CHAPTER 26

Purified Vero cell vaccine for humans

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The previous chapter described the rabies vaccine for human use prepared in human diploid cells. In spite of its safety and high immunogenicity, the relatively low titre of virus production by these cells constituted a limitation to the large-scale production of a comparatively cheap rabies vaccine of equal quality. The inactivated poliomyelitis vaccine was the first vaccine made using this cell substrate, and led to the revision of the requirements for the vaccine by the WHO Expert Committee on Biological Standardization (1).

The technique developed by van Wezel (2), consisting of the culture of cells on microcarriers, stimulated large-scale cultures of cells for human vaccine preparations. Following the production of the inactivated poliomyelitis vaccine in Vero cells (3), studies were carried out to develop a human rabies vaccine. The resulting vaccine, which required a purification step in order to remove the residual cellular DNA, is known as the purified Vero cell rabies vaccine (PVRV) (4–6).

Cell cultures

The Vero cell line was established in 1962, starting from a primary culture of vervet monkey (Cercopithecus aethiops) kidney cells (7). After further passages, the cell line was transferred to the American Type Culture Collection (ATCC) and in 1979 a primary cell bank (PCB) was established, starting from one ampuole of the cells at the 124th passage level (8 & 9). The PCB was used to prepare several manufacturers' working cell banks (MWCBs). The former was tested according to the existing requirements for continuous cell lines used for inactivated virus vaccine production (10); for the latter, the tests were completed taking into account the latest requirements available (16).

As potential tumorigenicity was the main concern associated with the use of these cell lines, studies were initiated using athymic nude mice and Syrian hamsters. The tests were negative in the hamsters, while the mice showed evidence of nodule formation at the inoculation site. However, even with well-known tumorigenic cells, such as HeLa or Hep 2 cells, no metastasis was observed in inoculated animals. When immunosuppressed newborn rats were inoculated with the tumorigenic cell lines, over 90% of the animals were positive for progressively growing tumours, and over 40% showed evidence of metastases in the lungs 3 weeks later (11). Inoculation with Vero cells had no effect until the 130th passage level. Accordingly, the 137th passage level was used for the MWC and the 142nd passage level was used for the production cell culture.

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The Vero cell line has been characterized according to the requirements for continuous cell lines used for inactivated virus production (7). The identity of the cell line was tested using isoenzyme analysis and DNA fingerprinting after restriction enzyme digestion. Tests for the presence of bacteria, fungi, mycoplasmas and viral contaminants (including retroviruses) in the MWCB and the production cell cultures were constantly negative.

Preparation of the vaccine

Seed lot of virus

For the production of the seed lot, the Pitman-Moore (PM) strain of fixed rabies virus is used, after adaptation to growth in WI-38 cells. The infectivity titre of the virus in the seed lot should be tested by intracerebral inoculation of mice and should be no less than $10^{6.7}$ LD$_{50}$ (median lethal dose) per ml.

Preparation of cells

Each batch of vaccine is produced starting from a working cell bank, consisting of a single ampoole of the MWCB at the 137th passage level. The cells are expanded by serial subculture up to the 112nd passage level. The area of microcarrier available to the cells increases from about 0.6 m$^2$ at the 137th passage level to 900 m$^2$ (3 g/litre) at the 142nd passage level. The culture medium consists of Eagle's minimum essential medium (EMEM; see Chapter 8, Annex 1), supplemented with 4–10% fetal calf serum (FCS) and neomycin, polymyxin B and dihydrostreptomycin sulfate.

After each passage (equivalent to a population doubling level of 2–4), the cells are removed from the microcarrier by trypsinization and homogenized in fresh EMEM supplemented with 4–10% FCS. The resulting cell suspension is then used as the inoculum for the next passage. At the 141st passage level, samples should be removed for testing for adventitious agents (see page 287).

Infection of cells

1. Inoculate freshly trypsinized cells at the 142nd passage level with the seed virus at an input multiplicity of infection of about 1 LD$_{50}$ per 1000 cells.
2. Seed the cells in culture flasks containing microcarriers and add culture medium (see above). Incubate at 31 °C for about 3 days until a complete monolayer is formed. Remove samples for testing for bacterial contaminants and adventitious agents.
3. Discard the culture medium and rinse the microcarriers several times with EMEM without serum.
4. Replace the culture medium by EMEM supplemented with 0.3% human albumin.
5. Incubate the flask for a further 6 days at 37 °C. Remove samples for testing for bacterial contaminants and adventitious agents.
6. Harvest the culture supernatants.

Generally, 5–6 harvests can be collected over a 3-week period.
Clarification of the virus
The harvested virus suspension is clarified by filtration through a 0.45-μm membrane filter.

Concentration of the virus
The virus suspension is concentrated 10–25-fold by ultrafiltration through membranes with a relative molecular mass cut-off of 10,000.

Inactivation of the virus
The virus suspension is inactivated using β-propiolactone (see Chapter 20). Samples are removed for testing for the absence of live virus (see page 288). After inactivation, the suspension is again concentrated by ultrafiltration and stored at -40°C.

Purification of the virus
When the above tests are completed satisfactorily, the aliquots of every individual harvest are thawed, pooled and purified by sucrose-density centrifugation at 90,000 g (Beckman rotors) for 3 hours. The purified fractions are diluted with phosphate-buffered saline (PBS) and clarified by filtration through a 0.45-μm membrane. The samples are stored at -40°C.

Production of the bulk vaccine
An aliquot of the bulk virus suspension is thawed and diluted with EMEM supplemented with 8% human serum albumin to a potency of 2.5 IU per ml, and then filtered through a 0.45-μm membrane.

Production of the final vaccine
The bulk vaccine is distributed in 1-ml amounts into vials, freeze-dried and sealed.

Control tests
Tests on the cell culture
Absence of extraneous agents
At least 10% of the cell suspension at the 141st passage level should be used to prepare control cultures. These cultures should be processed in the same way as the production cell cultures, but not inoculated with the virus. They should be observed for at least 7 weeks (until the final harvest of virus is completed) for evidence of any cytopathological changes.

At the end of the observation period, the control cell cultures should be examined. If this examination shows evidence of the presence of any extraneous agent, the virus grown in the inoculated cultures should not be used for vaccine production. The cell-culture supernatants should be pooled and tested for
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Infectivity in fresh Vero cells. A sample should be removed for haemadsorption tests using guinea-pig erythrocytes.

Tests on the final vaccine

The final vaccine should meet the requirements for human vaccines prepared in continuous cell lines published by WHO (10, 12, 13). Each final lot of vaccine should be tested for purity, safety, residual cellular DNA and potency.

Tests for residual DNA

Acceptable limits of cellular DNA per dose of the final vaccine should be determined by the rational control authority, taking into account the effect of the inactivation procedure on the biological activity of DNA as well as previous experience with DNA levels in rabies and other vaccines produced in various systems (12, 13). The quantity of residual cellular DNA is generally expressed in picograms (pg) per dose.

A recent collaborative study to examine the reliability of assays for the measurement of residual DNA in biological products derived from continuous cell lines revealed both a marked degree of inaccuracy and large differences in the estimates made by different laboratories (14). Accordingly, care is required in interpreting data obtained using hybridization techniques (15).

Potency test

The potency of each final lot of PVRV is determined by the NIH test (see Chapter 37). It should be no less than 2.5 IU per dose. The potency of the vaccine has been shown to be maintained for at least 36 months at 4°C, 30 months at 37°C, and at least 12 months at 45°C.

Expiry date

The vaccine may be used up to 36 months from the date of release from storage. It should be kept between 2°C and 8°C after release.

References


