

Brucella Abortus Coloured Antigen

1. **Synonym**- Brucella abortus Cotton Strain 99 coloured Antigen.

2. **Definition**- Brucella Abortus colored Antigen, is a suspension of pure smooth cultures of Brucella abortus strain 99 in phenolised glycerine saline, the bacteria being coloured by the addition of crystal violet and brilliant green. This antigen is used for plate test for serological diagnosis of brucella infection.

3. **Preparation**- Seventy-two hours old growth of Brucella Abortus strain ninety-nine in smooth form on potato infusion agar medium in Roux flasks is washed with phenolised glycerine saline (containing 12 per cent sodium chloride, 20 per cent glycerine and 0.5 per cent phenol). The washed growth is filtered through a pad of absorbent cotton wool and the suspension is coloured by the addition of 1 ml. each of 1 per cent aqueous solution of crystal violet and brilliant green for every 250 ml. of the suspension. The product is heated for sixty minutes in a water bath at 60° C before it is standardised.

4. **Standard**:

(a) *Description*- It is a greenish violet liquid containing dead bacteria in suspension.

(b) *Identification*-It gives specific agglutination when mixed with the serum of the animal infected with brucella organism.

(c) *Sterility Test*- Should comply with the tests for sterility described in the general monograph on 'Diagnostic Antigens'

(d) *Standardisation*- 0.5 ml. of the antigen is mixed with 4.5 ml of normal saline solution in Hopkins graduated tube. The mixture is centrifuged at 3000 r.p.m. for sixty minutes and the percentage of bacterial cells present in the original antigen is assessed by noting the height of the cell deposit. The antigen is then standardised so as to contain 10 per cent cell deposit.

5. **Labelling and Storage**- Should comply with the requirements of 'Labelling' and 'Storage' as laid down in the general monograph on 'Diagnostic Antigens'.

6. **Expiry Date**- The date of expiry of potency shall be not more than nine months from the date of manufacture when stored at 2 ° C to 4°C.

Brucella Abortus Plain Antigen

1. **Synonym**- Brucella Abortus Strain 99 Plain Antigen.

2. **Definition**- Brucella Abortus Plain Antigen is a suspension of a pure smooth culture of Brucella abortus strain 99 in phenol-saline.

3. **Preparation**- Seventy-two hours old growth of Br. Abortus strain 99 in smooth form on potato infusion agar medium in Roux flasks is washed with normal saline solution. The washed growth is filtered through a pad of absorbent cotton wool and the suspension is

kept at 60 ° C per cent. for sixty minutes on a water bath to kill the organisms. It is then preserved by the addition of phenol in a final concentration of 0.5 per cent.

4. Standard:

(a) *Description*- An opalescent liquid containing dead bacteria in suspension.

(b) *Identification* –It gives specific agglutination when mixed with the serum of animals infected with brucella organism.

(c) *Sterility Test*- Should comply with the tests for sterility described in the general monograph in ‘Diagnostic Antigen’.

(d) *Standardisation*- Mix the concentrated antigen well and dilute 1 ml. with 0.5 per cent phenol saline until it corresponds to about Tube 4 of Brown’s opacity tubes. Further dilutions of the antigen adjusted to opacity tube No. 4 are made. The particular dilution that gives 50 per cent agglutination with anti-brucella abortus serum (containing 1000 International Units) at 1 : 500 final serum dilution, is assessed as the diluting factor for the concentrated antigen. The bulk of the concentrated antigens is accordingly diluted for issue as standard antigen.

5. Labelling and Storage- Should comply with the requirements of ‘Labelling and Storage’ as laid down in the general monograph on ‘Diagnostic Antigen’.

6. Expiry Date- The date of expiry of potency shall be not more than nine months from the date of manufacture when stored at 2° C to 4° C.

Johnin

1. Definition- Johnin is a preparation of a fluid medium in which *Mycobacterium Paratuberculosis* has been grown in artificial culture and which has been freed by filtration from the bacilli.

2. Preparation- Young culture of selected strain of *Myco-Paratuberculosis* of bovine origin is grown on synthetic medium and incubated at 37 ° C for ten to twelve weeks. Flasks showing luxuriant and pure growth are steamed for three hours thereafter kept at room temperature overnight. The contents are filtered through fine meshed sieve. The filtrate is concentrated over a steam bath to one-tenth of its original volume and kept in cold storage for fourteen days before being filtered through Seitz filter. The product is dispensed in ampoules and hermetically sealed.

