1965 No. 1005

THERAPEUTIC SUBSTANCES

The Therapeutic Substances (Manufacture of Vaccines, Toxins and Antigens) Amendment Regulations 1965

Made - - - -	15*th April* 1965
Laid before Parliament	27*th April* 1965
Coming into Operation	1*st May* 1965

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:—

Citation, Commencement and Interpretation

1.—(1) These regulations may be cited as the Therapeutic Substances (Manufacture of Vaccines, Toxins and Antigens) Amendment Regulations 1965 and shall come into operation on 1st May 1965.

(2) The Therapeutic Substances (Manufacture of Vaccines, Toxins and Antigens) Regulations 1963 and 1964(**b**) and these regulations may be cited together as the Therapeutic Substances (Manufacture of Vaccines, Toxins and Antigens) Regulations 1963 to 1965.

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

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

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

(*a*) In regulation 5 (which relates to tests on guinea-pigs) for "XXV" there shall be substituted "XXVIII";

(*b*) After regulation 131 there shall be added the following regulations—

"PART XXVI

PROVISIONS RELATING TO MEASLES VIRUS VACCINE (LIVE ATTENUATED)

Definition and proper name

132. Measles virus vaccine (live attenuated) is a preparation of a live attenuated measles virus. The proper name of any such preparation is "measles virus vaccine (live attenuated)".

Strains of measles virus

133.—(1) The strains of measles virus used for the production of measles virus vaccine (live attenuated) shall be such as shall be approved by the licensing authority.

(a) 4 & 5 Eliz. 2. c. 25. (b) S.I. 1963/1459, 1964/1434 (1963 II, p. 2561; 1964 III, p. 3324).
(c) 52 & 53 Vict. c. 63.

(2) Production of the vaccine shall be based on a seed virus system and the final vaccine shall not represent more than 10 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 the same standards of purity as are required by these regulations to be applied to the final vaccine, and in addition, it shall be shown, by inoculation into the thalamus of monkeys, to be free of neuro-tropic properties.

Buildings and staff

134. Measles virus vaccine (live attenuated) shall be produced in completely self-contained premises, to which only persons 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.

Method of producing virus suspensions

135.—(1) Attenuated measles virus for the preparation of measles virus vaccine (live attenuated) shall be grown by aseptic methods in chick embryo tissue cultures using a suitable maintenance medium containing no penicillin.

(2) No extraneous protein capable of producing hypersensitivity when injected into man shall be added to the tissue cultures during the course of virus propagation, and if animal serum is added to initiate the cultures, it shall not be used in such quantity as will result in a concentration of more than one part per million of animal serum in the final vaccine.

(3) During the course of virus propagation the tissue culture vessels shall be incubated at a constant temperature which shall be accurately controlled.

(4) Virus suspensions shall be harvested not later than 14 days after virus inoculation. Bulk virus suspensions may be prepared from a number of single harvests and the tests specified in regulation 138 of these regulations shall be made either on the bulk virus suspensions or on the single harvests.

Condition of flocks of chickens providing chick embryo tissue

136. The chick embryo tissue shall be derived from embryonated chicken eggs from healthy flocks of chickens free from *Salmonella pullorum, Mycobacterium tuberculosis,* fowl pox, Rous sarcoma and the avian leucosis viruses, including the virus of neurolymphomatosis, and other adventitious agents.

Tests on chick embryo tissue cultures used for virus growth

137.—(1) Ten per cent of each batch of cultures intended for use for the propagation of virus shall be held as uninoculated control cultures. They shall be observed for a period of fourteen days after the time of inoculation of the remainder of the batch and shall show no evidence of virus contamination.

(2) At the time of virus harvest from the production cultures the fluids shall be withdrawn from the control cultures. These fluids shall be tested for and shall be shown to be free from:—

(*a*) avian adeno viruses and avian leucosis viruses, by inoculation into chick embryo and chick liver tissue cultures;

(*b*) Rous sarcoma virus, by inoculation into the chorioallantoic membranes of fertile eggs known to be sensitive to the virus;

(*c*) extraneous viruses other than those mentioned in sub-paragraphs (*a*) and (*b*) of this paragraph, by inoculation into human and simian tissue cultures.

