STATUTORY INSTRUMENTS

1963 No. 1459

THERAPEUTIC SUBSTANCES

The Therapeutic Substances (Manufacture of Vaccines, Toxins and Antigens) Regulations 1963

Made - - - -	23*rd August* 1963
Laid before Parliament	2*nd September* 1963
Coming into Operation	16*th September* 1963

The Joint Committee constituted by section 4(1) of the Therapeutic Substances Act 1956(a), in exercise of the powers conferred on them by section 5 of the said Act, after consultation with the Advisory Committee constituted under section 4(2) of the said Act, hereby make the following regulations :—

PART I

PRELIMINARY

Citation

1. These regulations may be cited as the Therapeutic Substances (Manufacture of Vaccines, Toxins and Antigens) Regulations 1963 and shall come into operation on 16th September 1963.

Interpretation

2.—(1) In these regulations, unless the context otherwise requires—

" the Act " means the Therapeutic Substances Act 1956 ;

" the general regulations " means the Therapeutic Substances (Manufacture and Importation) General Regulations 1963(b) ;

" the licensing authority " has the same meaning as in the general regulations ;

" the licensee " means a person to whom a licence for the manufacture of therapeutic substances has been granted under the general regulations.

(2) Any reference in these regulations to any provision of the general regulations shall be construed as a reference to that provision as amended, extended or re-enacted by any subsequent regulations made under part I of the Act.

(3) For the purposes of these regulations the cubic centimetre may be used wherever the millilitre is indicated.

(4) The Interpretation Act 1889(c) applies to the interpretation of these regulations as it applies to the interpretation of an Act of Parliament.

(a) 4 & 5 Eliz. 2. c. 25. (b) S.I. 1963/1450 (1963 II, p. 2483). (c) 52 & 53 Vict. c. 63.

PART II

GENERAL PROVISIONS RELATING TO VACCINES, TOXINS AND ANTIGENS

Tests for sterility

3. The substances to which these regulations relate, other than the substances to which parts IV, V and VII relate and smallpox vaccine prepared from vaccinal material obtained from the lesions produced by inoculation into the skin of healthy animals shall, except so far as any provision of these regulations otherwise provides, be tested for sterility in accordance with the general regulations.

Tests of combined antigens

4. Any combination of substances to which these regulations relate shall, if tested as a combined substance, conform to the standards prescribed by the general regulations and these regulations for the substances comprising the combination.

Tests on guinea-pigs

5. Where any provision of part IV or parts VII to XVIII of these regulations requires a substance to be injected into a guinea-pig for the purpose of any test, the guinea-pig—

(*a*) shall be healthy;

(*b*) shall weigh not less than 250 grammes unless any other requirement as to weight is specified in these regulations;

(*c*) shall not have been previously treated with any material which will interfere with the test.

Labelling

6.—(1) The particulars specified in regulation 6(1)(*a*), (*b*) and (*c*) of the general regulations shall be borne by the container of any substance to which these regulations relate.

(2) The particulars specified in sub-paragraphs (*e*), (*f*) and (*i*) of the said regulation 6(1) shall be borne either by the container of any such substance or by the package in which the substance is offered for sale.

(3) In addition to the particulars required by the said regulation 6(1), a statement of the proper dose for administration at one time into a human subject shall be borne by the container of any such substance.

PART III

PROVISIONS RELATING TO BACTERIAL AND VIRAL VACCINES

Definition

7.—(1) This part of these regulations applies to vaccines made from any micro-organism or virus pathogenic to man or other animal and to vaccines made from other micro-organisms or viruses having antigenic value.

(2) For the purposes of this part of these regulations a vaccine means a sterile suspension of a killed culture of the micro-organism or virus from which the vaccine derives its name or a sterile extract or derivative of a micro-organism or virus which has been prepared from a genuine culture of the micro-organism or virus.

Proper name

8.—(1) The proper name of any vaccine to which this part of these regulations applies and for which no proper name is provided by these regulations is the name of the micro-organism or virus from which it is made, followed by the word " vaccine ", or such other alternative name as the licensing authority may approve.

(2) The proper name of a combination of vaccines is such name as the licensing authority may approve.

Staff of establishment

9. The establishment in which vaccines are prepared shall be under the complete direction and control of an expert in microbiology.

Identity of cultures

10. Cultures used in the preparation of vaccines shall, before being manipulated into a vaccine, be thoroughly tested for identity by the generally accepted tests applicable to the particular micro-organism or virus. The records which the licensee is required to keep shall include a record of the origin, properties and characters of the cultures.

Method of preparation of oral vaccines in non-liquid form

11. In the case of a preparation of a vaccine intended for administration by any means other than injection into the tissues—

(*a*) any preparatory process of drying or concentration shall be performed with such precautions as are necessary to secure that the vaccine does not become contaminated in the process;

(*b*) the method of preparation shall be such as to secure that there shall be no bacterial growth arising from inside the preparation.

Tests

12.—(1) If any such preparation as is described in the last preceding regulation is in a solid form or is to be administered in a solid vehicle:—

(*a*) the tests referred to in regulation 8 of the general regulations may be applied to the vaccine from which the preparation is made instead of being applied to the preparation itself, but the ordinary standards of pharmaceutical cleanliness shall be observed in the manufacture of the preparation;

(*b*) the sterility tests described in the general regulations shall be applied with such modifications as the licensing authority consider appropriate for that preparation.

(2) In the case of any vaccine prepared from a micro-organism or virus which does not grow readily in nonviable culture media, each batch of the vaccine shall, in addition to being tested for sterility in accordance with the general regulations, be tested for the presence of living micro-organisms either in a similar manner on media which are specially favourable to the growth of the particular micro-organism or virus from which the vaccine was prepared or by injection into an animal of a species known to be susceptible to infection by the particular micro-organism or virus, and no material from any batch shall be issued unless the batch has passed one of these tests.

Containers

13. If a preparation of a vaccine intended for administration by any means other than injection into the tissues is in a solid form or is to be

administered in a solid vehicle, the container in which it is packed need not be sterile, but ordinary standards of pharmaceutical cleanliness shall be observed in the packing of the preparation.

Labelling

14.—(1) In addition to the particulars required by regulation 6(1) and (2) of these regulations, particulars of the composition of the vaccine shall be borne by the container of a vaccine to which this part of these regulations relates by reference either :—

(*a*) to the number of micro-organisms per millilitre ; or

(*b*) to the weight of dried substance of micro-organisms per millilitre ; or

(*c*) to the number of micro-organisms or weight of dried substance of micro-organisms used in preparing 1 millilitre of the finished product ; or

(*d*) to the immunising power of the vaccine in relation to a standard vaccine approved by the licensing authority ; or

(*e*) to any other criteria approved by the licensing authority.

In the case of a combined vaccine the reference to the number of micro-organisms per millilitre or to the weight of dried substance of micro-organisms shall distinguish between the several kinds of contributing micro-organisms.

(2) If the vaccine as issued for sale is combined with any substance other than a simple diluent, particulars of the exact nature and strength of that substance shall also be borne by the container.

Method of issue

15. Vaccines may be issued either singly or combined in any proportion in the same container.

PART IV

PROVISIONS RELATING TO BACILLUS CALMETTE-GUÉRIN VACCINE

Definition and proper names

16. Bacillus Calmette-Guérin vaccine is a preparation of the bacteria in a living pure culture of a strain of the bacillus of Calmette and Guérin, which bacillus is in this part of these regulations referred to by the initials B.C.G. The proper name of the vaccine when prepared in a liquid form is " B.C.G. vaccine ", and the proper name of the vaccine when prepared in a dried form is " dried B.C.G. vaccine ".

Characters of the strain of B.C.G.

17. The strain of B.C.G. employed in the preparation of vaccine shall be an authentic strain of the bacillus of Calmette and Guérin and shall be maintained so as to preserve its power of sensitising man to tuberculin and its relative non-pathogenicity to man and laboratory animals.

Staff of establishment

18.—(1) The establishment in which B.C.G. vaccine or dried B.C.G. vaccine is prepared shall be under the complete direction and control of an expert approved by the licensing authority.

(2) No worker shall be employed in the preparation of the vaccine unless, after full clinical, radiological and bacteriological examinations carried out by specialists approved by the licensing authority and repeated not less often than at 12-monthly intervals and whenever there is cause to suspect ill health, he is shown to be free from signs of active or potentially active tuberculous lesions. If an examination reveals signs of active or potentially active tuberculosis the worker shall be removed from the establishment, all strains of B.C.G. shall be re-examined and the premises in which the vaccine is manufactured shall be disinfected and all equipment used in the manufacture shall be sterilized. The licensee shall take all reasonably practicable precautions to secure that no person is employed in the establishment who is in close contact with a sufferer from any form of open tuberculosis.