3. Standards:-

(a) *Description*- A yellowish brown to brownish liquid.

(b) *Identification*- It produces hot, painful and oedemateus swelling at the site of inoculation in animals infected with *Myco-paratuberculosis* organism.

(c) *Sterility Test*- Should comply with the test for sterility described in the general monograph on ‘Diagnostic Antigens’.

(d) *Potency Test*- Two animals, previously infected with *Myco-paratuberculosis* and two healthy animals are each injected intrademally in the neck region with 0.1 ml. of the product. Forty-eight hours later, the injection is repeated at the same site. The product should produce a typical reaction in the infected animals in the form of a hot painful and oedemetous swelling at the site of inoculation persisting for at least forty-eight hours after the second injection. Control animals should not show such reaction.

4. Labelling and Storage- Should comply with the requirements of ‘Labelling’ and ‘Storage’ as laid down in general monograph on ‘Diagnostic Antigens’.

5. Expiry Date- The date of expiry of potency shall be not more than two years from the date of manufacture when stored at 2 ° C to 4 ° C.

Malleins

1. Definition- (i) Malleins are preparations of fluids media in which the *Actinobacillus mellei* has been grown in artificial culture and which have been freed by filtration from the bacilli.

(ii) For the purposes of this Schedule malleins are classified into (a) Mallein-subcutaneous and (b) Mallein intradermo-palpebral (I.D.P.)

2. Preparation:-

(a) Mallein Subcutaneous- Three to four weeks old pure growth of standard strain of *A. mallei* grown on synthetic medium is steamed for one hour in Koch's steam sterilizer. One part of 5 per cent phenol solution is added to every nine part of the dead culture which is then filtered through Seitz filter.

(b) Mallein Concentrated. - The procedure is the same as for Mallein Subcutaneous except, that the filtrate is evaporated in porcelain dish over steam to half the original volume before addition of phenol. Five per cent phenol solution is added in sufficient quantity to the concentrated product, to give a final concentrated of 0.5 per cent.

3. Standards:

(a) Description- A yellowish to brown viscous liquid.

(b) Identification- It produces hot tense, painful swelling when injected into the animals infected with *A. mallei* organisms.

(c) Sterility Test- Should comply with the tests for sterility described in the general monograph on 'Diagnostic Antigens'.

(d) Potency Test: -

(i) Mallein subcutaneous- Two ponies, previously sensitised with *A. Mallei* and controls, are injected each with 1 ml. of the product subcutaneously in the neck region. The animals are observed for local reaction and rise in temperature. Local reaction is manifested by a hot tense, painful swelling becoming prominent within twenty-four hours. The rise in temperature is observed by recording the body temperature at the time of inoculation and subsequently at short intervals. A rise in temperature of 1° C or more above normal is indicative of infection.

(ii) Mallein Intra-dermo-Palpebral (I.D.P.)- Two ponies, previously sensitized with *A. mallei* and two healthy ponies are injected intradermally with 0.2 ml. of the product near the rim of the lower eye lid of one eye. Typical reactions such as painful swelling of the palpebral tissue with mucopurulent discharge from the eye is indicative of infection. The two healthy ponies should not show such reactions.

Similar test in other eye is performed with a previously determined patient mallein using as a standard. When the local reactions produced by intradermo palpebral infections of the two preparations are comparable the batch is passed for issue.

4. Labelling and Storage- Should comply with the requirement of 'Labelling' and 'Storage' as laid down in the general monograph on 'Diagnostic Antigen'.

5. Expiry Date- The date of expiry of potency shall be not more than two years from the date of manufacture when stored at 2° C to 4° C.

Salmonella Abortus Equi 'H' Antigen

1. Synonym- Equine Abortion Diagnostic Antigen.

2. Definition- Salmonella Abortus Equi Antigen is suspension of a pure smooth culture of actively motile *Salmonella Abortus equi* in formal saline.

3. Preparation- Standard strain of *S.abortus equi* is grown on nutrient agar in Roux flasks at 37 ° C for twenty-four hours. The pure growth in Roux flasks is washed with normal saline and diluted to contain approximately 800 million organisms per ml. Solution of Formaldehyde I.P. is added to give a final concentration 0.5 per cent and the formalised product is incubated at 37 ° C for twenty-four hours. The final product is dispensed in suitable containers.

