1971 No. 118

THERAPEUTIC SUBSTANCES

The Therapeutic Substances (Manufacture of Vaccines, Toxins and Antigens) Amendment Regulations 1971

Made - - - -	22*nd January* 1971
Laid before Parliament	2*nd February* 1971
Coming into Operation	1*st March* 1971

The Joint Committee constituted by section 4(1) of the Therapeutic Substances Act 1956**(a)**, as amended by article 2(2) of and Schedule 1 to the Transfer of Functions (Wales) Order 1969**(b)**, in exercise of the powers conferred on them by sections 1 and 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:—

Citation, commencement and interpretation

1.—(1) These regulations may be cited as the Therapeutic Substances (Manufacture of Vaccines, Toxins and Antigens) Amendment Regulations 1971 and shall come into operation on 1st March 1971.

(2) The Therapeutic Substances (Manufacture of Vaccines, Toxins and Antigens) Regulations 1963 to 1967**(c)** and these regulations may be cited together as the Therapeutic Substances (Manufacture of Vaccines, Toxins and Antigens) Regulations 1963 to 1971.

(3) The Interpretation Act 1889**(d)** shall apply to the interpretation of these regulations as it applies to the interpretation of an Act of Parliament.

2. The Therapeutic Substances (Manufacture of Vaccines, Toxins and Antigens) Regulations 1963, as amended **(e)**, shall be further amended as follows:—

(*a*) part XX (which relates to Poliomyelitis Vaccine (Oral)) shall be deleted, and after regulation 95 there shall be inserted the following regulations—

"PART XX

PROVISIONS RELATING TO POLIOMYELITIS VACCINE (ORAL)

Definition and proper name

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

(a) 1956 c. 25. **(b)** S.I. 1969/388 (1969 I, p. 1070).
(c) S.I. 1963/1459, 1964/1434, 1965/1005, 1966/504, 1967/1196 (1963 II, p. 2561; 1964 III, p. 3324; 1965 I, p. 2454; 1966 I, p. 1046; 1967 II, p. 3502).
(d) 1889 c. 63.
(e) The relevant amending instruments are S.I. 1964/1434, 1965/1005, 1966/504, 1967/1196 (1964 III, p. 3324; 1965 I, p. 2454; 1966 I, p. 1046; 1967 II, p. 3502).

Buildings and Staff

97. Poliomyelitis vaccine (oral) shall be produced in a separate building or separate parts of a building to which, during production, only persons engaged in its production and testing have access. Procedures which potentially involve the presence of micro-organisms or the use of tissue culture cells other than those cell cultures in which it is proposed to produce the vaccine, shall not be carried out in the said separate building or parts of a building. If persons engaged in the production of the vaccine have been working with other infectious agents or with experimental animals, they shall not on the same day enter the areas where the vaccine is produced. The establishment for the production of the vaccine shall be under the complete control and direction of an expert in microbiology approved by the licensing authority.

Strains of poliomyelitis virus

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

(2) Production of the vaccine shall be based on a virus seed system and the final vaccine shall not contain any virus which is more than 3 subcultures from the strain culture on which the original laboratory and clinical tests were made, if that strain culture is type 1 or type 2, or more than 2 subcultures from the strain culture if it is type 3.

(3) The working seed lot of each strain used shall conform to the same standards of purity as are required by these regulations to be applied to the final vaccine.

Information and samples required by the licensing authority

99. Protocols made and samples taken during stages 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 cell cultures and virus suspensions intended for the preparation of poliomyelitis vaccine (oral) whether or not the cultures or the virus suspensions were used in preparing a finished batch.

Storage by manufacturer

100. Poliomyelitis vaccine (oral) during storage by the manufacturer shall be kept at a temperature not higher than −20°C.