Precautions to be observed in preparation and testing

19.—(1) The preparation of B.C.G. vaccine or dried B.C.G. vaccine shall be carried out in a special room or in special rooms forming topographically separate premises, to which only members of the staff authorised by the licensing authority shall have access. All media, glassware and other apparatus required for the preparation and testing of the vaccine shall be prepared and kept in these rooms. Effective precautions shall be taken to prevent the introduction of microbial contamination through the agency of air, water, equipment, material or personnel. No microbial culture other than that of B.C.G. shall be introduced into the said premises without the consent of the licensing authority.

(2) No animals shall be permitted in the said premises; the inoculations necessary for the testing of the parent strain of B.C.G. and the batches of vaccine shall be given to the animals in premises topographically and functionally separate from those in which the vaccine is prepared.

(3) The parent strain of B.C.G. shall be maintained under conditions which will preserve the properties defined in regulation 17 of these regulations.

(4) The growth from which the vaccine is made shall not be more than 14 days old, and shall be suspended in a concentration, approved by the licensing authority, in a sterile liquid medium designed to preserve the antigenicity and viability of the vaccine.

(5) Dried B.C.G. vaccine shall be dried by a method approved by the licensing authority. For the purposes of the tests described in regulations 20 to 24 of these regulations the dried vaccine shall be reconstituted in the form in which it is intended for use and all references to doses used in the said tests shall apply to the reconstituted vaccine.

Tests for sterility

20.—(1) B.C.G. vaccine and dried B.C.G. vaccine shall be tested for sterility in accordance with the general regulations.

(2) B.C.G. vaccine is hereby declared to be a substance to which paragraph 3 of schedule 2 to the general regulations applies.

(3) In addition to being tested for sterility in accordance with the said paragraph 3, B.C.G. vaccine shall be tested for the presence of organisms other than B.C.G. by inoculation of an animal by a method approved by the licensing authority. The licensee shall not issue the B.C.G. vaccine if there is within 48 hours evidence from any such test of contamination by any organism other than B.C.G.

Test for viability

21. Each batch of B.C.G. vaccine or dried B.C.G. vaccine shall be tested for viability by plating suitable dilutions on a suitable solid medium or by a method approved by the licensing authority.

Test for virulence

22. Each batch of B.C.G. vaccine or dried B.C.G. vaccine shall be tested for virulence by subcutaneous injection of 5 millilitres of vaccine into each of 6 guinea-pigs each weighing not more than 250 grammes. The batch shall be deemed to have passed the test if none of the animals dies within 42 days after the injection or if one only of the animals dies within that period and a post-mortem examination establishes that the animal is free from tuberculosis. If 2, but not more, of the animals die within the said period and a post-mortem examination establishes that both of them are free from tuberculosis, 6 further guinea-pigs may be similarly injected, and the batch shall be deemed to have passed the test if none of these animals dies within 42 days after injection or if one only of the animals dies within that period and a post-mortem examination establishes that the animal is free from tuberculosis.

Test for toxicity

23. Each batch of B.C.G. vaccine or dried B.C.G. vaccine shall be tested for toxicity by the intracutaneous injection in doses of 0·1 millilitre into a guinea-pig. Within three weeks after the injection the dose of vaccine shall produce a definite non-suppurating nodule but no local necrosis.

Test for skin-sensitising potency

24. Each batch of B.C.G. vaccine or dried B.C.G. vaccine shall be tested for skin sensitising potency by the subcutaneous or intramuscular injection of 0·5 millilitre into each of 2 guinea-pigs. The vaccine shall, within 4 weeks of the injection, induce in both guinea-pigs sensitivity to the intracutaneous injection of 10 international units of old tuberculin in a volume of 0·1 millilitre, of a degree which shall cause an inflammatory area of induration and oedema not less than 5 mm. in diameter, irrespective of the area of erythema.

Conditions of issue

25.—(1) No batch of B.C.G. vaccine shall be issued until it has passed the test prescribed by regulation 20(3) of these regulations.

(2) If the result of any of the tests required by regulation 20(3) and regulations 22, 23 and 24 of these regulations should after the issue of any B.C.G. vaccine indicate that a particular batch does not fulfil the requirements of these regulations, the licensee shall immediately inform the licensing authority.

(3) No batch of dried B.C.G. vaccine shall be issued until it has passed the tests prescribed by regulation 20(1) and regulations 21 to 24 of these regulations.

Labelling

26.—(1) In addition to the particulars required by regulation 6(1) of these regulations, the particulars specified in regulation 6(1)(*g*) of the general regulations shall be borne both by the container of B.C.G. vaccine or of dried B.C.G. vaccine and by the package in which the vaccine is offered for sale.

(2) For the purpose of the said sub-paragraph (*g*) the date to be specified shall be:—

(*a*) in the case of B.C.G. vaccine, 14 days from the date of manufacture;

(*b*) in the case of dried B.C.G. vaccine, 12 months from the date when the tests described in regulations 20 to 24 of these regulations are completed; or in either case such other date as the licensing authority may approve.

(3) In addition to the particulars required by regulation 6(1) and (2) and regulation 14 of these regulations, the particulars specified in regulation 6(1)(*d*) of the general regulations shall be borne by the container of B.C.G. vaccine or of dried B.C.G. vaccine.

(4) In addition to the particulars required by regulation 6(3) of these regulations, the following further particulars shall be borne either by the container of B.C.G. vaccine or of dried B.C.G. vaccine or by the package in which the vaccine is offered for sale:—

(*a*) the particulars specified in regulation 6(1)(*h*) of the general regulations;

(*b*) the route of injection;

(*c*) a statement that the contents have been standardised by viable count.

(5) In addition to the particulars required by regulation 6(3) of these regulations and by the foregoing paragraph the following particulars shall be borne by the container of dried B.C.G. vaccine or the package in which the vaccine is offered for sale:—

(*a*) the volume of fluid to be added to reconstitute the vaccine;

(*b*) a statement that when the container is opened any portion of the contents not used at once must be discarded.

(6) For the purposes of regulation 6(1)(*f*) of the general regulations, the date on which the manufacture of a batch of B.C.G. vaccine was completed shall be the date on which the B.C.G. culture was harvested for making the batch and the date on which the manufacture of a batch of dried B.C.G. vaccine was completed shall be the date on which the tests described in regulations 20 to 24 of these regulations were completed.

(7) Paragraph (4) of regulation 6 of the general regulations shall not apply to B.C.G. vaccine.

PART V

PROVISIONS RELATING TO TUBERCULOSIS VACCINES CONTAINING LIVING BACTERIA OTHER THAN B.C.G.

27. The definitions and proper names of tuberculosis vaccines containing living bacteria other than B.C.G., the characters of the strain of bacteria from which any such vaccine is made, the conditions under which the strain must be maintained in order to preserve these characters, the conditions under which the strain is to be harvested for the preparation of the vaccine, the tests for sterility, virulence, toxicity and immunising or sensitising potency, the condition of issue, the particulars to be borne by the container or by the package in which it is offered for sale and the dates of manufacture and expiry of the vaccine, shall be such as shall be approved by the licensing authority.

28. The provisions of regulation 18 and regulation 19(1) and (2) of these regulations shall apply to any such vaccine with any necessary modifications.

PART VI

PROVISIONS RELATING TO SMALLPOX VACCINE (VACCINIA VACCINE)

Definition and proper name

29. Smallpox vaccine is a preparation of the vaccinal material obtained from the lesions produced by inoculation of vaccinia virus into the skin of healthy animals or by inoculation of vaccinia virus into other tissues of a kind approved by the licensing authority. The proper name of the vaccine when prepared in a liquid form is " smallpox vaccine ", and the proper name of the vaccine when prepared in a dried form is " dried smallpox vaccine ".

Staff of establishment

30. The establishment in which smallpox vaccine or dried smallpox vaccine is prepared shall be under the complete direction and control of an expert approved by the licensing authority.

Buildings

31.—(1) Animals used in the production of smallpox vaccine or dried smallpox vaccine shall be housed under hygienic conditions in premises satisfactory for this purpose.

(2) A special room, with impervious walls and floor, which can be washed and, when necessary, chemically disinfected, shall be provided for the inoculation of the animals and the collection of the vaccinal material.