4. Standards:-

(a) *Description-* A slightly opalescent liquid containing dead bacteria in suspension.

(b) *Identification-* It gives specific agglutination when mixed with the serum of the animals infected with *S.abortus equi* organisms.

(c) *Sterility Test-* Should comply with the test for sterility described in the general monograph on 'Diagnostics Antigens'.

5. Labelling and storage- Should comply with the requirements of 'Labelling' and 'Storage' as laid down in the general monograph on 'Diagnostic Antigens'.

6. Expiry Date- The date of expiry of potency shall be not more than nine months from the date of manufacture when stored at 2 ° C to 4 ° C.

Salmonella Pullorum Coloured Antigen

1. Synonym- Bacillary White Diarrhoes (B.W.D) Antigen.

2. Definition- The antigen is a suspension in a solution containing 1 per cent Formaline, 1 per cent KH₂PO₄ and 0.85 per cent Sodium Chloride of pure smooth culture of standard strain of *Salmonella Pullorum*.

3 Preparation- Standard strain of *S. Pullorum* is grown on sulphur agar medium in Roux flasks for five days at 37 ° C. The pure growth is washed with 1.0 per cent Formal Saline.

Standardisation

The antigenic cell suspension is then centrifuged (preferably in cold centrifuge) for half an hour at 4000 rotations per minute and the packed cell volume determined. The packed cell is then re-suspended in a solution containing 1 per cent formalin, 1 per cent KH₂ PO₄ and 0.85 per cent sodium chloride, 1 ml. of packed cell is suspended in 10 ml. of the resuspendiary solution, mixed thoroughly and is passed through a cotton wool pad. The turbidity of the antigenic suspension is usually between 100 to 125 times Mac Farland scale standard and optimum 3 cc. of a 1 per cent aqueous solution of crystal violet are added to 100 ml. of the antigenic suspension. After making the dye the antigen is allowed to stand forty-eight hours before use. The average yield per Roux flasks of culture medium is about 50 ml. The antigen should be bottled in 10 ml. or 20 ml. quantity in amber-coloured bottles and corked with rubber caps and paraffin sealed and preserved until required for use within the expiry period. This antigen reacts instantly with the blood of all carrier birds and remains permanently negative with that of non-infected birds.

This antigen gives good reactions with positive sera whose titre is even as low as 1 : 20.

4. Standard:-

(a) *Description-* Violet coloured liquid containing dead bacteria in suspension.

(b) *Identification-* It gives specific agglutination when mixed with the serum of birds in infected with *S. Pullorum* infection. It is used for carrying out plate agglutination tests for serological diagnosis for *S. Pullorum* infection in birds.

(c) *Sterility Test-* Should comply with the test for sterility described in the general monograph on ' Diagnostic Antigen'. The tests shall be done before addition of 'Crystal Violet'.

5. Labelling and Storage- Should comply with the requirements of 'Labelling' and 'Storage' as laid down in the general monograph On 'Diagnostic Antigens'.

6. Expiry Date- A six month expiration date for this antigen is recommended. However, it is advisable to use fresh ones as far as possible. This antigen should be preserved at 4 ° C to 6 ° C in dark place in the refrigerator and should not be exposed to hot weather condition for longer than necessary before use in the field.

Salmonella Pullorum Plain Antigen

1. Synonym- Bacillary White Diarrhoes (B.W.D) Plain Antigen.

2. Definition- The antigen is a suspension of pure smooth culture of *Salmonella pullorum* in phenol saline.

3. Preparation- Forty-eight hours old pure culture of smooth strain of *S. Pullorum* is washed with 0.5 percent phenol saline and the pooled suspension is adjusted to contain approximately 800 million organisms per ml. by the addition of more carbol saline. The suspension is kept at room temperature of twenty-four hours, and dispensed in suitable containers.

4. Standard: -

(a) *Description-* An opalescent liquid containing dead bacteria in suspension.

(b) *Identification-* It gives specific agglutination when mixed with the serum of birds infected with *S. Pullorum*.

(c) *Sterility Test-* Should comply with the tests for sterility described in the general monograph on 'Diagnostic Antigen'.

5. Labelling and Storage- Should comply with the requirements of 'Labelling' and 'Storage' as laid down in the general monograph on 'Diagnostic Antigens'.