Labelling

101.—(1) In addition to the particulars required by regulation 6 of these regulations the following particulars shall be borne by the container of poliomyelitis vaccine (oral) or of preparations thereof:—

(*a*) a statement that the vaccine shall not be administered by injection;

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

(*c*) a statement identifying the tissue used for preparing the cell cultures in which the poliomyelitis virus was grown.

(2) The label of the container and the package of the vaccine shall bear the particulars required by regulations 6(1)(*g*) and (*h*) of the general regulations.

PART XXA

SPECIAL PROVISIONS RELATING TO POLIOMYELITIS VACCINE (ORAL) WHEN PREPARED IN MONKEY KIDNEY CELL CULTURES

Cells for vaccine production

102.—(1) Monkey kidney cell cultures used for the production of poliomyelitis vaccine (oral) shall be derived from a healthy stock of monkeys of suitable species free from *Mycobacterium tuberculosis*, B virus and from other adventitious pathogenic agents. Kidney tissue shall not be taken from a monkey that has been used previously for experimental purposes except with the approval of the licensing authority. The monkeys shall be kept in well constructed and ventilated animal rooms and shall be quarantined for an adequate period before being used. After they 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.

(2) If animal serum is added to initiate the cultures then, before virus propagation, the cultures shall be washed free from such serum. No extraneous protein shall be added to the tissue cultures during the course of virus propagation.

Testing of cell cultures

103.—(1) Twenty-five per cent of each batch of cell cultures intended for use for the propagation of virus shall be held as uninoculated control cultures and shall be incubated at the same temperature as the inoculated cultures. They shall be observed for 14 days from the time the production cultures are inoculated with virus, and shall show no evidence of the presence of adventitious agents. Not more than 20 per cent of the control cultures shall be discarded for accidental causes. At 7 and 14 days beyond the time of inoculation of the production cultures, two per cent of the fluids from the control cultures shall be inoculated into human, monkey and rabbit cell cultures, and the said cultures shall be incubated at the same temperature as the initial inoculated cultures and observed for 14 days during which time they shall show no evidence of the presence of adventitious agents.

(2) At the time of inoculation with virus all tissue cultures shall be examined for evidence of degeneration and if any show abnormal cytopathic change the whole batch concerned shall be discarded. The fluid removed from each culture bottle before inoculation with virus shall be tested for freedom from bacterial and mycotic contamination. The fluids from these cultures shall also be examined for freedom from adventitious agents by inoculating at least two per cent of the pooled cell culture fluid into a rabbit cell culture system and a further two per cent into human amnion cell cultures. Human amnion cell cultures may be substituted by any culture system shown to be as sensitive to measles virus as human amnion cell cultures. At the same time a further two per cent of pooled cell culture fluids shall be tested for freedom from adventitious agents on monkey tissue prepared from the same species but not the same animal as that used for the vaccine production. The cultures shall be maintained and observed over a period of at least 14 days. At the end of periods of 7 days and 14 days, sub-cultures shall be made from each of the inoculated tissues and examined over a further period of 14 days. No cultures shall show evidence of the presence of adventitious agents.

(3) At the time of virus harvest at least four per cent of the control cell cultures shall be examined for haemadsorption viruses using guinea-pig red

blood cells. Any remaining control tissue cultures shall be tested for haemadsorption viruses at the end of the observation period. No cultures shall show evidence of the presence of haemadsorption viruses.

Production of virus suspensions

104.—(1) Virus for the preparation of poliomyelitis vaccine (oral) produced in monkey kidney cell cultures shall be grown by aseptic methods. The kidneys from each monkey shall be processed separately.

(2) During the course of virus propagation the cell culture vessels shall be incubated at a range of temperature which shall be approved by the licensing authority and which shall at no time rise 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

105.—(1) Virus suspensions harvested from the cultures prepared from the kidneys of each monkey shall be kept separately during testing.

(2) Samples of the virus suspensions shall be taken immediately on harvesting and before clarification and shall be subjected to the tests described in paragraph (3) of this regulation. Between the time of harvesting and the time when the samples are tested they shall be kept under conditions that will preserve the agents that the tests are designed to detect.