(3) Laboratories in which vaccinal material, after harvesting, is manufactured into vaccine shall be in a building separated by a reasonable distance from stables or buildings housing animals. Such laboratories shall have impervious walls and floors and shall be capable of being readily disinfected.

Condition of animals

32. Only healthy animals or other healthy sources of tissue shall be used in the production of smallpox vaccine or dried smallpox vaccine. Each animal intended to be used as a source of the vaccine shall, before being passed for the production of the vaccine, be subjected to a period of observation in quarantine for at least 5 days. During the period of quarantine the animal shall remain free from any sign of disease and shall be thoroughly cleaned and groomed.

Precautions to be observed in preparation

33.—(1) The inoculation shall be made on such parts of the animal as are not liable to be soiled by the passage of faeces. The surface used for inoculation shall be shaved and cleaned. Immediately prior to the collection of vaccinal material the inoculated area of the skin shall be clean.

(2) The vaccinal material shall be collected immediately after the animal has been killed and a thorough post-mortem examination of the carcase shall be made by an expert. A complete record of the examination shall be kept, and shall be open to inspection by or on behalf of the licensing authority at any time. If the examination reveals any condition which indicates or suggests that the animal was suffering from any communicable disease (other than vaccinia) the vaccinal material obtained from that animal shall not be issued.

(3) All instruments and appliances used in the production of smallpox vaccine or dried smallpox vaccine shall be previously sterilised.

(4) All processes concerned with the manufacture of smallpox vaccine or dried smallpox vaccine shall be carried out with aseptic precautions.

(5) All vaccinal material used for the preparation of smallpox vaccine other than dried smallpox vaccine shall be subjected, after collection, to such treatment with glycerol, with or without some other disinfectant, as will bring the content of extraneous micro-organisms of the vaccine within the limit prescribed in regulation 35 of these regulations.

(6) When such treatment has been completed, smallpox vaccine shall be kept continuously in cold storage at a temperature not higher than −10°C. until it is withdrawn to be filled into containers for issue, after which process the filled containers shall immediately be returned to cold storage and kept continuously at a temperature not higher than −10°C. until required for issue:

Provided that the vaccine may be removed from one such cold store to another, if adequate precautions are taken during removal to guard against deterioration.

Stage at which tests to be applied

34. So much of regulation 8 of the general regulations as requires that the tests therein referred to shall be applied to samples taken from the final product after every manufacturing process has been completed shall not apply to smallpox vaccine.

Tests for bacterial contamination

35.—(1) Smallpox vaccine obtained from the lesions produced by inoculation into the skin of healthy animals shall be exposed to the action of glycerol, with or without some other disinfectant, under suitable conditions of temperature until tests made by means of plate culture have shown that the total number of living extraneous micro-organisms has been reduced to not more than 1,000 in 1 millilitre of the vaccine.

(2) Dried smallpox vaccine as reconstituted in the form in which it is to be inoculated shall be tested to ensure that it does not contain more than 1,000 living extraneous micro-organisms in 1 millilitre.

(3) The results of the tests required by the two foregoing paragraphs shall be recorded and the records shall be kept for inspection. The determination of the content of the living micro-organisms in the vaccine shall be made in a manner approved by the licensing authority, and the enumeration of colonies shall be made after incubation for 72 hours between 18°C. and 22°C. and for a further period of 48 hours between 32°C. and 37°C.

(4) The vaccinal material shall have been shown to contain no *Escherichia coli* or aerobic pathogenic organism which may prove harmful if introduced into the body by the process of vaccination.

(5) The vaccinal material shall have been shown to contain no anaerobic pathogenic organism which may prove harmful if introduced into the body by the process of vaccination.

Tests for potency

36.—(1) Each batch of vaccine shall be tested for potency—

(*a*) in the case of smallpox vaccine, after testing for bacterial contamination,

(*b*) in the case of dried smallpox vaccine, after reconstitution in the form in which it is to be inoculated,

so as to ensure its activity at the time of issue. These tests shall be applied not more than 12 months before a batch of vaccine is finally issued.

(2) The vaccine shall be tested:—

(*a*) on the skin of a rabbit, or of an animal of some other species approved by the licensing authority, in the manner described in paragraph (4) of this regulation, or in that manner with such modifications as may be approved by the licensing authority; or

(*b*) on material of such other kind, and in such manner, as may be approved by the licensing authority.

(3) For the purpose of the tests progressive dilutions of the vaccine shall be made in physiological saline solution or other suitable diluent. The dilutions shall be used for the test without filtration.

(4) If the test on the skin of a rabbit or other animal is used, the vaccine shall be tested by the application of the dilutions to the suitably prepared skin of the animal, in direct comparison with similar dilutions of a control vaccine of established potency approved by the licensing authority; and the vaccine shall pass the test if both the vaccine under test and the control vaccine—

(*a*) in a dilution of 1 in 1,000 produce confluent lesions characteristic of vaccinia;

(*b*) in a dilution of 1 in 10,000 produce not less than 10 characteristic discrete vesicles.

Labelling

37.—(1) In addition to the particulars required in regulation 6(3) of these regulations, the following further particulars shall be borne either by the container of smallpox vaccine or of dried smallpox vaccine or by the package in which the vaccine is offered for sale:—

(*a*) in the case of smallpox vaccine, a statement that the potency of the vaccine cannot be assured for more than 7 days from the date of completion of manufacture, unless—

(i) the vaccine is kept continuously at a temperature below 10°C., in which case its potency is assured for 14 days, or

(ii) it is kept continuously at a temperature not higher than −10°C., in which case its potency is assured for 12 months;

(*b*) in the case of dried smallpox vaccine, a statement that the potency of the reconstituted vaccine can be assured for not more than 7 days if the reconstituted vaccine is kept continuously at a temperature below 10°C.

(2) In its application to smallpox vaccine and dried smallpox vaccine issued in single dose containers regulation 6(1) of these regulations shall have effect as if the reference to the particulars specified in regulation 6(1)(*b*) and (*c*) of the general regulations were deleted; and the particulars specified in the said sub-paragraphs (*b*) and (*c*) shall be borne either by the container of any such substance or by the package in which it is offered for sale.

(3) For the purposes of regulation 6(1)(*f*) of the general regulations, the date on which the manufacture of the batch is completed shall be the date on which it is removed from cold storage for issue.

(4) Regulation 6(4) of the general regulations shall not apply to smallpox vaccine.

PART VII

PROVISIONS RELATING TO VACCINES CONTAINING LIVING BACTERIA, VIRUSES, OR OTHER POTENTIALLY INFECTIVE AGENTS, OTHER THAN VACCINES TO WHICH PARTS IV TO VI OF THESE REGULATIONS RELATE.

Extent of this part

38. This part of these regulations relates to every vaccine other than those to which parts IV to VI of these regulations relate, containing, or stated to contain, bacteria, viruses or other potentially infective agents in the living condition.

Proper name

39. The proper name of any vaccine to which this part of these regulations applies and for which no proper name is provided by these regulations is that which the licensing authority, in each particular case, shall approve.

Tests

40. Such a substance shall be tested in such manner as the licensing authority shall approve in each particular case for the purpose of determining—

(*a*) that it contains in living condition the bacteria, viruses or other potentially infective agents;

(*b*) that its administration is free from danger; and

(*c*) that it is free from living organisms other than those which it is stated to contain.

PART VIII

PROVISIONS RELATING TO THE REAGENTS USED IN THE SCHICK TEST FOR THE DIAGNOSIS OF SUSCEPTIBILITY TO DIPHTHERIA

Definitions and proper names

41.—(1) The reagents used in the Schick test are two, Schick toxin and Schick control. Their proper names respectively are "Schick test toxin" and "Schick control".

(2) Schick test toxin is a sterile filtrate from a culture on nutrient broth of the specific organism of diphtheria (*Corynebacterium diphtheriae*), diluted with an appropriate saline solution to the strength proper for use in the test.

(3) The dilution of Schick test toxin proper for the test is that in which 0·2 millilitre contains one test dose.

(4) A test dose for the purposes of the foregoing paragraph is such an amount of Schick test toxin—

(*a*) as, if injected intracutaneously into a guinea-pig will cause no local reaction if mixed with 1/750th or more of a unit of diphtheria antitoxin, and will cause a definite local reaction of the type known as "the positive Schick test reaction" if mixed with 1/1250th or less of such a unit;

(*b*) as, if so injected in a dilution of 1 in 50, will not cause a local reaction; and

(*c*) as, if so injected in a dilution of 1 in 25, will cause a definite local reaction of the type known as the "positive Schick test reaction".

