Chemicals used for disruption and purification. Tests for the chemicals used for disruption and purification are carried out on the monovalent virosomal preparation, the limits being approved by the competent authority.

Phospholipids. The content and identity of the phospholipids are determined by suitable immunochemical or physico-chemical methods.

Ratio of haemagglutinin to phospholipid. The ratio of haemagglutinin content to phospholipid content is within the limits approved for the particular product.

Virosome size. The average virosome diameter, determined by a suitable method such as photon-correlation spectroscopy, is not less than 100 nm and not greater than 300 nm. The polydispersity index is not greater than 0.4.

FINAL BULK VACCINE

Appropriate quantities of the monovalent virosomal preparations are blended to make the final bulk vaccine. Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The content is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility (*2.6.1*). Carry out the test for sterility, using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given under Tests and Assay may be released for use. Provided that the test for residual infectious virus has been performed with satisfactory results on each monovalent pooled harvest or, where appropriate, on the monovalent virosomal preparations, and that the tests for phospholipids, ratio of haemagglutinin to phospholipid, free formaldehyde, ovalbumin and total protein have been performed with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

IDENTIFICATION

The assay serves to confirm the antigenic specificity of the vaccine.

TESTS

Residual infectious virus. Inoculate 0.2 ml of the vaccine into the allantoic cavity of each of 10 fertilised eggs and incubate at 33-37 °C for 3 days. The test is not valid unless at least 8 of the 10 embryos survive. Harvest 0.5 ml of the allantoic fluid from each surviving embryo and pool the fluids. Inoculate 0.2 ml of the pooled fluid into a further 10 fertilised eggs and incubate at 33-37 °C for 3 days. The test is not valid unless at least 8 of the 10 embryos survive. Harvest about 0.1 ml of the allantoic fluid from each surviving embryo and examine each individual harvest for live virus by a haemagglutination test. If haemagglutination is found for any of the fluids, carry out for that fluid a further passage in eggs and test for haemagglutination; no haemagglutination occurs.

pH (*2.2.3*): 6.5 to 7.8

Phospholipids. The content and identity of the phospholipids is determined by a suitable immunochemical or physico-chemical method.

Ratio of haemagglutinin to phospholipid. The ratio of haemagglutinin content to phospholipid content is within the limits approved for the particular product.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Free formaldehyde (*2.4.18*): maximum 0.2 g/l, where applicable.

Ovalbumin. Not more than the quantity stated on the label and in any case not more than 1 µg per human dose, determined by a suitable immunochemical method (*2.7.1*) using a suitable reference preparation of ovalbumin.

Total protein. Not more than 40 µg of protein other than haemagglutinin per virus strain per human dose, and not more than a total of 120 µg of protein other than hemagglutinin per human dose.

Sterility (*2.6.1*). It complies with the test for sterility.

Virosome size. The average virosome diameter, determined by a suitable method such as photon-correlation spectroscopy, is not less than 100 nm and not greater than 300 nm. The polydispersity index is not greater than 0.4.

Bacterial endotoxins (*2.6.14*): less than 100 IU per human dose.

ASSAY

Determine the content of haemagglutinin antigen by an immunodiffusion test (*2.7.1*), by comparison with a haemagglutinin antigen reference preparation[4] or with an antigen preparation calibrated against it. Carry out the test at 20-25 °C. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated haemagglutinin antigen content. The lower confidence limit (P = 0.95) is not less than 80 per cent of the amount stated on the label for each strain.

LABELLING

The label states:

- that the vaccine has been prepared on eggs;
- the strain or strains of influenza virus used to prepare the vaccine;
- the method of inactivation;
- the haemagglutinin content, in micrograms per virus strain per dose;
- the maximum amount of ovalbumin;
- the season during which the vaccine is intended to protect.

01/2008:0159

INFLUENZA VACCINE (WHOLE VIRION, INACTIVATED)

Vaccinum influenzae inactivatum ex viris integris praeparatum

DEFINITION

Influenza vaccine (whole virion, inactivated) is a sterile, aqueous suspension of a strain or strains of influenza virus, type A or B, or a mixture of strains of the 2 types grown individually in fertilised hens' eggs and inactivated in such a manner that their antigenic properties are retained. The

stated amount of haemagglutinin antigen for each strain present in the vaccine is 15 µg per dose, unless clinical evidence supports the use of a different amount.

The vaccine is a slightly opalescent liquid.

PRODUCTION

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (*2.6.9*).

CHOICE OF VACCINE STRAIN

The World Health Organisation reviews the world epidemiological situation annually and if necessary recommends the strains that correspond to this epidemiological evidence.