6. Expiry Date- The date of expiry of potency shall be not more than nine months from the date of manufacture when stored at 2 ° C to 4 ° C.

Tuberculin

1. Definitions:

(i) Tuberculins are preparations of fluid media on which *Mycobacterium tuberculosis* has been grown in artificial culture and which has been freed by filtration from the bacilli.

(ii) For the purposes of the Schedule tuberculins are classified in (a) Tuberculin-Subcutaneous (b) Heat Concentrated Synthetic Medium (H.C.S.M) Tuberculin (c) Avian tuberculin.

2. Preparation:-

(a) *Tuberculin subcutaneous-* Flasks containing Henley and Dorset synthetic medium are inoculated with standard human strains of Myco-Tuberculosis previously grown on glycerol- beef broth medium for ten days. After ten to twelve weeks of incubation at 37 ° C, flasks containing pure growth are steamed for three hours. The contents are filtered through fine meshed sieve and the volume is made up to its original with phenolised distilled water such that the final concentration of phenol is 0.5 per cent. It is then filtered through Seitz filter.

(b) *Heat Concentrated Synthetic Medium (H.C.S.M) Tuberculin-* To the strained liquid obtained after sieving as in the method of preparation of Tuberculin subcutaneous, glycerol is added in the proportion of 122 ml. per litre of the original volume of medium used. The mixture is evaporated to one-fifth of the original volume on a steam bath. An equal volume of 1 per cent phenol in distilled water is added after the mixture is cooled. The product is stored at 47 ° C for fourteen days before it is filtered through Seitz filter. It is then dispensed in ampoules.

(c) *Avian Tuberculin Concentrated-* The procedure is the same as for Tuberculin Concentrated (H.C.S.M) except that standard strain of *Myco-tuberculosis (Avian)* is used in its preparation.

3. Standard:-

(a) *Description*- A yellowish brown viscous liquid.

(b) *Identification*- When injected intradermally into the animal infected with tuberculosis diffused swelling occurs depending upon the allergic status of the animal, the magnitude of dose and specificity of the product. In non-infected animals this reaction is not observed.

(c) *Sterility Test*- Should comply with the test for sterility described in the general monograph on 'Diagnostic Antigens'.

(d) *Potency Test*- (i) *Tuberculin subcutaneous*-Six large white guinea-pigs each weighing not less than 300-450 g. are individually inoculated intramuscularly with 0.5 mg. (Moist growth from solid plants) of *Mycobacterium tuberculosis* three weeks prior to test of each batch of tuberculin; the following dilutions of (a) test tuberculin and (b) standard tuberculin are used:-

1 in 200, 1 in 400, 1 in 800 and 1 in 1600.

The six sensitized guinea pigs are depilated on one flank and after about twenty-four hours each animal inoculated intradermally with 0.2 ml. of each dilution of the two tuberculins in two rows. The reactions are read after twenty-four and forty-eight hours. When the local reactions produced by the graded inter-dermal injections of the two preparations are comparable the brew is passed for issue.

(ii) *Heat Concentrated Synthetic Medium (H.C.S.M) Tuberculin*-Six adult white guinea-pigs each weighing not less than 300-450 g. and sensitized three weeks previously with 0.5 mg. (moist growth from solid plants) of *Myco-Tuberculosis* bovine type, injected intramuscularly are used for test of each batch. The following dilutions of (a) test tuberculin and (b) standard tuberculin are used: -

1 in 500, 1 in 1000, 1 in 2000 and 1 in 4000.

The six sensitized guinea pigs are depilated on one flank and after about twenty-four hours each animal is inoculated intradermally with 0.2 ml. of each dilution of the two tuberculins in two rows. The reactions are read after twenty-four and forty-eight hours. When the local reactions produced by the graded inter-dermal injections of the two preparations are comparable, the test tuberculin is passed for issue. The tuberculin is dispensed in ampoules.

(iii) *Avian Tuberculin*- Six adults fowls, with well developed wattles, sensitized at least three weeks previously by intramuscular injection with 10 mg. moist weight (from solid plants) of twenty-one days old culture of *Mycobacterium tuberculosis* (Avian Type) are used for potency test of each batch. In each fowl, one wattle is inoculated with 0.2 ml. of undiluted test tuberculin and the other wattle with similar quantity of undiluted standard tuberculin. The reactions in each fowl are read after twenty-four hours and forty-eight hours and if comparable the product is passed for issue.