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

(*a*) for identification of the poliomyelitis virus type by a suitable serological test;

(*b*) for freedom from bacterial and mycotic contamination by methods approved by the licensing authority;

(*c*) for freedom from mycoplasmal contamination by tests made on solid and in liquid media that have been shown to be capable of growing mycoplasma;

(*d*) for freedom from 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 manufacturer shall inform the licensing authority and the manufacture of all poliomyelitis vaccine shall be discontinued and shall be resumed only when a thorough investigation has been completed and the precautions necessary to prevent further contamination with B virus have been taken to the satisfaction of the licensing authority;

(*e*) for freedom from extraneous viruses. The poliomyelitis virus shall be neutralised by specific antisera prepared in animals other than monkeys. Such antisera shall be prepared using antigens grown in a non-simian cell line. The tests shall be made in monkey kidney cell cultures and in such other cell cultures as the licensing authority so requires. The monkey kidney cell cultures shall be prepared from the same species, but not from the same animal as that used for vaccine production.

(4) The samples shall also be tested as follows:—

(*a*) for freedom from other extraneous viruses by the inoculation of adult mice, new-born mice and guinea-pigs by methods approved by the licensing authority;

(*b*) for freedom from *Mycobacterium tuberculosis* by methods approved by the licensing authority.

The tests mentioned in this paragraph may if the licensing authority so allows, be carried out instead on samples taken from the bulk virus suspensions.

Preparation and testing of bulk virus suspensions

106.—(1) Bulk virus suspension shall be obtained by the combination of suitable virus suspensions. For the purpose of this regulation a batch of such bulk virus suspension is the quantity present in a bulk container from which final containers are to be filled. Samples of each such batch shall be taken immediately on pooling and before clarification. Between the time of taking and the time when the samples are tested the samples shall be kept under conditions suitable for the preservation of the agents that the tests are designed to detect.

(2) Samples of the bulk virus suspension shall be tested for freedom from B virus by the inoculation of rabbits by a method approved by the licensing authority. If the presence of B virus is shown by this test, the manufacture of all poliomyelitis vaccine shall be discontinued and shall be resumed only when a thorough investigation has been completed and the precautions necessary to prevent further contamination with B virus have been taken to the satisfaction of the licensing authority.

(3) If the contributory virus suspensions have not been tested individually in accordance with regulation 105(4), the samples of the bulk virus suspension shall be so tested.

(4) Bulk virus suspension shall be clarified by a method ensuring the removal of all cells and significant cell debris.

(5) Samples of the clarified bulk virus suspension shall be taken immediately after clarification and shall be kept frozen until tested as follows:—

(*a*) for identification of the poliomyelitis virus type by a suitable serological test;

(*b*) for concentration of live virus by titration by a method approved by the licensing authority;

(*c*) for genetic markers, if the licensing authority so requires, by tests made in parallel with similar tests on the virus seed used in preparing the vaccine, by methods approved by the licensing authority;

(*d*) for neurovirulence in monkeys by tests made in parallel with the virus seed used in preparing the vaccine, by methods approved by the licensing authority;

(*e*) for freedom from toxicity, if the licensing authority so requires, by tests made in both mice and guinea-pigs by methods approved by the licensing authority.

Further processing of bulk virus suspensions

107. Further processing, such as dilution and blending of types to form final vaccine, shall be done under aseptic conditions.

Tests of the final vaccine

108.—(1) Each batch of the final 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 issued for use.

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

(3) Samples of each batch shall be tested as follows:—

(*a*) for identification of the poliomyelitis virus type or types by suitable serological tests;

(*b*) for the concentration of live virus by titration by a method approved by the licensing authority;

(*c*) for freedom from bacterial and mycotic contamination by methods approved by the licensing authority;

(*d*) for freedom from toxicity by tests made in both mice and guinea-pigs by methods approved by the licensing authority.