(3) Between the time of withdrawing and the time of testing, the fluids withdrawn from the control cultures shall be kept under conditions which will preserve the agents which the tests are designed to detect.

(4) At the time of the last virus harvest twenty-five per cent of the control cultures shall be shown to be free from haemadsorbing viruses.

Testing of virus suspensions

138.—(1) Samples of the virus suspensions shall be taken immediately on harvesting, but before clarification as required by regulation 139 of these regulations, and shall be subjected to the tests described in paragraph (2) of this regulation. Between the time of harvesting and the time they are tested they shall be kept under conditions which will preserve the agents which the tests are designed to detect.

(2) The samples shall be tested for and shall be shown to be free from the following:—

(*a*) *Bacterial and mycotic contaminations.*

(*b*) *Mycoplasmal contamination:* The tests shall be made on solid and in liquid media which has been shown to be capable of detecting mycoplasma.

(*c*) *Extraneous viruses:*

(i) The tests shall be made in chick embryo, chick liver, monkey kidney and human tissue cultures after neutralization of the attenuated measles virus by specific antiserum prepared in animals other than chickens, using antigens grown in a non-avian cell line. For the purpose of these tests the expression "extraneous viruses" shall be deemed to include avian adeno viruses and avian leucosis viruses.

(ii) The tests shall be made by inoculation into the allantoic cavity and the yolk sac, as well as on to the chorioallantoic membranes, of fertile eggs after neutralization of the attenuated measles virus by specific antiserum.

(iii) The tests shall be made by inoculation into adult and new-born mice.

Further processing of virus suspensions

139. After testing in accordance with the last preceding regulation the virus suspensions shall be clarified by a method ensuring the removal of all tissue cells and a suitable stabilizer shall be added thereto. The vaccine shall then be distributed into final containers and dried from the frozen state before the containers are sealed.

Tests of the product in the final containers

140.—(1) Each batch of vaccine shall be subjected to the tests described in paragraph (3) of this regulation and these tests shall be carried out on vaccine obtained from final containers.

(2) For the purpose of this part of these regulations a batch of vaccine is the quantity of the finished biological preparation present in the container from which the final containers are filled at the time when the operation of filling is commenced.

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

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

(*b*) *Virus concentration :* The concentration of live attenuated measles virus shall be determined by titration in comparison with a reference preparation of measles virus vaccine (live attenuated) kept as a standard preparation at the National Institute of Medical Research, London.

(*c*) *Freedom from abnormal toxicity :* A human dose shall be inoculated intraperitoneally into each of 6 adult mice and a similar dose shall be inoculated subcutaneously into each of 3 guinea-pigs. None of the animals shall show signs of ill-health for the seven days following the inoculation.

Information and samples required by the licensing authority

141. 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 in accordance with regulations 137 and 138 of these regulations whether or not the chick embryo tissue cultures or the virus suspensions were used in the preparation of a finished batch. The licensing authority may require a manufacturer to submit samples taken at any stage in the preparation of the vaccine.

Conditions of issue

142. No batch of measles virus vaccine (live attenuated) shall be issued for sale until it has passed the tests for such vaccine prescribed by these regulations and has been shown :—

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

(*b*) to have a virus titre approved by the licensing authority.

Labelling

143.—(1) In addition to the particulars required by regulation 6(2) of these regulations, the following particulars shall be borne by the package in which a container of measles virus vaccine (live attenuated) is offered for sale :—

(*a*) a statement that the vaccine should be reconstituted only by using the reconstituting fluid accompanying the vaccine in a separate container within the same package ;

(*b*) a statement that the vaccine should be used within one hour of reconstitution ; and

(*c*) the particulars specified in regulation 6(1)(*g*) and (*h*) of the general regulations.

(2) Regulation 14 of these regulations shall have effect in relation to measles virus vaccine (live attenuated) as if references therein to particulars being required to be borne by the container of such vaccine were references to particulars being required to be borne by the container of such vaccine or by the package in which such container is offered for sale.

PART XXVII

PROVISIONS RELATING TO MEASLES VIRUS VACCINE (INACTIVATED)

Definition and proper name

144. Measles virus vaccine (inactivated) is a preparation of an inactivated measles virus. The proper name of any such preparation is " measles virus vaccine (inactivated) ".