4. Labelling and Storage- Should comply with the requirements of 'Labelling' and 'Storage' as laid down in the general monograph on 'Diagnostic Antigens'.

5. Expiry Date- The date of expiry of potency shall be not more two years months from the date of manufacture when stored at 2 ° C to 4 ° C.

PART IV**GENERAL**

1. For the purpose of this Schedule any test or method of testing described in the ¹[British Pharmacopoeia (Veterinary)] shall be deemed to be a method approved by the Licensing Authority.
2. The Licensing Authority shall publish in the official Gazette from time to time particulars of any test or method of testing approved by him.]

1. Subs. by G.S.R. 647(E) dated 28-10-1998.

¹[SCHEDULE F (II)]

(See Rule 124-C)

STANDARDS FOR SURGICAL DRESSINGS

Synonyms:

Bandage Cloth, Bleached Bandage Cloth, Rolled Bandage, Open Wove Bandage, Cotton Bandage Cloth.

Bandage Cloth consists of cotton cloth of plain weave made from machine spun yarn of suitable count to comply with a bleached count between 20 tex and 25 tex for warp and between 25 tex and 30 tex for weft. The fabric contains no filling, sizing or dressing material. It may be supplied uncut and folded or cut to suitable size and rolled.

Description for uncut bandages:

Uncut bandages are cotton cloth of plain weave, in one continuous length showing no joints or seams, with well-formed selvages. The cloth is bleached to a good white, is clean and odourless and reasonably free from weaving defects and from seed and leaf debris.

Description for cut bandage:

Same as for uncut bandages, except for selvages which shall not be included in cut bandages. In addition, both the extremes and edges of cut bandages shall be straight and evenly cut, with reasonable freedom and loose threads.

Threads per dm: – Warp not less than 150 and weft not less than 85.

Weight in g/m²: - 57 ± 5 .

Length and Width: - The length and width shall not be less than 99 per cent each of the length and width stated on the label. For cut bandages, each of the bandages in a packing complies with this requirement.

Foreign matter: - Not more than 2 per cent.

Fluorescence:

When viewed under screened ultra-violet light, not more than occasional points of fluorescence are observed.

Packing, Labelling and Storage:

Bandage Cloth shall be packed securely so as to allow normal handling and transport without tearing and exposing the contents. In packages of cut and rolled bandages, each bandage shall also individually be wrapped in a suitable paper. The net content is stated on the label in terms of length and width. Bandage Cloth must be stored in packed condition protected from dust. The packings of Bandage Cloth shall be labelled prominently with the words “Non-Sterile”.

Absorbent Gauze.

Synonyms.- Gauze; Unmedicated Gauze; absorbent Cotton Gauze.

Absorbent Gauze is cotton fabric of plain weave, supplied in various widths and lengths. The Gauze is bleached and free from any sizing, dressing or filling material. The yarn used is machine spun cotton yarn, of suitable count to comply with a bleached count between 17 and 25 tex in the finished fabric.

1. Ins. by G.S.R. No.318(E), dt. 1-5-1985.

Description:

Cotton cloth, plain weave, with a simple selvedge present on both sides to prevent unravelling of yarn. The cloth is bleached to a good white, is clean, odourless, reasonably free from fabric defects and adhering sand debris from cotton seeds and leaves, or any other foreign matter.

Threads per dm.:- Warp not less than 75 and weft not less than 55.

Weight in g/m²: - 30 ± 5 .

Length and width:- Not less than 98 per cent each of the length and width stated on the label.

Absorbency:- Average sinking time not more than 10 seconds.

Fluorescence:- When viewed under screened ultra-violet light not more than occasional points of fluorescence are observed.

Foreign matter:- Not more than 1 per cent.

Sterility:- If sterile, the contents comply with the test for sterility.

Packing, Labelling and Storage:

Absorbent Gauze is folded and packed with such materials and so securely as to protect its absorbency and allow normal handling and transport without tearing and exposing the contents. The net content is stated on the label in terms of length and width. The packages shall be labelled prominently with the words "Non-Sterile". If sterile, it shall be so stated on the label, and the packing method and material shall be such as to maintain the sterility. The Absorbent Gauze must also comply with the Sterility Test. Absorbent Gauze must be stored in packed conditions protected from moisture and dust.

