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 season during which the vaccine is intended to protect.

**MEASLES, MUMPS AND RUBELLA VACCINE (LIVE)**

Vaccinum morbillorum, parotiditis et rubellae vivum

**DEFINITION**

Measles, mumps and rubella vaccine (live) is a freeze-dried preparation of suitable attenuated strains of measles virus, mumps virus and rubella virus.

The vaccine is reconstituted immediately before use, as stated on the label, to give a clear liquid that may be coloured owing to the presence of a pH indicator.

**PRODUCTION**

The three components are prepared as described in the monographs on Measles vaccine (live) (0213), Mumps vaccine (live) (0536) and Rubella vaccine (live) (0162) and comply with the requirements prescribed therein.

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).

**FINAL BULK VACCINE**

Virus harvests for each component are pooled and clarified to remove cells. A suitable stabiliser may be added and the pooled harvests diluted as appropriate. Suitable quantities of the pooled harvest for each component are mixed. Only a final bulk vaccine that complies with the following requirement may be used in the preparation of the final lot.

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

**FINAL LOT**

For each component, a minimum virus concentration for release of the product is established such as to ensure, in the light of stability data, that the minimum concentration stated on the label will be present at the end of the period of validity.

Only a final lot that complies with the requirements for minimum virus concentration of each component for release, with the following requirement for thermal stability and with each of the requirements given below under Identification and Tests may be released for use. Provided that the tests for bovine serum albumin and, where applicable, for ovalbumin have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

**Thermal stability.** Maintain samples of the final lot of freeze-dried vaccine in the dry state at 37 °C for 7 days. Determine the virus concentration as described under Assay in parallel for the heated vaccine and for unheated vaccine stored at 5 ± 3 °C. For each component, the virus concentration of the heated vaccine is not more than 1.0 \(\log_{10}\) lower than that of the unheated vaccine.

**IDENTIFICATION**

When the vaccine reconstituted as stated on the label is mixed with antibodies specific for measles virus, mumps virus and rubella virus, it is no longer able to infect cell cultures susceptible to these viruses. When the vaccine reconstituted as stated on the label is mixed with quantities of specific antibodies sufficient to neutralise any two viral components, the third viral component infects susceptible cell cultures.

**TESTS**

**Bacterial and fungal contamination.** The reconstituted vaccine complies with the test for sterility (2.6.1).

**Bovine serum albumin.** Not more than 50 ng per single human dose, determined by a suitable immunochemical method (2.7.1).

**Ovalbumin.** If the mumps component is produced in chick embryos, the vaccine contains not more than 1 µg of ovalbumin per single human dose, determined by a suitable immunochemical method (2.7.1).

**Water (2.5.12).** Not more than 3.0 per cent, determined by the semi-micro determination of water.

**ASSAY**

A. Mix the vaccine with a sufficient quantity of antibodies specific for mumps virus. Titrate the vaccine for infective measles virus at least in triplicate, using at least five cell cultures for each 0.5 \(\log_{10}\) dilution step or by a method of equal precision. Use an appropriate virus reference preparation to validate each assay. The estimated measles virus concentration is not less than that stated on the label; the minimum measles virus concentration stated on the label is not less than \(1 \times 10^3\) CCID\(_{50}\) per single human dose. The assay is not valid if the confidence interval \((P = 0.95)\) of the logarithm of the virus concentration is greater than ± 0.3. Measles vaccine (live) BRP is suitable for use as a reference preparation.

B. Mix the vaccine with a sufficient quantity of antibodies specific for measles virus. Titrate the vaccine for infective mumps virus at least in triplicate, using at least five cell cultures for each 0.5 \(\log_{10}\) dilution step or by a method of equal precision. Use an appropriate virus reference preparation to validate each assay. The estimated mumps virus concentration is not less than that stated on the label; the minimum mumps virus concentration stated on the label is not less than \(5 \times 10^3\) CCID\(_{50}\) per single human dose. The assay is not valid if the confidence interval \((P = 0.95)\) of the logarithm of the virus concentration is greater than ± 0.3. Mumps vaccine (live) BRP is suitable for use as a reference preparation.