(5) Schick control is prepared from the same batch of Schick test toxin as that with which it is issued for sale, by destroying the specific toxicity. This is effected by heating the toxin at a temperature not lower than 70°C. and not higher than 85°C. for not less than 5 minutes. Schick control is issued in a dilution not weaker than that in which the corresponding toxin is used in the test.

PART IX

GENERAL PROVISIONS RELATING TO DIPHTHERIA VACCINE

Definitions and proper names

42.—(1) Diphtheria vaccine is diphtheria toxin (that is to say, the sterile filtrate from a culture in a suitable culture medium of *Corynebacterium diphtheriae*), or material derived therefrom, of which either—

(*a*) the specific toxicity has been completely removed by the action of chemical substances ; or

(*b*) the specific toxicity has been reduced to a low level by the action of chemical substances and the remaining toxicity has been completely removed by the addition of an appropriate quantity of diphtheria antitoxin.

(2) The proper name of a preparation in relation to which no special provisions are hereinafter made applicable, or of a mixture of diphtheria vaccine with another therapeutic substance, is " diphtheria vaccine " together with such other words or abbreviations as may be approved by the licensing authority as descriptive of that preparation or mixture.

Quality

43. No batch of diphtheria vaccine shall be issued unless it is an efficient immunising antigen.

Test for toxicity

44. 5 millilitres of the preparation, or, if a different volume is specified in the next following 5 parts of these regulations or approved by the licensing authority in relation to any preparation, the volume so specified or approved, shall be injected subcutaneously or intraperitoneally into each of not less than 5 guinea-pigs. The injection shall not cause the death of any of the guinea-pigs from specific intoxication within 30 days.

Definition of toxin test dose and flocculation equivalent

45.—(1) For the purpose of testing diphtheria vaccine for minimum potency as an immunising antigen, a toxin test dose is a quantity of diphtheria toxin which contains the 1/1000th part of a specific flocculation equivalent (0·001 Lf) and such an amount of free toxin, that, when 1/20th of this quantity (0·00005 Lf) or less is injected intracutaneously into guinea-pigs they will 48 hours after injection regularly exhibit local reactions of the Schick positive type.

(2) For the purpose of a test of diphtheria vaccine involving the determination of a specific flocculation equivalent, the diphtheria antitoxin used shall be a suitable sample which has been standardised, by comparative tests, in terms of the Preparation of Diphtheria Antitoxin (Reagent for the Flocculation Test) supplied by the National Institute for Medical Research, London.

Labelling

46. In addition to the particulars required by regulation 6(3) of these regulations, the particulars specified in regulation 6(1)(*g*) and (*h*) of the general regulations shall be borne either by the container of diphtheria vaccine or by the package in which it is offered for sale.

PART X

SPECIAL PROVISIONS RELATING TO DIPHTHERIA VACCINE (FORMOL TOXOID)

Definition and proper name

47. Diphtheria vaccine (formol toxoid) is diphtheria toxin, or material derived therefrom, the specific toxicity of which has been completely removed by the action of formaldehyde. The proper name of the substance is "diphtheria vaccine F.T.".

Quality

48. Diphtheria vaccine F.T. shall contain not less than 50 flocculation equivalents (50 Lf) per millilitre.

Tests for minimum potency as an immunising antigen

49. 0·05 millilitre of the diphtheria vaccine F.T. diluted with physiological saline solution to a volume of 1 millilitre shall be injected into each of 10 white or light coloured guinea-pigs on each of 2 occasions separated by an interval of not more than 4 weeks. The guinea-pigs shall be tested for immunity to diphtheria toxin not later than 3 weeks after the second injection. The test for immunity shall be made by the intracutaneous injection into each guinea-pig of 0·2 millilitre of a dilution of diphtheria toxin containing the equivalent of 5 toxin test doses (0·005 Lf). If, 48 hours after injection, more than 2 of the 10 guinea-pigs exhibit a positive Schick reaction, the batch shall be treated as insufficiently potent and shall not be used.

PART XI

SPECIAL PROVISIONS RELATING TO DIPHTHERIA VACCINE (ALUM PRECIPITATED TOXOID)

Definitions and proper name

50. Diphtheria vaccine (alum precipitated toxoid) is a preparation made by adding a solution of alum in suitable quantity to diphtheria vaccine F.T., and collecting, adequately washing and resuspending the resulting precipitate in physiological saline solution. The proper name of the substance is "diphtheria vaccine A.P.T.".

Test for specific combining power with diphtheria antitoxin

51. Diphtheria vaccine A.P.T. shall be tested for its specific combining power with diphtheria antitoxin by dissolving the suspended precipitate of alum toxoid by the addition of sodium citrate or other suitable salt. 1 millilitre of the final preparation shall represent not less than 50 specific flocculation equivalents (50 Lf) of the toxoid used in its preparation.

Test for minimum potency as an immunising antigen

52. 0·02 millilitre of diphtheria vaccine A.P.T. diluted with physiological saline solution to 1 millilitre shall be injected into each of 10 guinea-pigs on each of 2 occasions separated by an interval of not more than 4 weeks.

The guinea-pigs shall be tested for immunity not later than 3 weeks after the second injection, by determining the diphtheria antitoxin content of the serum of each guinea-pig separately. If the geometric mean of the antitoxin contents so determined is less than 2 units per millilitre the batch of diphtheria vaccine A.P.T. shall be rejected as insufficiently potent and shall not be issued.

Test for toxicity

53. The volume of diphtheria vaccine A.P.T. to be used for the purpose of the test for toxicity shall be 1 millilitre.

PART XII

SPECIAL PROVISIONS RELATING TO DIPHTHERIA VACCINE (PURIFIED TOXOID, ALUMINIUM PHOSPHATE)

Definition and proper name

54. Diphtheria vaccine (purified toxoid, aluminium phosphate) is a preparation made by adding a suitable quantity of dissolved purified diphtheria toxoid to a suspension of hydrated aluminium phosphate in physiological saline, so adjusted that the required amount of the toxoid is adsorbed by the aluminium phosphate. The proper name of the substance is " diphtheria vaccine P.T.A.P.".

Tests

55. The provisions of regulations 51, 52 and 53 of these regulations shall apply to diphtheria vaccine P.T.A.P., with the substitution for references to diphtheria vaccine A.P.T. of references to diphtheria vaccine P.T.A.P.

PART XIII

SPECIAL PROVISIONS RELATING TO DIPHTHERIA VACCINE (TOXOID-ANTITOXIN MIXTURE)

Definition and proper name

56. Diphtheria vaccine (toxoid-antitoxin mixture) is a preparation made from diphtheria toxin the specific toxicity of which has been either completely removed by the action of formaldehyde or has been so reduced by the action of formaldehyde that the content of unaltered toxin is not greater than 5 toxin test doses (0·005 Lf) per millilitre, and to which diphtheria antitoxin has been added in an amount sufficient to neutralise completely any remaining unaltered toxin but insufficient to satisfy completely the combining power of the toxoid. The proper name of the substance is " diphtheria vaccine T.A.M.".

Quality

57. 1 millilitre of the final preparation shall represent not less than 3 specific flocculation equivalents (3 Lf) of the toxoid used in its preparation.

Test for minimum potency as an immunising antigen

58. 0·1 millilitre of diphtheria vaccine T.A.M. diluted with physiological saline solution to 1 millilitre shall be injected into each of 10 guinea-pigs on each of 2 occasions separated by an interval of not more than 4 weeks. The guinea-pigs shall be tested for immunity to diphtheria toxin not later than 3 weeks after the second injection. The test for immunity shall be

made by the intracutaneous injection into each guinea-pig of 0·2 millilitre of a dilution of diphtheria toxin containing the equivalent of one toxin test dose (0·001 Lf). If, 48 hours after injection, more than 2 of the 10 guinea-pigs exhibit a positive Schick reaction, the batch of diphtheria vaccine T.A.M. shall be rejected as insufficiently potent and shall not be issued.

PART XIV

SPECIAL PROVISIONS RELATING TO DIPHTHERIA VACCINE (TOXOID-ANTITOXIN FLOCCULES)

Definition and proper name

59. Diphtheria vaccine (toxoid-antitoxin floccules) is a preparation made by adding to diphtheria toxin, the specific toxicity of which has been either completely removed by the action of formaldehyde or has been so reduced by the action of formaldehyde that the content of unaltered toxin is not greater than 5 toxin test doses (0·005 Lf) per millilitre, an amount of diphtheria antitoxin equivalent to about 80 per cent. of the toxoid so produced, and collecting and adequately washing the resultant specific precipitate and resuspending it in physiological saline solution. The proper name of the substance is " diphtheria vaccine T.A.F.".