PART XXB

SPECIAL PROVISIONS RELATING TO POLIOMYELITIS VACCINE (ORAL) WHEN PREPARED IN HUMAN DIPLOID CELL CULTURES

Cells for vaccine production

109.—(1) Human diploid cell cultures used for the production of poliomyelitis vaccine (oral) shall satisfy the criteria for the acceptability of a passaged human diploid cell population as approved by the licensing authority.

(2) The production of the cell cultures shall be based on a cell seed culture system.

Testing of cell cultures

109A. The human diploid cell cultures used for vaccine production shall be tested for normal characteristics and for freedom from extraneous agents by methods approved by the licensing authority.

Production of virus suspensions

109B.—(1) During the course of virus propagation the cell culture vessels shall be incubated at a range of temperature which shall be approved by the licensing authority and which shall at no time rise above 35 °C.

(2) 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

109C.—(1) Virus suspensions harvested from the cultures derived from a single ampoule of the cell seed shall be kept separately during testing.

(2) Samples of the virus suspensions shall be taken immediately on harvesting and before clarification and shall be subjected to the tests described in paragraph (3) of this regulation. Between the time of harvesting and the time when the samples are tested the samples shall be kept under conditions that will preserve the agents that the tests are designed to detect.

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

(*a*) for identification of the poliomyelitis virus type by a suitable serological test;

(*b*) for freedom from bacterial and mycotic contamination by methods approved by the licensing authority;

(*c*) for freedom from mycoplasmal contamination by tests made on solid and in liquid media that have been shown to be capable of growing mycoplasma;

(*d*) for freedom from 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 manufacturer shall inform the licensing authority and the manufacture of all poliomyelitis vaccine shall be discontinued and shall be resumed only when a thorough investigation has been completed and the precautions necessary to prevent further contamination with B virus have been taken to the satisfaction of the licensing authority;

(*e*) for freedom from extraneous viruses. The poliomyelitis virus shall be neutralised by specific antisera prepared in animals other than humans or monkeys. Such antisera shall be prepared using antigens grown in human cells obtained from a different anatomical source from the cells used for vaccine production. At least 5 ml of each virus suspension, but not less than a total of 50 ml or 500 doses, whichever is the greater, from all the virus suspensions forming a single final batch of vaccine shall be tested in monkey kidney cell cultures. The cell cultures shall be examined for evidence of degeneration twice weekly for at least 14 days. At seven days, and at the end of the observation period, two per cent of the fluid from each culture bottle shall be sub-cultured into monkey kidney and human amnion cell cultures. These sub-cultures shall be observed for at least 14 days. Human amnion cell cultures may be substituted by any culture system shown to be as sensitive to measles virus as human amnion cell cultures.

(4) The samples shall also be tested as follows:—

(*a*) for freedom from other extraneous viruses by the inoculation of adult mice, new-born mice and guinea-pigs by methods approved by the licensing authority;

(*b*) for freedom from *Mycobacterium tuberculosis* by methods approved by the licensing authority.

The tests mentioned in this paragraph may, if the licensing authority so allow, be carried out instead on samples taken from the bulk virus suspensions.

Preparation and testing of bulk virus suspensions

109D.—(1) Suitable virus suspensions from the cell cultures prepared from each ampoule of the cell seed shall be pooled to form a bulk virus suspension, the volume of which shall not be less than 10 litres. Samples of the bulk virus suspension thus obtained shall be taken immediately on pooling and before clarification. Between the time of taking the samples and the time when the samples are tested the samples shall be kept under conditions suitable for the preservation of the agents that the tests are designed to detect.

(2) If the contributory virus suspensions have not been tested individually in accordance with regulation 109C(4) the samples of the bulk virus suspension shall be so tested.

(3) Bulk virus suspension shall be clarified by a method ensuring the removal of all cells and significant cell debris.