METHODS OF TEST**Defect in fabric:**

The sample is unfolded, opened and held against diffused daylight or spread on black topped table to locate and identify prominently visible defects in yarn and fabric construction. The fabric shall be reasonably free from holes, slubs, snarls and naps as well as the following:

-

Odour:- Misty odour, or any objectionable smell like that of chemicals or materials used in sizing and bleaching.

Skewness:- (For Bandage Cloth only) A condition where warp and weft do not keep at right angles to each other.

Defective Selvedge. - The selvedge tearing and allowing yarn to unravel and loop formation at selvedge.

Cracks:- Prominent steaks of space or gaps between warp or weft yarns.

Double ends:- More warp threads woven as one, due to wrong draw.

Sloughing:- Entanglement in the fabric of a bulk of yarn that has slipped off the weft yarn due to loose widening.

Measurement of length and width.

Length is the distance from end to end, along one edge of the fabric, and width is the perpendicular distance from one edge to the opposite edge.

Length. - Fix a meter scale to a table or mark off the division of one metre on a table edge. Starting from one end, spread the fabric flat on that table in a single layer keeping one

selvedge parallel to the scale; smoothen the fabric without stretching it, to avoid creases, and mark off with a coloured pencil, on the selvedge exactly one metre. Shift the fabric and measure the same way the second metre and so on for the entire length of the fabric making a mark at each metre. Note down the total length in metres. Repeat this at the opposite selvedge, as well as on the fabric folded approximately about the middle. The average of the three readings is the length. For cut bandages, one measurement at the middle of the bandages by folding it length-wise will suffice.

Width. - Lay the portion of the fabric to be measured flat and smooth on the tables, but do not stretch fabric except sufficiently to render it creaseless. At the place where mark had been made on the selvedge in measuring the length measure the perpendicular distance to the opposite selvedge with a metre scale. Note the width, repeat this at every mark made in measuring the length. The average of all the readings is the width of the fabric. For cut bandages, width shall be measured at every 50 cms., and average reported as width.

Threads per dm.- (For examples not less than 15 m. in length).

Weft. - At the third metre from one extreme locate three places one at about 5 cm. below the top selvedge, a second in the middle and third at about 5 cm. below the top selvedge, a second in the middle and third about 5 cm. above the bottom selvedge, all three in a vertical row. Take a rectangular plate (made of suitable material such as plastic or aluminium) with the rectangular opening of 5 cm. x 10 cm. cut in it. Keep the plate on the fabric horizontally so that the left 5 cm. side and bottom (10 cm. side) edges of the opening coincides with a weft and warp yarns respectively; count the number of weft yarns within the opening for the length of 10 cm.. Repeat this at the other two selected places, and note down the average of three readings. Repeat this at every third metre in the sample and calculate the average weft per dm.

Warp.- Keep the rectangular plate, this time vertically with left (10 cm. side) and bottom (5 cm. side) edge of opening coinciding with a weft and warp yarn respectively. Count the number of warp yarns within the opening for 10 cm. and note down. Repeat this for about 10 selected places in the samples taking care that the same set of warp yarns is not counted more than twice and calculate the average warp per dm. Magnifying glass mounted on stand may be used for counting.

For samples less than 15 m. in length, locate as many different places as the dimension of the fabric permits, the total being not less than 10 for each sample and calculate the warp and weft per dm. as above.

For cut bandages, all the warp threads in the samples are counted, taking care to leave 5 mm at the cut edge, and weft is counted at every 50 cm. at any place about the middle of the bandage.

Weight per unit area.

For samples not less than 15 m. in length, cut out pieces of fabric from the entire length of sample, representative places being taken from areas at every third or fourth metre so that the total area of all the pieces so collected is not less than 3 sq. metre. Weigh the pieces accurately, measure the dimension of each of the pieces and calculate the area and weight of all the pieces. From the average area and average of weight thus obtained, calculate the area per sq. metre.

For samples less than 15 m. length, take pieces in such a manner that the total area of the selected pieces is not less than 20 per cent of the total area of the sample.

For cut bandages, pieces of 50 cm. in length, cut from 5 different cut bandages in a packing should be taken and weight calculated as an average of 5 readings.