Such strains are used in accordance with the regulations in force in the signatory States of the Convention on the Elaboration of a European Pharmacopoeia. It is now common practice to use reassorted strains giving high yields of the appropriate surface antigens. The origin and passage history of virus strains shall be approved by the competent authority.

SUBSTRATE FOR VIRUS PROPAGATION

Influenza virus seed to be used in the production of vaccine is propagated in fertilised eggs from chicken flocks free from specified pathogens (SPF) (*5.2.2*) or in suitable cell cultures (*5.2.4*), such as chick-embryo fibroblasts or chick kidney cells obtained from SPF chicken flocks (*5.2.2*). For production, the virus of each strain is grown in the allantoic cavity of fertilised hens' eggs from healthy flocks.

VIRUS SEED LOT

The production of vaccine is based on a seed-lot system. Working seed lots represent not more than 15 passages from the approved reassorted virus or the approved virus isolate. The final vaccine represents 1 passage from the working seed lot. The haemagglutinin and neuraminidase antigens of each seed lot are identified as originating from the correct strain of influenza virus by suitable methods.

Only a working virus seed lot that complies with the following requirements may be used in the preparation of the monovalent pooled harvest.

Bacterial and fungal contamination. Carry out the test for sterility (*2.6.1*), using 10 ml for each medium.

Mycoplasmas (*2.6.7*). Carry out the test for mycoplasmas, using 10 ml.

VIRUS PROPAGATION AND HARVEST

An antimicrobial agent may be added to the inoculum. After incubation at a controlled temperature, the allantoic fluids are harvested and combined to form a monovalent pooled harvest. An antimicrobial agent may be added at the time of harvest. At no stage in the production is penicillin or streptomycin used.

MONOVALENT POOLED HARVEST

To limit the possibility of contamination, inactivation is initiated as soon as possible after preparation. The virus is inactivated by a method that has been demonstrated on 3 consecutive batches to be consistently effective for the manufacturer. The inactivation process shall have been shown to be capable of inactivating the influenza virus without destroying its antigenicity; the process should cause minimum alteration of the haemagglutinin and neuraminidase antigens. The inactivation process shall also have been shown to be capable of inactivating avian leucosis viruses and mycoplasmas. If the monovalent pooled harvest is stored after inactivation, it is held at 5 ± 3 °C. If formaldehyde solution is used, the concentration does not exceed 0.2 g/l of CH_2O at any time during inactivation; if betapropiolactone is used, the concentration does not exceed 0.1 per cent *V/V* at any time during inactivation.

Before or after the inactivation process, the monovalent pooled harvest is concentrated and purified by high-speed centrifugation or other suitable method.

Only a monovalent pooled harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Haemagglutinin antigen. Determine the content of haemagglutinin antigen by an immunodiffusion test (*2.7.1*), by comparison with a haemagglutinin antigen reference preparation or with an antigen preparation calibrated against it[(5)]. Carry out the test at 20-25 °C.

Neuraminidase antigen. The presence and type of neuraminidase antigen are confirmed by suitable enzymatic or immunological methods on the first 3 monovalent pooled harvests from each working seed lot.

Sterility (*2.6.1*). Carry out the test for sterility, using 10 ml for each medium.

Residual infectious virus. Carry out the test described below under Tests.

FINAL BULK VACCINE

Appropriate quantities of the monovalent pooled harvests are blended to make the final bulk vaccine.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility (*2.6.1*). Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Tests and Assay may be released for use. Provided that the test for residual infectious virus has been performed with satisfactory results on each monovalent pooled harvest and that the tests for free formaldehyde, ovalbumin and total protein have been performed with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

IDENTIFICATION

The assay serves to confirm the antigenic specificity of the vaccine.

TESTS

Residual infectious virus. Inoculate 0.2 ml of the vaccine into the allantoic cavity of each of 10 fertilised eggs and incubate at 33-37 °C for 3 days. The test is not valid unless at least 8 of the 10 embryos survive. Harvest 0.5 ml of the allantoic fluid from each surviving embryo and pool the fluids. Inoculate 0.2 ml of the pooled fluid into a further 10 fertilised eggs and incubate at 33-37 °C for 3 days. The test is not valid unless at least 8 of the 10 embryos survive. Harvest about 0.1 ml of the allantoic fluid from each surviving embryo and examine each individual harvest for

(5) Reference haemagglutinin antigens are available from the National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, Great Britain.

live virus by a haemagglutination test. If haemagglutination is found for any of the fluids, carry out for that fluid a further passage in eggs and test for haemagglutination; no haemagglutination occurs.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Free formaldehyde (*2.4.18*): maximum 0.2 g/l, where applicable.