C. Mix the vaccine with a sufficient quantity of antibodies specific for mumps virus. Titrate the vaccine for infective rubella virus at least in triplicate, using at least five cell cultures for each 0.5 \(\log_{10}\) dilution step or by a method of equal precision. Use an appropriate virus reference preparation to validate each assay. The estimated rubella virus concentration is not less than that stated on the label; the minimum rubella virus concentration stated on the label is not less than \(1 \times 10^3\) CCID\(_{50}\)
MEASLES VACCINE (LIVE)

Vaccinum morbillorum vivum

DEFINITION
Measles vaccine (live) is a freeze-dried preparation of a suitable attenuated strain of measles virus. The vaccine is reconstituted immediately before use, as stated on the label, to give a clear liquid that may be coloured owing to the presence of a pH indicator.

PRODUCTION
The production of vaccine is based on a virus seed-lot system and, if the virus is propagated in human diploid cells, a cell-bank system. The production method shall have been shown to yield consistently live vaccines of adequate immunogenicity and safety in man. Unless otherwise justified and authorised, the virus in the final vaccine shall have undergone no more passages from the master seed lot than are used to prepare the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy; even with authorised exceptions, the number of passages beyond the level used for clinical studies shall not exceed five.

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).

SUBSTRATE FOR VIRUS PROPAGATION
The virus is propagated in human diploid cells (5.2.2) or in cultures of chick-embryo cells derived from a chicken flock free from specified pathogens (5.2.2).

SEED LOT
The strain of measles virus used shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation. To avoid the unnecessary use of monkeys in the test for neurovirulence, virus seed lots are prepared in large quantities and stored at temperatures below −20 °C if freeze-dried, or below −60 °C if not freeze-dried. Only a seed lot that complies with the following requirements may be used for virus propagation.

Identification. The master and working seed lots are identified as measles virus by serum neutralisation in cell culture, using specific antibodies.

Virus concentration. The virus concentration of the master and working seed lots is determined to monitor consistency of production.

Extraneous agents (2.6.16). The working seed lot complies with the requirements for seed lots.

Neurovirulence (2.6.18). The working seed lot complies with the test for neurovirulence of live virus vaccines. Macaca and Cercopithecus monkeys susceptible to measles virus are suitable for the test.

PROPAGATION AND HARVEST
All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are handled. Suitable animal (but not human) serum may be used in the growth medium, but the final medium for maintaining cell growth during virus multiplication does not contain animal serum. Serum and trypsin used in the preparation of cell suspensions and culture media are shown to be free from extraneous agents. The cell culture medium may contain a pH indicator such as phenol red and suitable antibiotics at the lowest effective concentration. It is preferable to have a substrate free from antibiotics during production. Not less than 500 ml of the production cell culture is set aside as uninfected cell cultures (control cells). The viral suspensions are harvested at a time appropriate to the strain of virus being used.

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

Identification. The single harvest contains virus that is identified as measles virus by serum neutralisation in cell culture, using specific antibodies.

Virus concentration. The virus concentration in the single harvest is determined as prescribed under Assay to monitor consistency of production and to determine the dilution to be used for the final bulk vaccine.

Extraneous agents (2.6.16). The single harvest complies with the tests for extraneous agents.

Control cells. If human diploid cells are used for production, the control cells comply with a test for identification. They comply with the tests for extraneous agents (2.6.16).

FINAL BULK VACCINE
Virus harvests that comply with the above tests are pooled and clarified to remove cells. A suitable stabiliser may be added and the pooled harvests diluted as appropriate.

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

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

FINAL LOT
A minimum virus concentration for release of the product is established such as to ensure, in the light of stability data, that the minimum concentration stated on the label will be present at the end of the period of validity.

Only a final lot that complies with the requirements for minimum virus concentration for release, with the following requirement for thermal stability and with each of the requirements given below under Identification and Tests may be released for use. Provided that the test for bovine serum albumin has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.