Absorbency. - Take a glass trough of approximate size length 30 cm. x width 30 cm. x depth 25 cm. with straight thick walls and flat bottom. Fill it almost full with distilled water leaving only about 5 cm. from the top rim of trough. Maintain the water at $27^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

Cut out from any five places located equidistant down the length of the entire sample, square pieces, each weighting one gm. (± 10 per cent). Fold each piece in such a manner that a square of approximately 5 cm. x 5 cm. is obtained. Keep one of the folded test specimen between two glass plates and place 1 kg weight on the top for 10 minutes. Remove the weight. Lift the specimen with forceps and gently place it on to the surface of water (the specimen should be lightly pinched in the middle with a blunt forceps having no serrations). As soon as the specimen touches the water surface start a stopwatch which is stopped when the entire sample disappears below the surface of the water. Record the time taken. Repeat the test with the other four-test specimens. Calculate the average time in seconds.

Foreign Matter

Dry about 5 g. of the sample to constant weight at 105° C and weigh the dried sample accurately. Extract the dried sample with chloroform for one hour in an apparatus for the continuous extraction of drugs. Remove the extracted sample to a beaker and allow the evaporation of residual chloroform. Wash the material 12 times with hot water, using about 1000 ml. for each washing and wringing the material by hand after each washing; pass all water through a fine sieve (100 mesh). Place the washed material and any loose threads or fibres from sieve in a beaker, cover with a 0.5 per cent aqueous solution of diastase and maintain at 50°C until free from starch. Allow to cool, filter the solution through a sieve; return sample and loose fibre to a beaker. Repeat the washing process as before with hot water. Dry the material to constant weight at 105°C, and determine the loss in weight. Calculate the percentage of foreign matter, which is equal to the loss in weight, with reference to the sample dried to constant weight, at 105° C.

If the sample is tested with iodine and is known to be free from starch, the treatment with solution of diastase and the second series of washing with hot water may be omitted.

Cloth for manufacture of Plaster of Paris Bandages, cut and uncut.

Synonyms.- Bleached Bandage Cloth for Plaster of Paris, Rolled Bandage for Plaster of Paris.

Cloth for Plaster of Paris Bandages shall consist of cotton cloth of leno weave made from yarn of suitable count. It may be supplied cut or uncut of various widths and lengths.

Description

(a) For *uncut bandages.*- Cotton cloth of leno weave, in one continuous length showing no joints or seams, and with selvages. The cloth is bleached to a good white, is clean and odourless and reasonably free from weaving defects as well as from seed and leaf debris; the cloth may be dressed if necessary and if so, shall not dust off when unrolled.

(b) For *cut bandages.* - Same as for uncut bandages except for selvages which shall not be included and the bandages shall be cut evenly with straight edges and be reasonably free from loose threads.

Threads per dm:

Warp. - Average not less than 150/dm; and *Weft.*- average not less than 75/dm.

Weight in gm/m²: - 35 ± 5

Length and width:

The length and width for uncut bandages shall not be less than 98 per cent each of the length and width stated. For cut bandages a tolerance of ± 5 cm. in length and ± 0.5 cm. in width may be allowed, and each of the bandages in packing complies with these requirements.

Fluorescence

When viewed under screened ultra-violet light not more than occasional points of fluorescence are observed.

Packing, Labelling and Storage

Bandage Cloth for Plaster of Paris shall be packed securely so as to allow normal handling and transport without tearing and exposing the contents. In packages of cut and rolled bandages, each bandage shall also individually be wrapped in suitable paper. The package shall be labelled as “Cloth for Plaster of Paris Bandage”. The net content is stated on the label in terms of number of rolls and length and width. Bandage Cloth for Plaster of Paris must be stored in packed condition protected from dust.

¹**[SCHEDULE F (III)]**

(See rule 124-D)

STANDARDS FOR UMBILICAL TAPES

(A) Standards for Sterilised Umbilical Polyester Tape.

Description. - A uniform strand of Polyester yarn prepared by braiding and may be finished with a suitable silicone finishing material, white to yellowish-white in colour. Tape shall be sterilized by Gamma Radiation or other suitable method approved by the Licensing Authority.

Other requirements. - The Umbilical Polyester Tape shall conform to the claims made on the label in respect of length and width.

Tensile strength. - The Umbilical Polyester Tape shall have Tensile strength of not less than 4 kg. on straight pull.

