

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

Sterility (2.6.1). The final bulk vaccine complies with the test for sterility, carried out using 10 ml for each medium.

FINAL LOT

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for free formaldehyde (where applicable) and antimicrobial preservative content (where applicable) have been carried out on the final bulk vaccine with satisfactory results, they may be omitted on the final lot. If the assay is carried out *in vivo*, then provided it has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

IDENTIFICATION

The assay or, where applicable, the electrophoretic profile, serves also to identify the vaccine.

TESTS

Aluminium (2.5.13): maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (2.4.18): maximum 0.2 g/l.

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

Sterility (2.6.1). The vaccine complies with the test for sterility.

Pyrogens (2.6.8). The vaccine complies with the test for pyrogens. Inject the equivalent of one human dose into each rabbit.

ASSAY

The vaccine complies with the assay of hepatitis B vaccine (rDNA) (2.7.15).

LABELLING

The label states:

- the amount of HBsAg per container,
- the type of cells used for production of the vaccine,
- the name and amount of the adsorbent used,
- that the vaccine must be shaken before use,
- that the vaccine must not be frozen.

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INFLUENZA VACCINE (SPLIT VIRION, INACTIVATED)

Vaccinum influenzae inactivatum
ex virorum fragmentis praeparatum

DEFINITION

Influenza vaccine (split 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 two types grown individually in fertilised hens' eggs, inactivated and treated so that the integrity of the virus particles has been disrupted without diminishing the antigenic properties of

the haemagglutinin and neuraminidase antigens. 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 new strains corresponding to prevailing 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 (5.2.2) or in suitable cell cultures (5.2.4), such as chick-embryo fibroblasts or chick kidney cells obtained from chicken flocks free from specified pathogens (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 three 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 a

temperature of 5 ± 3 °C. If formaldehyde solution is used, the concentration does not exceed 0.2 g/l of CH₂O 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 procedure, the monovalent pooled harvest is concentrated and purified by high-speed centrifugation or other suitable method and the virus particles are disrupted into component subunits by the use of approved procedures. For each new strain, a validation test is carried out to show that the monovalent bulk consists predominantly of disrupted virus particles.

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⁽¹⁾. Carry out the test at 20 °C to 25 °C.

For some vaccines, the physical form of the haemagglutinin particles prevents quantitative determination by immunodiffusion after inactivation of the virus. For these vaccines, a determination of haemagglutinin antigen is made on the monovalent pooled harvest before inactivation. The production process is validated to demonstrate suitable conservation of haemagglutinin antigen and a suitable tracer is used for formulation, for example, protein content.

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.

Viral inactivation. Carry out the test described below under Tests.

Chemicals used for disruption. Tests are carried out on the monovalent pooled harvest for the chemicals used for disruption, the limits being approved by the competent authority.

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 viral inactivation 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

Viral inactivation. Inoculate 0.2 ml of the vaccine into the allantoic cavity of each of 10 fertilised eggs and incubate at 33 °C to 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 °C to 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.

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.

Ovalbumin. Not more than 1 µg of ovalbumin per human dose, determined by a suitable technique using a suitable reference preparation of ovalbumin.

Free formaldehyde (2.4.18): maximum 0.2 g/l.

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.

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 °C to 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.

For some vaccines, quantitative determination of haemagglutinin antigen with respect to available reference preparations is not possible. An immunological identification of the haemagglutinin antigen and a semi-quantitative determination are carried out instead by suitable methods.

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,

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

- the haemagglutinin content in micrograms per virus strain per dose,
- the season during which the vaccine is intended to protect.

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INFLUENZA VACCINE (SURFACE ANTIGEN, INACTIVATED)

Vaccinum influenzae inactivatum ex corticis antigeniis praeparatum

DEFINITION

Influenza vaccine (surface antigen, inactivated) is a sterile 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, inactivated and treated so that the preparation consists predominantly of haemagglutinin and neuraminidase antigens, without diminishing the antigenic properties of these antigens. 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 may contain an adjuvant.

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 new strains corresponding to prevailing 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 one 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 CH₂O 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. Virus particles are disrupted into component subunits by approved procedures and further purified so that the monovalent bulk consists mainly of haemagglutinin and neuraminidase antigens.

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⁽²⁾. 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.

Viral inactivation. Carry out the test described below under Tests.

Purity. The purity of the monovalent pooled harvest is examined by polyacrylamide gel electrophoresis or by other approved techniques. Mainly haemagglutinin and neuraminidase antigens shall be present.

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

FINAL BULK VACCINE

Appropriate quantities of the monovalent pooled harvests are blended to make the final bulk vaccine. An adjuvant may be added.

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

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