Method of preparation

60. No procedure shall be used at any stage of the preparation which leads to the liberation of toxoid from its combination.

Quality

61. 1 millilitre of the final suspension shall represent not less than 100 specific flocculation equivalents (100 Lf) of the original toxoid used in its preparation.

Tests for minimum potency as an immunising antigen

62. 0·1 millilitre of the diphtheria vaccine T.A.F. diluted with physiological saline solution to 1 millilitre shall be injected into each of at least 9 guinea-pigs on each of 2 occasions separated by an interval of not more than 4 weeks. Tests for immunity to diphtheria toxin shall be made not later than 3 weeks after the second injection by the separate but simultaneous intracutaneous injection, into each of not less than 9 of the guinea-pigs of 0·2 millilitre of dilutions of diphtheria toxin containing respectively the equivalent of 1 toxin test dose (0·001 Lf) and 2 toxin test doses (0·002 Lf) of diphtheria toxin. If, 48 hours after injection, not more than $\frac{1}{3}$ of the guinea-pigs tested exhibit a positive reaction to the equivalent of one toxin test dose of diphtheria toxin, or alternatively, not more than $\frac{2}{3}$ of the guinea-pigs tested exhibit a positive reaction to the equivalent of 2 toxin test doses of diphtheria toxin, the batch shall be accepted as sufficiently potent.

PART XV

PROVISIONS RELATING TO STAPHYLOCOCCUS TOXOID

Definition and proper name

63. Staphlylococcus toxoid is staphylococcus alpha toxin produced by the growth of *Staphylococcus aureus* the specific toxicity of which has been reduced to a low value by the action of chemical substances in such a manner that it retains efficient properties as an immunising antigen. Its proper name is " staphylococcus toxoid ".

Staphylococcus toxoid may be issued either—

(*a*) undiluted; or

(*b*) already diluted with a saline solution to the strength suitable for injection.

Tests

64. Staphylococcus toxoid shall be submitted to the tests prescribed in this regulation and shall not be issued unless it passes all the tests.

(*a*) *Tests to determine that the specific toxicity of the toxin used in its preparation has been sufficiently reduced*

(i) 1 volume of the undiluted staphylococcus toxoid shall be added to 4 volumes of physiological saline solution; equal volumes of this dilution of staphylococcus toxoid and of a 2 per cent. suspension of washed red blood cells of a rabbit shall be mixed; when the mixture is heated to 37°C. for one hour there shall be no haemolysis.

(ii) 0·2 millilitre of the undiluted staphylococcus toxoid shall be injected intracutaneously into a healthy rabbit or guinea-pig; this injection may cause a slight local reaction but shall not produce necrosis.

(iii) 2 rabbits shall be injected intravenously with doses of staphylococcus toxoid calculated at the rate of 2·5 millilitres per kilogram body weight; this injection shall not cause the death of either rabbit within 3 days.

(*b*) *Test for non-specific toxicity*

3 healthy mice shall be injected intraperitoneally with 0·5 millilitre of the undiluted toxoid; this injection shall not cause the death of any of the animals within 7 days.

(*c*) *Tests for potency as an immunising antigen*

1 millilitre of the undiluted staphylococcus toxoid shall be injected into each of not less than 9 guinea-pigs on each of 2 occasions separated by an interval of not more than 4 weeks; at a date not later than 2 weeks after the second injection the staphylococcus alpha antitoxin present in the serum of each guinea-pig shall be determined.

If the serum of each of $\frac{2}{3}$ or more of the guinea-pigs tested contains 0·5 unit or more of staphylococcus alpha antitoxin per millilitre of serum or, alternatively, if the serum of each of $\frac{1}{3}$ or more of the guinea-pigs tested contains 1 unit or more of staphylococcus alpha antitoxin per millilitre of serum, the toxoid shall be accepted as sufficiently potent.

Labelling

65. In addition to the particulars required by regulation 6(3) of these regulations the particulars specified in regulation 6(1)(*g*) and (*h*) of the general regulations shall be borne either by the container of staphylococcus toxoid or by the package in which it is offered for sale.

PART XVI

PROVISIONS RELATING TO TETANUS VACCINE

Definition and proper name

66. Tetanus vaccine is tetanus toxin produced by the growth of *Clostridium tetani* the specific toxicity of which has been completely removed by the action of chemical substances in such a manner that it retains efficient

properties as an immunising antigen. The proper name of the substance in the form of a simple solution is "tetanus vaccine in simple solution". The proper name of the substance in any form other than that of simple solution is "tetanus vaccine" together with a phrase indicating the nature of the additional process to which it has been subject, e.g. "alum precipitated tetanus vaccine".

Tests of tetanus vaccine

67. Tetanus vaccine shall be submitted to the tests prescribed in this regulation and shall not be issued unless it passes all the tests.

(*a*) *Tests for sterility*

In addition to being tested for sterility in accordance with the general regulations, tetanus vaccine shall be tested for sterility on media and under conditions approved by the licensing authority as being specially favourable for the growth of *Clostridium tetani*.

(*b*) *Tests to determine that the specific toxicity of the toxin used in its preparation has been completely removed*

A quantity of the tetanus vaccine equivalent to 5 times the maximum dose stated on the label shall be injected subcutaneously into each of not less than 5 guinea-pigs each weighing not more than 350 grammes. If this injection produces any symptom of tetanus in any of the animals injected within 21 days of injection the tetanus vaccine shall be held not to have passed the test.

(*c*) *Tests for potency as an immunising antigen*

The tests shall be carried out on not less than 9 guinea-pigs each weighing not more than 350 grammes. Each guinea-pig shall receive the tetanus vaccine by injection, either in one dose equivalent to 5 times the dose or minimum dose stated on the label or in 2 doses each equivalent to $\frac{1}{10}$ of the dose or minimum dose and separated by an interval of not more than 4 weeks. The tetanus antitoxin present in the serum of each guinea-pig shall be determined, in the case of those which received the said one dose not more than 6 weeks after the injection and in the case of those which received the said 2 doses not later than 2 weeks after the second injection. If the serum of each of $\frac{2}{3}$ or more of the guinea-pigs tested contains 0·05 international unit or more of tetanus antitoxin per millilitre of serum, or alternatively, if the serum of each of $\frac{1}{3}$ or more of the guinea-pigs tested contains 0·5 international unit or more of tetanus antitoxin per millilitre of serum, the tetanus vaccine shall be accepted as sufficiently potent.

Tests of tetanus vaccine in forms other than simple solution

68.—(1) When tetanus vaccine is prepared for administration in a form other than simple solution, the tetanus vaccine from which such a form is prepared shall be submitted to the tests for sterility and for absence of specific toxicity referred to or prescribed in paragraphs (*a*) and (*b*) of the last foregoing regulation.

(2) Such a form, after precipitation or other process used for its final preparation, shall again be subjected to the said sterility tests with such modifications as the nature of the product may require to make the test effective.

(3) Such a form, after the precipitation or other process used for its final preparation, shall be subjected to the tests for absence of specific toxicity

and for potency as an immunising antigen prescribed in paragraphs (*b*) and (*c*) of the last foregoing regulation, with the modification that the dose injected in the test for absence of specific toxicity shall be the dose or maximum dose stated on the label and in the test for potency as an immunising antigen, when a single dose is administered, shall be the dose or minimum dose stated on the label.

Labelling

69. In addition to the particulars required by regulation 6(3) of these regulations, the particulars specified in regulation 6(1)(*g*) and (*h*) of the general regulations shall be borne either by the container of tetanus vaccine or by the package in which it is offered for sale.

PART XVII

SPECIAL PROVISIONS RELATING TO OLD TUBERCULIN

Definition and proper name

70. Old tuberculin is the concentrated filtrate from the growth of *Mycobacterium tuberculosis* in a suitable nutrient broth. For its preparation the bacillus shall be grown at approximately 37°C. for a period, usually not less than 6 weeks, sufficient to allow the surface of the fluid medium to become covered by a thick growth of the bacillus. At the end of this period the fluid medium, from which the bacilli may or may not have been previously separated by filtration, shall be concentrated by evaporation to $\frac{1}{10}$ of its original volume, and then filtered. If the required test for potency shows that the preparation so concentrated is more potent than the standard preparation, the potency may be reduced to the required degree by appropriate dilution. If the test shows that the potency is less than that of the standard preparation it shall not be increased by further evaporation. The proper name of the preparation is "old tuberculin" with or without a suffix such as T., or P.T. The suffix T. will indicate that a strain of *Mycobacterium tuberculosis* var. *hominis* was used in preparing the tuberculin, and the suffix P.T. that a strain of *Mycobacterium tuberculosis* var. *bovis* was used.