(4) Samples of the clarified bulk virus suspension shall be taken immediately after clarification and shall be kept frozen until tested as follows:—

(*a*) for identification of the poliomyelitis virus type by a suitable serological test;

(*b*) for concentration of live virus by titration by a method approved by the licensing authority;

(*c*) for genetic markers, if the licensing authority so requires, by tests made in parallel with similar tests on the virus seed used in preparing the vaccine, by methods approved by the licensing authority;

(*d*) for neurovirulence in monkeys by tests made in parallel with the virus seed used in preparing the vaccine, by methods approved by the licensing authority;

(*e*) for freedom from toxicity, if the licensing authority so require, by tests made in both mice and guinea-pigs by methods approved by the licensing authority.

Further processing of bulk virus suspensions

109E. Further processing, such as dilution and blending of types to form final vaccine, shall be done under aseptic conditions.

Tests of the final vaccine

109F.—(1) Each batch of the final 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 issued for use.

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

(3) Samples of each batch shall be tested as follows:—

(*a*) for identification of the poliomyelitis virus type or types by suitable serological tests;

(*b*) for the concentration of live virus by titration by a method approved by the licensing authority;

(*c*) for freedom from bacterial and mycotic contamination by methods approved by the licensing authority;

(*d*) for freedom from toxicity by tests made in both mice and in guinea-pigs by methods approved by the licensing authority.";

(*b*) After regulation 172 there shall be inserted the following regulations—

"PART XXX

PROVISIONS RELATING TO RUBELLA VACCINE (LIVE ATTENUATED)

Definition and proper name

173.—(1) Rubella vaccine (live attenuated) is a freeze-dried preparation of a live attenuated rubella virus. The proper name of any such preparation is "rubella vaccine (live attenuated)".

Buildings and staff

174. Rubella vaccine (live attenuated) shall be produced in a separate building or separate parts of a building to which, during production, only persons engaged in its production and testing have access. Procedures which potentially involve the presence of micro-organisms or the use of tissue culture cells other than those cell cultures in which it is proposed to produce the vaccine shall not be carried out in the said separate building or parts of a building. If persons engaged in the production of the vaccine have been working with other infectious agents or with experimental animals, they shall not on the same day enter the areas where the vaccine is produced. The establishment for the production of the vaccine shall be under the complete control and direction of an expert in microbiology approved by the licensing authority.

Preparation and testing of vaccine in final containers

175. Bulk virus suspension shall be distributed into final containers and dried from the frozen state. For the purpose of this regulation a batch of the vaccine in final containers is the total number of the filled final sealed containers prepared by freeze-drying in a single operation. Samples of each such batch shall be tested as follows:—

(*a*) for concentration of live attenuated rubella virus by titration in comparison with a reference preparation of rubella virus kept as a standard preparation at the National Institute for Medical Research (Hampstead Laboratories) London;

(*b*) for freedom from bacterial and mycotic contamination by methods approved by the licensing authority;

(*c*) for freedom from toxicity by tests made in both mice and guinea-pigs by methods approved by the licensing authority.

Information and samples required by the licensing authority

176. Protocols made and samples taken during stages of production and testing of each batch of vaccine shall be submitted to the licensing authority. Information shall be submitted concerning the results of tests made on all cell cultures and virus suspensions intended for the preparation of rubella vaccine (live attenuated) whether or not the cultures or the virus suspensions were used in preparing a finished batch.

Storage by Manufacturer

177. Rubella vaccine (live attenuated) during storage by the manufacturer shall be kept at a temperature not higher than −20°C.

Labelling

178.—(1) In addition to the particulars required by regulation 6 of these regulations the following particulars shall be borne by the container of rubella vaccine (live attenuated) or of preparations thereof:—

(*a*) a statement that the vaccine shall be reconstituted by using only the reconstituting fluid supplied by the manufacturers;

(*b*) a statement identifying the tissue used for preparing the cell cultures in which the rubella virus was grown;

(*c*) the strain type of the rubella virus included in the vaccine;

(*d*) the virus titre of the rubella virus included in the vaccine;

(*e*) a statement specifying the route of administration of the vaccine;

(*f*) a statement that the vaccine after reconstitution shall be used within the period of time approved by the licensing authority.