Strains of measles virus

145.—(1) The strains of measles virus used for the production of measles virus vaccine (inactivated) shall be such as shall be approved by the licensing authority.

(2) Production of the vaccine shall be based on a seed virus system.

(3) The working seed lot of each strain shall conform to the same standards of purity as are required by these regulations to be applied to the virus suspensions before inactivation.

Staff of establishment

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

Method of producing virus suspensions

147.—(1) Measles virus for the preparation of measles virus vaccine (inactivated) shall be grown by aseptic methods in monkey kidney or chick embryo tissue cultures using a suitable maintenance medium containing no serum and no penicillin.

(2) No extraneous protein capable of producing hypersensitivity when injected into man shall be added to the tissue cultures during the course of virus propagation, and if animal serum is added to initiate the cultures, it shall not be used in such quantity as will result in a concentration of more than one part per million of animal serum in the final vaccine.

(3) Bulk virus suspensions may be prepared from a number of single harvests and the tests specified in regulation 149 of these regulations shall be made either on the bulk virus suspensions or on the single harvests.

(4) After harvesting, the virus suspensions shall be clarified by a method ensuring the removal of all tissue cells.

Condition of monkeys and flocks of chickens providing tissue

148.—(1) The kidney tissue shall be derived from healthy monkeys of suitable species. The monkeys shall be quarantined for an adequate period before being used, and when 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) The chick embryo tissue shall be derived from embryonated chicken eggs from healthy flocks of chickens free from *Salmonella pullorum, Mycobacterium tuberculosis,* fowl pox, Rous sarcoma and the avian leucosis viruses, including the virus of neurolymphomatosis, and other adventitious agents.

Testing of virus suspensions

149.—(1) Samples of the virus suspensions shall be taken either before or immediately after clarification as required by regulation 147(4) of these regulations and shall be subjected to the tests described in paragraphs (2) and (3) of this regulation. Between the time of taking the samples and the time they are tested they shall be kept under conditions which will preserve the agents which the tests are designed to detect.

(2) Samples of virus suspensions from monkey kidney tissue cultures shall be tested for and shall be shown to be free from the following :—

(*a*) *Bacterial and mycotic contaminations.*

(*b*) *Mycoplasmal contamination :* The tests shall be made on solid and in liquid media which has been shown to be capable of detecting mycoplasma.

(*c*) *B virus :* The tests shall be made by the inoculation of rabbits. 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*) *Extraneous viruses other than B virus :*

(i) The tests shall be made in monkey kidney tissue cultures prepared from the same species, but not from the same animal, as that used for vaccine production, and, where the tissue culture system used for vaccine production is not susceptible to S.V.40 virus, in an additional tissue culture system susceptible to that virus. The tests shall be made by suitable methods after neutralization of the measles virus by specific antiserum which shall be prepared in animals other than man, monkeys or chickens, using antigens grown in non-simian cell lines.

(ii) The tests shall be made by inoculation into adult mice, newborn mice and guinea-pigs.

(*e*) *Mycobacterium tuberculosis :* The tests shall be made by inoculation into guinea-pigs and by inoculation of a suitable culture medium.

(3) Samples of virus suspensions from chick embryo tissue cultures shall be tested for and shall be shown to be free from the following :—

(*a*) *Bacterial and mycotic contaminations.*

(*b*) *Mycoplasmal contamination :* The tests shall be made on solid and in liquid media which has been shown to be capable of detecting mycoplasma.

(*c*) *Extraneous viruses :*

(i) The tests shall be designed to ensure freedom from avian adeno viruses, avian leucosis viruses and other extraneous viruses. The tests shall be made in chick embryo and chick liver tissue cultures after neutralization of the measles virus by specific antiserum which shall be prepared in animals other than chickens, using antigens grown in non-avian cell lines.

(ii) The tests shall be made by inoculation into adult mice, newborn mice and embryonated eggs.