Standard preparation

71. The standard preparation of old tuberculin is one kept at the National Institute for Medical Research, London.

Unit of standardisation

72. The unit of old tuberculin for the purposes of this part of these regulations is the specific biological activity contained in such an amount of the standard preparation as the Medical Research Council may from time to time indicate as exactly equivalent to the unit accepted for international use.

Tests for potency

73.—(1) Each batch of old tuberculin shall be tested for potency in comparison with the standard preparation by a method approved by the licensing authority.

(2) Old tuberculin shall contain 100,000 units per millilitre.

Test for non-specific toxicity

74. Each batch of old tuberculin shall be tested for the absence of non-specific toxicity by the subcutaneous injection of 0·5 millilitre into a

guinea-pig weighing not more than 350 grammes and shall pass the test if the injection does not produce any serious effects within 4 days.

Labelling

75.—(1) In addition to the particulars required by regulation 6(1) and (2) and regulation 14 of these regulations, the following particulars shall be borne by the container of old tuberculin: —

(*a*) the particulars specified in regulation 6(1)(*d*) of the general regulations;

(*b*) the number of international units of old tuberculin per millilitre of the preparation.

(2) In addition to the particulars required by regulation 6(3) of these regulations, the particulars specified in sub-paragraphs (*g*) and (*h*) of regulation 6(1) of the general regulations shall be borne either by the container of old tuberculin or by the package in which it is offered for sale.

PART XVIII

PROVISIONS RELATING TO PURIFIED PROTEIN DERIVATIVE OF TUBERCULIN

Definition and proper name

76. Purified protein derivative of tuberculin is prepared by fractional precipitation with ammonium sulphate, trichloroacetic acid or other suitable protein precipitant of a fluid medium on which *Mycobacterium tuberculosis* var. *hominis* has been grown, and from which the bacilli have been removed by filtration. The proper name of the substance is " tuberculin P.P.D.".

Test for potency

77.—(1) Each batch of tuberculin P.P.D. shall be tested for potency in comparison with the standard preparation of old tuberculin by a method approved by the licensing authority.

(2) Tuberculin P.P.D. shall not be issued if it contains less than 40,000 units of old tuberculin per milligram.

Test for non-specific toxicity

78. Each batch of tuberculin P.P.D. shall be tested for the absence of non-specific toxicity by the subcutaneous injection of 0·5 millilitre of a solution containing the equivalent of 100,000 units of old tuberculin per millilitre into a guinea-pig weighing not more than 350 grammes, and shall pass the test if the injection does not produce any serious effects within 4 days.

Labelling

79.—(1) In addition to the particulars required by regulation 6(1) and (2) and regulation 14 of these regulations, the following particulars shall be borne by the container of tuberculin P.P.D.: —

(*a*) the particulars specified in regulation 6(1)(*d*) of the general regulations;

(*b*) the potency in units of old tuberculin per millilitre in the case of liquid preparations, the number of such units per tablet in the case of tablets, and the number of such units per milligram in the case of dried or solid preparations.

(2) In addition to the particulars required by regulation 6(3) of these regulations, the particulars specified in sub-paragraphs (*g*) and (*h*) of regulation 6(1) of the general regulations shall be borne either by the container of tuberculin P.P.D. or by the package in which it is offered for sale.

PART XIX

PROVISIONS RELATING TO POLIOMYELITIS VACCINE (INACTIVATED)

Definition and proper name

80. This part of these regulations applies to any inactivated vaccine prepared from the poliomyelitis virus (types 1, 2 and 3). The proper name of any such vaccine is "poliomyelitis vaccine (inactivated)".

Strains of poliomyelitis virus

81. The strains of poliomyelitis virus used for preparation of poliomyelitis vaccine (inactivated) shall be such as shall be approved by the licensing authority.

Staff of establishment

82. The establishment in which poliomyelitis vaccine (inactivated) is prepared shall be under the complete direction and control of an expert approved by the licensing authority.

Production records

83. The licensee shall forward to the licensing authority protocols of all batches of vaccine, including batches the manufacture of which the licensee does not complete, whether the batch passes or fails any of the tests laid down in the subsequent provisions of this part of these regulations.

Provisions as to tissue for production of poliomyelitis virus

84.—(1) The tissue used for the production of poliomyelitis virus shall be the kidney tissue of healthy cynomolgus or rhesus monkeys or of monkeys of other suitable species.

(2) The kidney of a monkey shall not be used for this purpose—

(*a*) if the monkey has previously been used for any experimental purpose; or

(*b*) if on post-mortem examination under the supervision of a qualified pathologist any significant gross pathological lesion is revealed:

Provided that if the only experimental purpose for which a monkey has been used is the tests required by regulation 91 of these regulations its kidney may be used if since the beginning of those tests it has shown no clinical abnormality.

Method of producing virus suspension

85.—(1) Poliomyelitis virus for preparing poliomyelitis vaccine (inactivated) shall be grown with aseptic techniques in tissue cultures containing—

(*a*) a suitable nutrient fluid;

(*b*) viable cells, trypsinised or non-trypsinised, from the kidneys of monkeys;

(*c*) suitable antibiotics in the smallest effective concentration; and

(*d*) if the licensee desires, phenol red in a concentration not exceeding 0·002 per cent.:

Provided that if penicillin or streptomycin is used as the antibiotic not more than 200 units per millilitre shall be used.

(2) No extraneous protein which is capable of producing allergic effects on injection into human subjects (such as embryonic extracts which have not been subjected to ultrafiltration, and animal serum) shall be added to the tissue culture prepared as aforesaid, and if animal serum is used at any

stage in the manufacture, it shall not be used in such quantity that the final medium will contain more than one part of animal serum in one million.

(3) The virus suspension shall be filtered at suitable intervals by a suitable method.

(4) The total volume of the virus suspension shall not exceed such volume as may for the time being be approved by the licensing authority.

(5) After harvesting but before filtration the virus suspension shall be tested by suitable methods for the presence of B virus, *Mycobacterium tuberculosis* and L.C.M. virus.

Tests of virus suspension

86. After filtration the virus suspension shall be tested by a suitable method to determine the minimum infective dose for tissue culture and it shall not be used in the next stage of the preparation of poliomyelitis vaccine (inactivated) unless it contains at least $10^{7\cdot5}$, $10^{7\cdot0}$ or $10^{7\cdot5}$ such doses per millilitre for types 1, 2 and 3 respectively.

Method of inactivating virus suspension

87.—(1) All virus infectivity in the virus suspension shall be destroyed by a suitable method, by the use of formaldehyde or by some other means approved by the licensing authority.

(2) The course of the inactivation procedure shall be checked from time to time by suitable methods, and the minimum periods of inactivation shall be estimated from the results of these checks.

Tests for inactivation

88.—(1) When the minimum period of inactivation has been estimated in relation to a virus suspension, a sample of 500 millilitres of the suspension shall be withdrawn 3 days before the end of that period, and a further similar sample shall be withdrawn at the end of that period. Each sample shall be tested by suitable methods for the presence of active virus.

(2) The suspension shall be regarded as inactivated if neither sample is shown to contain active virus. If the sample withdrawn at the end of the period of inactivation does not, but the sample withdrawn 3 days before the end of that period does, contain active virus the suspension may be subjected to inactivation in accordance with the last preceding paragraph for a further 3 days. The suspension shall be regarded as inactivated if at the end of that period a further sample of 500 millilitres, withdrawn and tested in the same way as the other samples, contains no active virus.

Preparation of vaccine

89. Poliomyelitis vaccine (inactivated) shall be made by mixing inactivated suspensions of poliomyelitis virus of types 1, 2 and 3 in suitable proportions.

Tests for safety of vaccine

90.—(1) Before distributing the vaccine into containers and before the addition of a bacteriostatic a sample of at least 1,500 millilitres of the vaccine shall be tested by suitable methods for the presence of active poliomyelitis virus and may also be tested for the presence of other virus, including simian viruses. If the licensing authority so requires, a similar sample shall be made available to the licensing authority.

(2) After the vaccine has been distributed into containers, a sample from the containers shall also be tested for the presence of active poliomyelitis virus by suitable methods by inoculation into suitable monkeys. If the

vaccine has not previously been tested for the presence of other virus, including simian viruses, the sample shall be tested for the presence of any such virus if the licensing authority so require.

