Bacterial endotoxins (2.6.14): less than 2 IU of endotoxin per human dose.

ASSAY
Determine the antigen content of the vaccine using a suitable immunochemical method (2.7.1) by comparison with the reference preparation. The acceptance criteria are approved for a given reference preparation by the competent authority.

LABELLING
The label states:
- the biological origin of the cells used for the preparation of the vaccine,
- that the carrier contains influenza proteins prepared in eggs,
- that the vaccine is not to be frozen,
- that the vaccine is to be shaken before use.

01/2005:1056

HEPATITIS B VACCINE (rDNA)

Vaccinum hepatitis B (ADNr)

DEFINITION
Hepatitis B vaccine (rDNA) is a preparation of hepatitis B surface antigen (HBsAg), a component protein of hepatitis B virus; the antigen may be adsorbed on a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate. The antigen is obtained by recombinant DNA technology.

PRODUCTION
GENERAL PROVISIONS
The vaccine shall have been shown to induce specific, protective antibodies in man. The production method shall have been shown to yield consistently vaccines that comply with the requirements for immunogenicity and safety. The production method is validated to demonstrate that the production method is validated to demonstrate that the vaccine is to be shaken before use.

The vaccine may contain the product of the S gene (major protein), a combination of the S gene and pre-S2 gene products (middle protein) or a combination of the S gene, the pre-S2 gene and pre-S1 gene products (large protein).

Reference preparation. The reference preparation is part of a representative batch shown to be at least as immunogenic in animals as a batch that, in clinical studies in young, healthy adults, produced not less than 95 per cent seroconversion, corresponding to a level of HBsAg neutralising antibody recognised to be protective, after a full-course primary immunisation. An antibody level not less than 10 mIU/ml is recognised as being protective.

CHARACTERISATION OF THE SUBSTANCE
Development studies are carried out to characterise the antigen. The complete protein, lipid and carbohydrate structure of the antigen is established. The morphological characteristics of the antigen particles are established by electron microscopy. The mean buoyant density of the antigen particles is determined by a physico-chemical method, such as gradient centrifugation. The antigenic epitopes are characterised. The protein fraction of the antigen is characterised in terms of the primary structure (for example, by determination of the amino-acid composition, by partial amino-acid sequence analysis and by peptide mapping).

CULTURE AND HARVEST
Identity, microbial purity, plasmid retention and consistency of yield are determined at suitable production stages. If mammalian cells are used, tests for extraneous agents and mycoplasmas are performed in accordance with Tests for extraneous agents in viral vaccines for human use (2.6.16), but using 200 ml of harvest in the test in cell culture for other extraneous agents.

PURIFIED ANTIGEN
Only a purified antigen that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Total protein. The total protein is determined by a validated method. The content is within the limits approved for the specific product.

Antigen content and identification. The quantity and specificity of HBsAg is determined in comparison with the International Standard for HBsAg subtype ad or an in-house reference, by a suitable immunochemical method (2.7.1) such as radio-immunoprecipitation (RIA), enzyme-linked immunosorbent assay (ELISA), immunoblot (preferably using a monoclonal antibody directed against a protective epitope) or single radial diffusion. The antigen/protein ratio is within the limits approved for the specific product.

The molecular weight of the major band revealed following sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) performed under reducing conditions corresponds to the value expected from the known nucleic acid and polypeptide sequences and possible glycosylation.

Antigenic purity. The purity of the antigen is determined by comparison with a reference preparation using liquid chromatography or other suitable methods such as SDS-PAGE with staining by acid blue 92 and silver. A suitable method is sensitive enough to detect a potential contaminant at a concentration of 1 per cent of total protein. Not less than 95 per cent of the total protein consists of hepatitis B surface antigen.

Composition. The content of proteins, lipids, nucleic acids and carbohydrates is determined.

Host-cell- and vector-derived DNA. If mammalian cells are used for production, not more than 10 pg of DNA in the quantity of purified antigen equivalent to a single human dose of vaccine.

Caesium. If a caesium salt is used during production, a test for residual caesium is carried out on the purified antigen. The content is within the limits approved for the specific product.

Sterility (2.6.7). The purified antigen complies with the test for sterility, carried out using 10 ml for each medium. Additional tests on the purified antigen may be required depending on the production method used: for example, a test for residual animal serum where mammalian cells are used for production or tests for residual chemicals used during extraction and purification.

FINAL BULK VACCINE
An antimicrobial preservative and an adjuvant may be included in the vaccine. Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.
**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**

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

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**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 vitro, 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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**01/2005:0158**