(2) the label of the container and the package of the vaccine shall bear the particulars required by regulations 6(1)(*g*) and (*h*) of the general regulations.

PART XXXA

SPECIAL PROVISIONS RELATING TO RUBELLA VACCINE (LIVE ATTENUATED) WHEN PREPARED IN RABBIT KIDNEY CELL CULTURES

Cells for vaccine production

179.—(1) Rabbit kidney cell cultures used for the production of rubella vaccine (live attenuated) shall be derived from a healthy stock of rabbits free from *Mycobacterium tuberculosis*, myxomatosis, fibromatosis and rabbit pox viruses and from other adventitious pathogenic agents.

(2) If animal serum is added to initiate the cultures then, before virus propagation, the cultures shall be washed free from such serum. No extraneous protein shall be added to the cell cultures during the course of virus propagation.

Strain of rubella virus

180.—(1) The strain of rubella virus used for the production of rubella vaccine (live attenuated) shall be approved by the licensing authority.

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

(3) The working seed lot of the strain used shall conform to the same standards of purity as are required by these regulations to be applied to the final vaccine and it shall be shown to be free from neurovirulence.

Testing of cell cultures

181.—(1) Ten per cent of each batch of cultures intended for use for the propagation of rubella virus shall be held as uninoculated control cultures and shall be incubated at the same temperature as the inoculated cultures. They shall be observed for the time rubella virus is grown in the production tissue, or for 14 days beyond the time of inoculation of production tissue with rubella virus, whichever is the longer, and shall show no evidence of virus contamination.

(2) At the time of virus harvest from the production cultures the fluids from the control tissue cultures shall be examined for freedom from adventitious agents by inoculating at least two per cent of pooled control tissue culture fluids into each of a human and a simian tissue culture system. At the same time a further two per cent of pooled control tissue culture fluids shall be tested for freedom from adventitious virus agents on rabbit tissue prepared from the same species but not the same animal as that used for the vaccine production. The tests shall be done by methods approved by the licensing authority.

(3) If a multiple harvest production system is used, samples from the control tissue cultures shall be taken about midway between the first and last virus harvests and used for the tests described under paragraph (2) of this regulation.

(4) At the end of the period for which the control tissue is observed the above tests shall be repeated using two per cent of the pooled control tissue culture fluids on each tissue used in testing. Subcultures shall be made from the inoculated tissues at 14 days, and each sub culture observed for a further 14 days.

(5) At the time of virus harvest at least ten per cent of the control tissue cultures shall be examined for haemadsorption viruses using guinea-pig red blood cells. Any remaining control tissue cultures shall be tested for haemadsorption viruses at the end of the observation period.

(6) The tissue cultures for virus propagation are satisfactory only if at least 80 per cent of the remaining control tissue cultures survive the observation period and none of the tests show evidence of virus contamination.

Production of virus suspensions

182. Virus for the preparation of rubella vaccine (live attenuated) produced in primary rabbit kidney cell cultures shall be grown by aseptic methods.

Testing of virus suspensions

183.—(1) Samples of the virus suspensions shall be taken immediately on harvesting and before clarification or the addition of stabiliser and shall be subjected to the tests described in paragraphs (3), (4) and (5) of this regulation. Between the time of harvesting and the time when the samples are tested the samples shall be kept under conditions that will preserve the agents that the tests are designed to detect.

(2) Where multiple harvests have been made they shall be pooled for the purpose of the tests described in paragraphs (3), (4) and (5) of this regulation.

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

(*a*) for identification of rubella virus by a suitable serological test;

(*b*) for freedom from bacterial and mycotic contamination by methods approved by the licensing authority;

(*c*) for freedom from mycoplasmal contamination by tests made on solid and in liquid media that have been shown to be capable of growing mycoplasma.