(3) The vaccine shall also be tested for abnormal toxicity by inoculation of mice and guinea-pigs, using methods approved by the licensing authority.

Tests for antigenicity of vaccine

91. After the filling of the containers a sample of the vaccine shall be tested in 12 healthy rhesus monkeys, each weighing between 2 and 4 kilogrammes. Each monkey shall receive the poliomyelitis vaccine (inactivated) by intramuscular injections in 3 doses, each of one millilitre with an interval of 7 days between the doses. The neutralising antibody to each of the 3 poliomyelitis virus types present in the serum of each monkey shall be determined in sera withdrawn not more than 2 weeks after the third injection by a method approved by the licensing authority. If the geometric mean of the antibody contents so determined to any type is less than 0·5 unit per millilitre the batch shall be rejected as insufficiently potent and shall not be issued. The standard antisera for use in this test are kept at the National Institute for Medical Research, London.

Condition of issue

92. No batch of poliomyelitis vaccine (inactivated) shall be issued for sale until it has passed the tests contained in this part of these regulations.

Bacteriostatic

93. If poliomyelitis vaccine (inactivated) is used in multidose containers, the bacteriostatic added shall be one which, at the concentration used, prevents the growth of micro-organisms and does not cause any loss of potency of the vaccine before the date after which the vaccine may not be used.

Labelling

94. In addition to the particulars required by regulation 6(3) of these regulations, a statement of the nature and maximum concentration of any antibiotics used in preparing the vaccine shall be borne either by the container of poliomyelitis vaccine or by the package in which it is offered for sale.

Method of issue

95. Poliomyelitis vaccine shall not be issued for sale combined with any other material without the consent of the licensing authority.

PART XX

PROVISIONS RELATING TO POLIOMYELITIS VACCINE (ORAL)

Definition

96. Poliomyelitis vaccine (oral) is a preparation of live, attenuated poliomyelitis virus (types 1, 2 and 3) containing any one of the three types or a combination of them. The proper name of any such preparation is "poliomyelitis vaccine (oral)" followed by words descriptive of the type or types of poliomyelitis virus contained in the preparation.

Strains of poliomyelitis virus

97.—(1) The strains of poliomyelitis virus used for the production of poliomyelitis vaccine (oral) shall be such as shall be approved by the licensing authority.

(2) Production of the vaccine shall be based on a seed virus system and the final vaccine shall not represent more than 5 subcultures from the strain culture on which the original laboratory and clinical tests were made.

(3) The working seed lot of each strain shall conform to such standards of purity that if tested in accordance with regulations 101, 102 and 103 of these regulations it would pass the tests, and it shall not differ significantly in respect of neurovirulence and other characteristics (e.g. genetic markers) from the strain culture on which the original laboratory and clinical tests were made.

Buildings and staff

98. Poliomyelitis vaccine (oral) shall be produced in completely self-contained premises, to which only members of the staff engaged in its production and testing shall have access. The establishment shall be under the complete control and direction of an expert approved by the licensing authority.

Condition of monkeys

99. Only healthy monkeys of suitable species shall be used as the source of kidney tissue for the production of poliomyelitis vaccine (oral) and no monkey which has been used previously for experimental purposes shall be used except with the approval of the licensing authority. The monkeys shall be kept in well constructed and ventilated animal rooms under conditions approved by the licensing authority. They shall be quarantined for an adequate period before being used for the production of vaccine. When the monkeys are killed, they shall be examined thoroughly by a qualified person experienced in the diagnosis of disease in monkeys, particularly for evidence of tuberculosis and B virus infection.

Method of producing virus suspension

100.—(1) Virus for the preparation of poliomyelitis vaccine (oral) shall be grown by aseptic methods in monkey kidney cell cultures, using a suitable maintenance medium containing no serum. The kidneys from each monkey shall be processed separately.

(2) During the course of virus propagation the cell culture vessels shall at no time be at a temperature above 35°C.

(3) Virus suspensions shall be harvested not later than 4 days after virus inoculation or at such time as the licensing authority may approve.

Testing of virus suspensions

101.—(1) Samples of the virus suspension shall be taken immediately on harvesting, and shall be subjected to the tests described in paragraph (3) of this regulation. Between the time of harvesting and the time when they are tested they shall be kept under conditions which will preserve the agents which the tests are designed to detect.

(2) During testing the virus suspensions harvested from the cultures prepared from the kidneys of each monkey shall be kept separate.

(3) The samples shall be tested as follows:—

(*a*) *Identification:* The poliomyelitis virus type shall be identified by a suitable serological test.

(*b*) *Bacterial and mycotic sterility:* Tests shall be made for bacterial and mycotic sterility by suitable methods.

(*c*) *Presence of B virus:* Tests shall be made for the presence of B virus by the inoculation of rabbits or rabbit kidney cell cultures by a method approved by the licensing authority. If the presence of B virus is shown by this test, the manufacture of the vaccine shall be discontinued and shall be resumed only when a thorough investigation has been completed and the necessary precautions have been taken to the satisfaction of the licensing authority.

(*d*) *Presence of extraneous viruses:* Tests shall be made by suitable methods for the presence of extraneous viruses after neutralization of the poliomyelitis virus by specific antisera, which shall be prepared in animals other than monkeys, using antigens grown in a non-simian cell line. The tests shall be made in monkey kidney cell cultures prepared from the same species, but not from the same animal, as that used for vaccine production, and in such other cell cultures as the licensing authority may from time to time specify.

(4) In addition to the tests described in the last preceding paragraph, the virus suspensions may be subjected to the following tests:—

(*a*) *Presence of extraneous viruses:* Tests for the presence of extraneous viruses other than B virus may be made by the inoculation of adult mice, new-born mice and guinea-pigs by methods approved by the licensing authority.

(*b*) *Presence of Mycobacterium tuberculosis:* Tests may be made for the presence of *Mycobacterium tuberculosis* by the inoculation of guinea-pigs and of a suitable culture medium by methods approved by the licensing authority.

Preparation and testing of bulk virus suspensions

102.—(1) The virus suspensions referred to in the last preceding regulation shall be pooled to form a bulk virus suspension. Samples of the bulk virus suspension shall be taken immediately on pooling and between the time of taking and the time when they are tested they shall be kept under conditions suitable for the preservation of the agents which the tests are designed to detect.

(2) The samples shall be tested for the presence of B virus by the inoculation of rabbits by methods approved by the licensing authority. If the presence of B virus is shown by this test, the manufacture of the vaccine shall be discontinued and shall be resumed only when a thorough investigation has been completed and the necessary precautions have been taken, to the satisfaction of the licensing authority.

(3) If the virus suspensions have not been tested in accordance with regulation 101(4) of these regulations, the samples of the bulk virus suspension shall be subjected to the tests described in that paragraph.

Filtration of the bulk virus suspension and testing

103.—(1) Bulk virus suspensions shall be filtered through a bacteria-retaining filter.

(2) Samples of the filtered virus suspension shall be taken immediately after filtering, and shall be subjected to the tests described in paragraph (3) of this regulation. Until the time when they are tested they shall be kept frozen.

(3) The samples shall be tested as follows:—

(*a*) *Identification:* The poliomyelitis virus type shall be identified by a suitable serological test.

(*b*) *Virus concentration:* The concentration of live virus shall be determined by titration using a method and a cell line approved by the licensing authority.

(*c*) *Genetic markers:* Tests for genetic markers shall be made, when the licensing authority so require, in parallel with the seed virus used in preparing the vaccine.

(*d*) *Neurovirulence:* The bulk virus suspension shall be tested for neurovirulence in monkeys by methods approved by the licensing authority, in parallel with the seed virus issued in preparing the vaccine.

Further processing of bulk virus suspensions

104. Further processing, such as diluting, blending of types and filling of final containers, shall be performed under conditions approved by the licensing authority.

Tests of the product in the form in which it is to be distributed for use

105.—(1) Each batch of vaccine shall be subjected to the tests described in paragraph (3) of this regulation. The tests shall be carried out on the vaccine in the form in which it is to be distributed for use, or at an earlier stage of preparation if the licensing authority so allow.

(2) For the purposes of this regulation a batch of vaccine is that quantity which is filled out into final containers in one continuous operation.

(3) The batches shall be tested as follows:—

(*a*) *Identification:* The poliomyelitis virus type or types shall be identified by suitable serological tests.

(*b*) *Virus concentration:* The concentration of live virus shall be determined by titration.