Ovalbumin. Not more than the quantity stated on the label and in any case not more than 1 µg per human dose, determined by a suitable immunochemical method (*2.7.1*) using a suitable reference preparation of ovalbumin.

Total protein. Not more than 6 times the total haemagglutinin content of the vaccine as determined in the assay, but in any case, not more than 100 µg of protein per virus strain per human dose and not more than a total of 300 µg of protein per human dose.

Sterility (*2.6.1*). It complies with the test for sterility.

Bacterial endotoxins (*2.6.14*): less than 100 IU per human dose.

ASSAY

Determine the content of haemagglutinin antigen by an immunodiffusion test (*2.7.1*), by comparison with a haemagglutinin antigen reference preparation or with an antigen preparation calibrated against it[1]. Carry out the test at 20-25 °C. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated haemagglutinin antigen content. The lower confidence limit (P = 0.95) is not less than 80 per cent of the amount stated on the label for each strain.

LABELLING

The label states:

- that the vaccine has been prepared on eggs,
- the strain or strains of influenza virus used to prepare the vaccine,
- the method of inactivation,
- the haemagglutinin content in micrograms per virus strain per dose,
- the maximum amount of ovalbumin,
- the season during which the vaccine is intended to protect.

01/2008:2308

INFLUENZA VACCINE (WHOLE VIRION, INACTIVATED, PREPARED IN CELL CULTURES)

Vaccinum influenzae inactivatum ex cellulis virisque integris praeparatum

DEFINITION

Influenza vaccine (whole virion, inactivated, prepared in cell cultures) is a sterile, aqueous suspension of a strain or strains of influenza virus, type A or B, or a mixture of strains of the 2 types grown individually in cell cultures and inactivated in such a manner that their antigenic properties are retained. The stated amount of haemagglutinin antigen for each strain present in the vaccine is 15 µg per dose, unless clinical evidence supports the use of a different amount. The vaccine is a slightly opalescent or opalescent liquid. The vaccine may contain an adjuvant. This monograph applies to vaccines produced in diploid or continuous cell lines of mammalian origin.

PRODUCTION

GENERAL PROVISIONS

Production of the vaccine is based on a virus seed-lot system and a cell-bank system. The production method shall have been shown to yield consistently vaccines that comply with the requirements for immunogenicity, safety and stability.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (*2.6.9*).

The production method is validated to demonstrate suitable reduction of residual host-cell protein. With the agreement of the competent authority and for each specific product, routine testing for residual host-cell proteins may be omitted based on the results of validation studies for the product. Guidance on the principles of such validation studies is given, for example, in the monograph on *Products of recombinant DNA technology (0784)*, in particular in the chapters on "Validation of the production process - extraction and purification" and "Production consistency - Host-cell-derived proteins".

CHOICE OF VACCINE STRAIN

The World Health Organisation reviews the world epidemiological situation annually and if necessary recommends new strains corresponding to this epidemiological evidence.

Such strains are used in accordance with the regulations in force in the signatory states of the Convention on the Elaboration of a European Pharmacopoeia. It is now common practice to use reassorted strains giving high yields of the appropriate surface antigens. The origin and passage history of virus strains shall be approved by the competent authority.

SUBSTRATE FOR VIRUS PROPAGATION

Influenza virus used in the preparation of seed lots is propagated in fertilised eggs from chicken flocks free from specified pathogens (SPF) (*5.2.2*) or in suitable cell cultures (*5.2.3*), such as chick-embryo fibroblasts, chick kidney cells obtained from SPF chicken flocks (*5.2.2*), or a diploid or continuous cell line. The final passage for establishment of the working seed lot is prepared in the cell line used for routine production. For this production, the virus of each strain is propagated in a diploid or continuous cell line (*5.2.3*).

VIRUS SEED LOT

The production of vaccine is based on a seed-lot system. Each of the strains of influenza virus used shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation. Working seed lots represent not more than 15 passages from the approved reassorted virus or the approved virus isolate. The final vaccine represents 1 passage from the working seed lot.

Only a seed lot that complies with the following requirements may be used for virus propagation.

Identification. The haemagglutinin and neuraminidase antigens of each master and working seed lot are identified as originating from the correct strain of influenza virus by suitable methods.

Virus concentration. The virus concentration of each working seed lot is determined. Where applicable, the virus concentration of each master seed lot is determined.