Method of inactivating virus suspensions

150. All virus infectivity in the virus suspensions shall be destroyed by a suitable method.

Tests after virus inactivation

151.—(1) After virus inactivation a sample of the vaccine equivalent to at least 500 human doses shall be tested for, and shall be shown to be free from, measles and other viruses. The tests shall be made after neutralization of the inactivating agent, in the tissue culture system used for production of the vaccine and also in a tissue culture system susceptible to measles virus, S.V.40 virus and other simian viruses.

(2) If the vaccine has been produced from chick embryo tissue cultures, in addition to the test required by paragraph (1) of this regulation, a sample equivalent to at least 200 human doses shall be tested by inoculation into embryonated eggs.

Tests of the product in the final containers

152.—(1) Each batch of vaccine shall be subjected to the tests described in paragraph (3) of this regulation and these tests shall be carried out on vaccine obtained from final containers.

(2) For the purpose of this part of these regulations a batch of vaccine is the quantity of the finished biological preparation present in the container from which the final containers are filled at the time when the operation of filling is commenced.

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

(*a*) *Freedom from abnormal toxicity:* A human dose shall be inoculated intraperitoneally into each of 6 adult mice and a similar dose shall be inoculated subcutaneously into each of 3 guinea-pigs. None of the animals shall show signs of ill-health for the seven days following the inoculation.

(*b*) *Antigenicity:* The antigenicity of the vaccine shall be compared with and shall not be less than that of a reference vaccine held by the National Institute of Medical Research, London. The test shall be made by measuring the antibody responses of suitable animals.

Information and samples required by the licensing authority

153. 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 in accordance with regulations 149 and 151 of these regulations, whether or not the virus harvest or the material after virus inactivation was used in the preparation of a finished batch. The licensing authority may require a manufacturer to submit samples taken at any stage in the preparation of the vaccine.

Conditions of issue

154. No batch of measles virus vaccine (inactivated) shall be issued for sale until—

(*a*) it has passed the tests prescribed by these regulations ; and

(*b*) where it is prepared for use in multi-dose containers and a bacteriostatic is added, such bacteriostatic shall, at the concentration used, prevent the growth of micro-organisms and shall not cause any loss of antigenicity of the vaccine before the date after which the vaccine may not be used.

Labelling

155.—(1) In addition to the particulars required by regulation 6(2) of these regulations, particulars of any antibiotic used in preparing the vaccine shall be borne either by the container of any measles virus vaccine (inactivated) or by the package in which such vaccine is offered for sale.

(2) Regulation 14 of these regulations shall have effect in relation to measles virus vaccine (inactivated) as if references therein to particulars being required to be borne by the container of such vaccine were references to particulars being required to be borne by the container of such vaccine or by the package in which such container is offered for sale.

Method of issue

156. Measles virus vaccine (inactivated) shall not be issued for sale combined with any other material without the consent of the licensing authority.

PART XXVIII

PROVISIONS RELATING TO OIL ADJUVANT INFLUENZA VIRUS VACCINE (INACTIVATED)

Definition and proper name

157. Oil adjuvant influenza virus vaccine (inactivated) is influenza virus vaccine (inactivated) contained in the aqueous phase of a water in oil emulsion, such emulsion having been effected by an emulsifying agent. The proper name of the substance is " oil adjuvant influenza virus vaccine (inactivated) ".

Staff of establishment

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

Quality and nature of substances used in preparation of vaccine.

159.—(1) The oil and the emulsifying agent used for the preparation of oil adjuvant influenza virus vaccine (inactivated) shall be such as shall be approved by the licensing authority.

(2) Each batch of oil and of emulsifying agent used for the preparation of oil adjuvant influenza virus vaccine (inactivated) shall be subjected to the biological tests described in paragraph (4) of this regulation.

(3) For the purpose of this regulation a batch of oil or of emulsifying agent is that amount of oil or of emulsifying agent which is intended for use in the preparation of a final bulk vaccine as defined in regulation 160(2) of these regulations.