(*c*) *Sterility:* Tests for bacterial and mycotic sterility shall be made by methods approved by the licensing authority.

(*d*) *B virus:* At least 100 doses shall be tested for the presence of B virus by the inoculation of not less than 5 rabbits.

(*e*) *Toxicity:* Tests for toxicity shall be made in guinea-pigs and mice by methods approved by the licensing authority.

Information and samples required by the licensing authority

106. Protocols of production and testing of each batch of vaccine shall be submitted to the licensing authority. Information shall also be submitted concerning the results of tests made on all monkey kidney cell cultures and virus suspensions intended for the preparation of poliomyelitis vaccine (oral), whether or not the cultures or the virus suspensions were in fact used in preparing a finished batch. The licensing authority may require a manufacturer to submit samples taken at any stage in the preparation of the vaccine.

Storage by manufacturer

107. Poliomyelitis vaccine (oral) shall be stored by the manufacturer at a temperature of not more than −20°C.

Conditions of issue

108.—(1) No batch of poliomyelitis vaccine (oral) shall be issued for sale until it has passed the tests prescribed by these regulations, and has been shown:—

(*a*) to be free from extraneous micro-organisms;

(*b*) not to differ significantly in respect of neurovirulence for monkeys and other characteristics from the strain culture on which the original laboratory and clinical tests were made.

(2) Poliomyelitis vaccine (oral) shall not be issued more than 2 years after the test described in regulation 105(3)(*b*) of these regulations has been completed.

Labelling

109.—(1) In addition to the particulars required by regulation 6 of these regulations the following particulars shall, subject to the provisions of paragraph (3) of this regulation, be borne by the container of poliomyelitis vaccine (oral): —

(*a*) a statement that the vaccine is a living poliomyelitis virus and is not intended for use by injection;

(*b*) the virus titre of each type of poliomyelitis virus included in the vaccine.

(2) In addition to the particulars mentioned in paragraph (1) of this regulation the following information shall be borne either by the container of poliomyelitis vaccine (oral) or by the package in which it is offered for sale, that is to say, information, in terms approved by the licensing authority, as to the ranges of temperature recommended for storage and the periods for which the vaccine may be expected to retain its potency if stored at such temperatures.

(3) Regulation 14 of these regulations shall not apply to poliomyelitis vaccine (oral) and, in its application to such vaccine, regulation 6(2) of these regulations shall have effect as if the reference to the particulars specified in regulation 6(1)(*f*) and (*i*) of the general regulations were deleted.

(4) In their application to poliomyelitis vaccine (oral) issued in single dose containers, paragraph (1) of this regulation and regulation 6(1) and (2) of these regulations shall have effect as if they provided that the particulars mentioned therein should be borne by the package in which the containers are offered for sale.

PART XXI

PROVISIONS RELATING TO PERTUSSIS VACCINE

Definition and proper name

110. Pertussis vaccine is a sterile suspension in isotonic solution of killed *Bordetella pertussis*. The proper name of the substance in the form of a simple suspension is "pertussis vaccine" and in any form other than that of a simple suspension is "pertussis vaccine" together with a phrase indicating the nature of the additional process to which it has been subjected, e.g. "pertussis vaccine, alum precipitated".

Standard preparations

111. The standard preparation of pertussis vaccine is a dried pertussis vaccine kept at the National Institute for Medical Research, London, and the standard preparation of opacity is a watery suspension of powdered glass kept at the National Institute for Medical Research.

Test for opacity

112. Pertussis vaccine in simple suspension, and pertussis vaccine used for the preparation of any form other than a simple solution, diluted if necessary so that one human dose is contained in 1 millilitre, shall when

diluted with an equal volume of saline solution have an opacity which is not greater than that of the standard preparation of opacity.

Test for potency as an immunizing agent

113. Pertussis vaccine shall be tested by a method approved by the licensing authority in parallel with the standard preparation of pertussis vaccine for its power of protecting mice against an intracerebral challenge injection with a suitable strain of *Bordetella pertussis*. The pertussis vaccine shall be accepted as sufficiently potent if the test shows that, when used at the same concentration (organisms per millilitre) as the standard preparation, its potency is at least equal to that of the standard preparation.

Test for non-specific toxicity

114. The vaccine shall be tested for non-specific toxicity by the intraperitoneal inoculation of mice and the subcutaneous inoculation of guinea-pigs by methods approved by the licensing authority.

PART XXII

PROVISIONS RELATING TO INFLUENZA VIRUS VACCINE (INACTIVATED)

Definition and proper name

115. Influenza virus vaccine (inactivated) is a sterile suspension in isotonic solution of influenza virus, inactivated so that it is non-infective but retains its antigenic properties. The proper name of the substance is " influenza virus vaccine (inactivated) ".

Strains of influenza virus

116. The strains of influenza virus used for the preparation of influenza virus vaccine (inactivated) shall be such as shall be approved by the licensing authority.

Staff of establishment

117. The establishment in which influenza virus vaccine (inactivated) is prepared shall be under the complete direction and control of an expert approved by the licensing authority.

Preparation of vaccine

118.—(1) Influenza virus for preparing influenza virus vaccine (inactivated) shall be grown in the allantoic cavity of fertile chick embryos which are 10 to 13 days old and the allantoic fluid shall be harvested after 2 to 3 days' incubation at 33°C. to 37°C.

(2) All virus activity in the pooled bacteriologically sterile fluids shall be destroyed by the use of formaldehyde or by some other means approved by the licensing authority.

(3) The virus shall then be purified by high-speed centrifugation or by other suitable means and suspended in neutral isotonic buffered salt solution to which thiomersal or any other suitable bactericide has been added.

(4) Vaccine containing more than one strain of influenza virus shall be made by mixing purified inactivated suspensions of each strain in proportions approved by the licensing authority.

Test for inactivation

119. The vaccine shall be tested for the presence of active virus. Each of at least 6 fertile eggs which are 10 to 13 days old shall be inoculated by the allantoic route with 0·1 millilitre of vaccine. The suspension shall be regarded as inactivated if at the end of 3 days' incubation at 33°C. to 37°C. no growth of virus has occurred in any of the eggs.

Test for haemagglutinating activity

120. The vaccine shall be tested for its haemagglutinating activity. Serial two fold dilutions of the vaccine shall be prepared in physiological saline buffered at neutral pH, and to 0·25 millilitre of each dilution an equal volume of 0·5 per cent. fowl red blood cells shall be added; pooled cells from 3 or more suitable fowls shall be used. The mixtures shall be held for one hour at 20°C. to 25°C. and the end point of the titration shall be taken as the dilution of vaccine producing 50 per cent. haemagglutination. The vaccine shall be accepted as satisfactory if the end point of the titration is produced by vaccine diluted initially at least 1/2500 but no more than 1/5000, by the pattern test. Other methods of measuring the haemagglutinating activity may be used if approved by the licensing authority.

Tests for non-specific toxicity

121. The vaccine shall be tested for non-specific toxicity by the inoculation of mice and guinea-pigs by methods approved by the licensing authority.

Labelling

122. In addition to the particulars required by regulation 6(1) and (2) of these regulations, a statement of the strain or strains used for the preparation of the vaccine shall be borne by the container of influenza virus vaccine (inactivated).

These regulations were made by the aforesaid Joint Committee on 23rd August 1963.

J. Enoch Powell,
Michael Noble,
Wm. J. Morgan,
Members of the Joint Committee.

B. H. Betts,
Clerk to the Joint Committee.

EXPLANATORY NOTE

(This Note is not part of the Regulations, but is intended to indicate their general purport.)

These regulations, made under Part I of the Therapeutic Substances Act 1956 form part of a comprehensive code relating to the manufacture and importation of therapeutic substances and are to be read in conjunction with the Therapeutic Substances (Manufacture and Importation) General Regulations 1963.

These regulations consolidate with minor amendments (except those relating to B.C.G. vaccine, smallpox vaccine and poliomyelitis vaccine (inactivated), which have been substantially revised, and poliomyelitis vaccine (oral), pertussis vaccine and influenza virus vaccine (inactivated), which are additional provisions) those requirements of the Therapeutic Substances Regulations 1952 to 1957 which apply to vaccines, toxins and antigens and in particular provide for:—

(*a*) tests appropriate to the particular substance;
(*b*) standards of quality;
(*c*) requirements as to labelling;
(*d*) precautions to be observed in preparation and testing; and
(*e*) requirements as to staff, buildings and equipment and as to the condition of any animals used in the preparation of the substance.