(4) For the purpose of the tests described in this paragraph the rubella virus in the samples shall be neutralised by specific antiserum prepared in animals other than humans, monkeys or rabbits and the samples shall be tested as follows:—

(*a*) for freedom from extraneous virus by the inoculation of at least 10 ml onto a suitable monkey kidney tissue culture known to be sensitive to SV40 virus. The culture shall be observed for at least 7 days and at the end of that period a sub culture shall be made from the inoculated tissue and observed for 7 days. A further sub culture shall then be made from this sub culture and observed for 7 days. Additional rubella virus antiserum prepared in animals other than humans, monkeys or rabbits may be added at the time of each sub-culture. At the end of each seven day observation period each sub-culture shall be challenged with a suitable virus to prove the absence of rubella virus;

(*b*) by repeating the test described under paragraph 4(*a*) of this regulation using a human tissue culture system instead of monkey kidney tissue;

(*c*) for freedom from other extraneous viruses by the inoculation of at least 5 ml in 0.5 ml quantities into each of the allantoic cavity and yolk sac in separate groups of embryonated eggs; and

(*d*) by the inoculation of at least 5 ml in 0.1 ml quantities onto the chorioallantoic membrane of embryonated eggs.

(5) The samples shall also be tested as follows:—

(*a*) for freedom from other extraneous viruses by the inoculation of adult mice, new-born mice, guinea-pigs and rabbits by methods approved by the licensing authority;

(*b*) for freedom from *Mycobacterium tuberculosis* by methods approved by the licensing authority.

These tests described in this paragraph may, if the licensing authority so allows, be carried out instead on samples taken from the bulk virus suspension.

Preparation and testing of bulk virus suspension

184.—(1) Bulk virus suspension shall be obtained by the combination of suitable virus suspensions. For the purpose of this regulation a batch of such bulk virus suspension is the quantity present in a bulk container from which final containers are to be filled. Samples of each such batch shall be taken immediately on pooling and before clarification or addition of stabiliser. Between the time of taking and the time when the samples are tested the samples shall be kept under conditions suitable for the preservation of the agents that the tests are designed to detect.

(2) If the contributory virus suspensions have not been tested individually in accordance with regulation 183(5) the samples of the bulk virus suspension shall be so tested.

(3) Bulk virus suspension shall be clarified by a method ensuring the removal of all cells and significant cell debris.

(4) Any stabiliser or nutrient added at any stage in the production shall be approved by the licensing authority.

PART XXXB

SPECIAL PROVISIONS RELATING TO RUBELLA VACCINE (LIVE ATTENUATED) WHEN PREPARED IN HUMAN DIPLOID CELL CULTURES

Cells for vaccine production

185.—(1) Human diploid cell cultures used for the production of rubella vaccine (live attenuated) shall satisfy the criteria for the acceptability of a passaged human diploid cell population as approved by the licensing authority.

(2) The production of the cell cultures shall be based on a cell seed culture system.

Strain of rubella virus

186.—(1) The strain of rubella virus used for the production of rubella vaccine (live attenuated) shall be approved by the licensing authority.

(2) Production of the vaccine shall be based on a virus seed system and the final vaccine shall not contain any virus which is more than 10 subcultures from the strain culture on which the original laboratory and clinical tests were made.

(3) The working seed lot of the strain used shall conform to the same standards of purity as are required by these regulations to be applied to the final vaccine, and it shall be shown to be free from neurovirulence.

Testing of cell cultures

187. The human diploid cell cultures used for vaccine production shall be tested for normal characteristics and for freedom from extraneous agents by methods approved by the licensing authority.

Production of virus suspensions

188.—(1) During the course of virus propagation the cell culture vessels shall be incubated at a range of temperature which shall be approved by the licensing authority.

(2) Virus suspensions shall be harvested not later than 28 days after virus inoculation or at such time as the licensing authority may approve.