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

(*a*) mice weighing not less than 9 grammes and not more than 11 grammes each shall be weighed individually to the nearest 0·1 gramme and shall be marked for identification. Two days later the mice shall be weighed and only those which have gained weight in that time shall be used in the test. Samples of each batch of oil and of emulsifying agent shall be tested separately in not less than 10 of the mice, each mouse being inoculated intraperitoneally with 0·25 millilitre of the batch under test. The weight of each mouse shall

be observed for seven days following the inoculation and the mean weight gain of these mice shall be compared with the mean weight gain of a similar group of mice which have been inoculated intraperitoneally with 0·5 millilitre of physiological saline. No oil or emulsifying agent which depresses the mean weight gain per cent of the first group of mice more than ten per cent below the mean weight gain per cent of the second group of mice shall be used;

(*b*) the first group of mice referred to in sub-paragraph (*a*) of this paragraph shall be killed between the tenth and fourteenth day following inoculation. If, on inspection of the peritoneum of any of the mice, visible lesions are found, the oil or the emulsifying agent shall not be used.

(5) Only influenza virus vaccine (inactivated) which has been prepared and tested in accordance with regulations 116, 118, 119 and 120 of these regulations and with this regulation shall be used in the preparation of oil adjuvant influenza virus vaccine (inactivated):

Provided that, for the purpose of regulation 120 of these regulations (test for haemagglutinating activity) influenza virus vaccine (inactivated) shall be accepted as satistactory if the end point of the titration is produced by vaccine diluted initially at least 1/5,000 but no more than 1/10,000 by the pattern test.

(6) The protein nitrogen content of the influenza virus vaccine (inactivated) used for the preparation of oil adjuvant influenza virus vaccine (inactivated) shall be estimated by a method approved by the licensing authority and shall not exceed 1 microgramme of protein nitrogen for every 250 haemagglutinating units of vaccine.

Tests on final bulk vaccine

160.—(1) Each final bulk vaccine shall be subjected to the tests described in paragraph (3) of this regulation.

(2) For the purpose of this regulation a final bulk vaccine is the quantity of the finished biological preparation present in the container from which the final containers are filled at the time when the operation of filling is commenced.

(3) The final bulk vaccines shall be tested as follows:—

(*a*) *Stability:* A sample shall be centrifuged at a force of at least 1500 g for a period of at least 10 minutes at the end of which time the sample shall show not more than ten per cent separation.

(*b*) *Antigenicity:* Samples, each equivalent to a human dose, shall be inoculated intramuscularly into suitable laboratory animals. The animals shall show a satisfactory antibody response.

Tests of the product in the final containers

161.—(1) Each batch of vaccine shall be subjected to the tests described in paragraph (3) of this regulation, such tests being designed to show freedom from abnormal toxicity.

(2) For the purpose 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*) A sample equivalent to a human dose shall be inoculated intraperitoneally into each of 3 guinea-pigs. None of the animals shall show signs of ill-health for the 7 days following the inoculation.

(*b*) A sample equivalent to a human dose shall be subjected to tests similar to those described in regulation 159(4) of these regulations for oils and emulsifying agents.

Containers

162. The vaccine shall not be distributed otherwise than in containers in which the volume or amount of such vaccine does not exceed by more than ten per cent the dose required by regulation 6(3) of these regulations to be stated on the container.

Labelling

163.—(1) In addition to the particulars required by regulation 6(1) of these regulations, the container of an oil adjuvant influenza virus vaccine (inactivated) shall bear a statement:—

(*a*) of the strain or strains of the micro-organisms used for the preparation of the vaccine; and

(*b*) that the contents constitute a single dose.

(2) In addition to the particulars required by regulation 6(2) of these regulations the package in which a container of oil adjuvant influenza virus vaccine (inactivated) is offered for sale shall bear the particulars specified in regulation 6(1)(*g*) and (*h*) of the general regulations.

(3) Regulation 14 of these regulations shall not apply to oil adjuvant influenza virus vaccine (inactivated).".

These regulations were made by the aforesaid Joint Committee on 15th April 1965.

Signed { *Kenneth Robinson,*
William Ross,
Wm. J. Morgan,

Members of the Joint Committee.

Signed *Wilfred Turner,*
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 extend the provisions of the Therapeutic Substances (Manufacture of Vaccines, Toxins and Antigens) Regulations 1963 and 1964 to make special provisions for—

(i) measles virus vaccine (live attenuated);

(ii) measles virus vaccine (inactivated);

(iii) oil adjuvant influenza virus vaccine (inactivated),

including provisions for their definition and method of preparation, and for testing their antigenicity and freedom from toxicity.