Packing and labelling. - The Umbilical Polyester Tape shall be packed in sealed Polythene bags or sealed plastic containers which ensure that when packed, the tape is sterile. The packing shall protect the tape from contamination and damage. Every packing offered for sale shall bear a clear and permanent marking with the following particulars: -

- (i) The proper name of the drug i.e. Umbilical Polyester Tape ‘Sterile’
- (ii) Manufacturer’s name and address.
- (iii) Batch number.
- (iv) Licence number under which the tape is manufactured.
- (v) Date of manufacture and date of expiry.
- (vi) Length and width of the Tape.

Storage condition. - It should be stored in a cool place protected from light.

(B) Standards for Sterilised Umbilical Cotton Tape—

Description. - A uniform strand of cotton yarn prepared by braiding and may be finished with a suitable silicone finishing material, white to yellowish-white in colour. The tape shall be sterilized by Gamma Radiation or by any other suitable method approved by the Licensing Authority.

Other Requirement. - The Umbilical Cotton Tape shall conform to the claims made on the label in respect of length and width.

Tensile strength. - The Umbilical Cotton Tape shall have a Tensile strength of not less than 4 kg. on straight pull.

Packing and labelling. - The Umbilical Cotton Tape shall be packed in sealed Polythene bags or sealed plastic containers which ensure that when packed the tape is sterile. The packing shall protect the tape from contamination and damage. Every packing offered for sale shall bear a clear and permanent marking with the following particulars:-

- (i) The proper name of drug i.e. Umbilical Cotton Tape “Sterile”.
- (ii) Manufacturer’s name and address.

1. Ins. by G.S.R. No.1115(E), dt. 30-9-1986.

- (iii) Batch number.
- (iv) Licence number under which the tape is manufactured.
- (v) Date of manufacture and the date of expiry.
- (vi) Length and width of the Tape.

Storage condition.- It should be stored in a cool place protected from light.]

¹[SCHEDULE FF

(See rule 126-A)

Standards for ophthalmic preparations.

Part-A. Ophthalmic Solutions and suspensions.

Ophthalmic Solutions and Suspensions shall-

- (a) be sterile when dispensed or when sold in the unopened container of the manufacturer, except in case of those ophthalmic solutions and suspensions which are not specifically required to comply with the test for 'Sterility' in the Pharmacopoeia;
- (b) contain one or more of the following suitable substances to prevent the growth of micro-organisms:-
 - (i) Benzalkonium Chloride, 0.01 per cent (This should not be used in solutions of nitrates or salicylates).
 - (ii) Phenyl mercuric nitrate, 0.001 per cent.
 - (iii) Chlorbutanol 0.5 per cent.
 - (iv) Phenyl ethyl alcohol 0.5 per cent.

Provided that solutions used in surgery shall not have any preservative and be packed in single dose container.

Provided further that the Licensing Authority may in his discretion authorise the use of any other preservative or vary the concentration prescribed on being satisfied that its use affords equal guarantee for preventing the growth of micro-organisms:-

- (c) be free from foreign matter;
- (d) be contained in bottles made of either neutral glass or soda glass specially treated to reduce the amount of alkali released when in contact of aqueous liquids, or in suitable plastic containers which would not in any way be incompatible with the solutions;

The droppers to be supplied with the containers of ophthalmic solutions and suspensions shall be made of neutral glass or of suitable plastic material and when supplied separately shall be packed in sterile cellophane, or other suitable packings;

- (e) In addition to complying with the provisions of labelling laid down in the rules the following particulars shall also be shown on the label:-

1. Added by Notification No. F-1-13/69-D , dt.. 3-1-1970.

(1) *of the containers*

- (i) The statement 'Use the solution within one month after opening the container'.
- (ii) Name and concentration of the preservative, if used.
- (iii) The words 'NOT FOR INJECTION'.

(2) *of container or carton or package leaflet*

- (i) Special instructions regarding storage, wherever applicable.
- (ii) A cautionary legend reading as

“WARNING (i) if irritation persists or increases, discontinue the use and consult the physician.

(ii) Do not touch the dropper tip or other dispensing tip to any surface since this may contaminate solutions”.

Part B: Ophthalmic Ointments

Ophthalmic Ointments shall-

- (a) be sterile when dispensed or when sold in the unopened container of the manufacturer;
- (b) be free from foreign matter;
- (c) in addition to complying with the provisions for labelling laid down in the rules the following particulars shall be shown on the container or carton or package leaflet-
 - (i) Special instructions regarding storage wherever applicable;
 - (ii) A cautionary legend reading

“Warning :- If irritation persists or increases discontinue the use and consult Physicians”].