Testing of virus suspensions

189.—(1) Virus suspensions harvested from the cultures derived from a single ampoule of the cell seed shall be kept separately during testing.

(2) Samples of the virus suspensions shall be taken immediately on harvesting and before clarification or the addition of stabiliser and shall be subjected to the tests described in paragraphs (4), (5) and (6) of this regulation. Between the time of harvesting and the time when the samples are tested the samples shall be kept under conditions that will preserve the agents that the tests are designed to detect.

(3) Where multiple harvests have been made they shall be pooled for the purpose of the tests described in paragraphs (4), (5) and (6) of this regulation.

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

(*a*) for identification of rubella virus by a suitable serological test;

(*b*) for freedom from bacterial and mycotic contamination by methods approved by the licensing authority;

(*c*) for freedom from mycoplasmal contamination by tests made on solid and in liquid media that have been shown to be capable of growing mycoplasma.

(5) For the purpose of the tests described in this paragraph the rubella virus in the samples shall be neutralised by specific antiserum prepared in animals other than humans or monkeys and the samples shall be tested as follows:—

(*a*) for freedom from extraneous virus by the inoculation of at least 10 ml onto a suitable monkey kidney tissue culture known to be sensitive to SV40 virus. The cultures shall be observed for at least 7 days and at the end of that period a subculture shall be made from the inoculated tissue and observed for 7 days. A further subculture shall then be made from this subculture and observed for 7 days. Additional rubella virus antiserum prepared in animals other than humans and monkeys may be added at the time of each subculture. At the end of each 7 day observation period each subculture shall be challenged with a suitable virus to prove the absence of rubella virus;

(*b*) by repeating the test described under paragraph (5)(*a*) of this regulation using a human tissue culture system instead of monkey kidney tissue.

(6) The samples shall also be tested as follows:—

(*a*) for freedom from other extraneous viruses by the inoculation of adult mice, new-born mice and guinea-pigs by methods approved by the licensing authority;

(*b*) for freedom from *Mycobacterium tuberculosis* by methods approved by the licensing authority.

The tests described in this paragraph may, if the licensing authority so allows, be carried out instead on samples taken from the bulk virus suspension.

Preparation and testing of bulk virus suspension

190.—(1) Suitable virus suspensions from the cell cultures from each ampoule of the cell seed shall be pooled to form a bulk virus suspension, the volume of which shall not be less than 3 litres. Samples of the bulk virus suspension thus obtained shall be taken immediately on pooling and before clarification or the addition of stabiliser. Between the time of taking and the

time when the samples are tested the samples shall be kept under conditions suitable for the preservation of the agents that the tests are designed to detect.

(2) If the contributory virus suspensions have not been tested individually in accordance with regulation 189(6) the samples of the bulk virus suspension shall be so tested.

(3) Bulk virus suspensions shall be clarified by a method ensuring the removal of all cells and significant cell debris.

(4) Any stabiliser or nutrient added at any stage in the production shall be approved by the licensing authority.";

(*c*) In regulation 5 (which relates to tests on guinea-pigs) after the figure "XXIX" there shall be inserted the figures "XXA, XXB, XXXA and XXXB".

These regulations were made by the aforementioned Joint Committee on 22nd January 1971.

Keith Joseph,
Peter Thomas,
Gordon Campbell,
W. K. Fitzsimmons,
Members of the Joint Committee.

L. H. Hayward,
Clerk to the Joint Committee.

EXPLANATORY NOTE

(*This Note is not part of the Regulations.*)

These Regulations amend the provisions of the Therapeutic Substances (Manufacture of Vaccines, Toxins and Antigens) Regulations 1963 to 1967 by replacing Part XX (which made provisions for Poliomyelitis Vaccine (Oral)) by new special provisions and by extending the regulations to make special provisions for Rubella Vaccine (Live Attenuated).

SI 1971/ 118
ISBN 0-11-010118-9

