CHAPTER 27

# Purified chick-embryo cell vaccine for humans

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This vaccine is prepared in primary chick embryo cells derived from specific pathogen-free (SPF) eggs. It is a freeze-dried preparation consisting of purified and concentrated rabies virus antigen inactivated with β-propiolactone.

## History

Early tissue-culture studies revealed that the low egg passage (LEP) Flury strain of rabies virus showed favourable characteristics as a vaccine strain (*1*). On the basis of these results, an inactivated vaccine for veterinary use was developed in 1973 with the Flury-LEP strain propagated in a primary chick-embryo cell system. The first steps towards the production of a tissue-culture vaccine for human use were begun with the concentration and purification of rabies virus by ultracentrifugation in a sucrose-density gradient (*2*). The antibody-binding test was modified to permit quantitative determination of inactivated rabies virus antigen (*3*). During the 1980s, a variety of other laboratory tests were carried out on pilot batches of the purified chick-embryo cell (PCEC) vaccine (*4, 5*) and clinical trials were initiated in humans (*6, 7*). The efficacy of the vaccine was also extensively tested under field conditions (*8–13*).

## Preparation of the vaccine

### *Seed lot of virus*

For the production of the master seed lot, the Flury-LEP strain of fixed rabies virus is used, after adaptation to growth in primary SPF chick embryo cells.

The origin of this virus is shown below.

59th passage level in primary chick embryo cells (obtained from ATCC)[3]

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primary hamster kidney cells (90 passages)

↓

human diploid cell strain WI-38 (12 passages)

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primary chick embryo cells (25 passages) (LEP-C25)

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[3] American Type Culture Collection.

The master seed lot (LEP-C25) represents the 25th passage level of the virus in primary SPF chick embryo cells. The working seed lot (LEP-C26) represents the 26th passage level of the virus.

Both the master seed lot and the working seed lot have been shown to be free from foreign viruses, mycoplasms, bacteria and fungi by procedures recommended for rabies vaccines for human use (*14, 15*). The master seed lot and the working seed lot are stored at − 80 °C and − 190 °C respectively.

### *Cell cultures*

The virus is cultivated in primary SPF chick embryo cells, which are propagated and controlled according to the recommendations published by WHO (*14–16*) and the regulations of national authorities.

Fertile hens' eggs incubated for 7–9 days at 36.5 °C are used. The eggs are obtained from an SPF flock and transferred to the laboratory 1 day before inoculation.

1. On the 7th day of incubation, candle the eggs and mark the edge of the air sac. Discard any eggs that contain dead or underdeveloped embryos.
2. Disinfect the shell with 70% ethanol and briefly flame it.
3. Place the eggs in a tray in a biological safety cabinet (class II), and remove the live embryos by one of the methods described in Chapter 23 (page 263).
4. Decapitate the embryos (discarding the heads) and place them in a chilled container.
5. Weigh the container with the collected embryos. Sufficient chick embryos should be collected to provide cell culture for one batch of vaccine.
6. Add 0.25% trypsin solution to the embryos. Wash the resulting cell suspension with PBS and resuspend in Eagle's minimum essential medium (see Chapter 8, Annex 1) supplemented with 0.3% human albumin to a final concentration of $0.8–1.2 \times 10^6$ cells per ml. Reserve at least 5% of the cell suspension to prepare control cultures (see page 293).

### *Infection of cells and harvest of the virus*

With the exception of step 3, the following steps should be carried out in a biological safety cabinet (class II).

1. Inoculate freshly trypsinized cells in suspension with the working stock virus at an input multiplicity of infection predetermined to infect most cells after 3–4 days.
2. Seed the cells into flat-bottomed culture flasks and Leighton tubes and add culture medium.
3. Incubate for 3–4 days at 34–36 °C until a complete rabies-infected monolayer can be observed by fluorescent microscopy on the Leighton tubes.
4. Harvest the culture supernatant containing the virus. Remove samples for testing for bacterial contaminants and adventitious agents.
5. Add fresh culture medium and incubate the flasks for a further 3–4 days at 34–36 °C. Harvest the culture supernatant. Remove samples for testing for bacterial contaminants and adventitious agents.

### *Clarification of the virus*

The harvested virus should be filtered to remove cell debris.

### *Inactivation of the virus*

The virus is inactivated with β-propiolactone (see Chapter 20, page 235). After inactivation, the virus suspension is kept between 2 and 6 °C.

### *Concentration and purification of the virus*

The virus suspension is concentrated and purified by centrifugation at 90000 *g* in a sucrose-density gradient (0–60%). A band is formed at about 36% sucrose. This band, which contains the virus, is collected in fractions at the end of the centrifuge run. The virus is stored at − 70 °C until all the control tests are completed (see below). The potency of the concentrated and purified virus suspension should be between 150 and 500 IU/ml.

### *Preparation of the final vaccine*

The purified and concentrated virus suspension is mixed with a stabilizer solution containing degraded gelatin in TEN (trometamol–edetic acid[1]–sodium chloride) buffer solution and distributed in 1.0-ml amounts into vials, which are freeze-dried under vacuum and sealed. The final vaccine should have a minimum potency, as determined by the NIH test (see Chapter 37) and the modified antibody-binding test (see Chapter 43), of 2.5 IU per dose.

## Control tests

### *In-process controls*

Representative samples are removed at each stage of the production process ("in-process controls") for testing according to *The International Pharmacopoeia* (*17*) and the requirements for rabies vaccines for human use published by WHO (*14*, *15*).

### *Potency tests*

Studies of tests for evaluating the potency of rabies vaccines have shown that the NIH test measures only a fraction of the potency of PCEC vaccine compared with other potency tests. Since Challenge Virus Standard (CVS), a derivative of the Pasteur strain, is recommended as the challenge strain for the NIH test, vaccines prepared with viruses other than the Pasteur strain (such as the PCEC vaccine) may appear to be of lower potency than expected when tested in mice challenged intracerebrally with CVS. However, this finding does not appear to influence the

[1] Also known as ethylenediamine tetraacetate or EDTA.

efficacy of PCEC vaccine in the field. Various comparative studies on PCEC vaccine have revealed that the ratio of potency values as determined by the NIH test and other tests is 1:2.2 (*18–20*).

### *Stability test*

The stability of the PCEC vaccine was evaluated by the NIH test using different lots of vaccine which were stored at different temperatures. The results are shown in Table 27.1.

The shelf-life of the vaccine is controlled by storing samples of the final vaccine at different temperatures and determining the potency by the NIH test and the modified antibody-binding test conducted in parallel.

### *Tests on the cell culture*

At least 5% of the working cell suspension is used to prepare control cultures. These cultures are processed in the same way as the production cell cultures, but not inoculated with the virus. They are tested as described in Chapter 25.

## Administration of the vaccine

The vaccine dose is 1.0 ml administered intramuscularly into the deltoid region of the arm or, in the case of small children, into the anterolateral aspect of the thigh.

### *Pre-exposure immunization*

One dose of vaccine is administered intramuscularly on days 0, 7 and 28.

### *Post-exposure treatment*

One dose of vaccine is given intramuscularly on days 0, 3, 7, 14 and 30.

## Expiry date

The vaccine may be used up to 3 years from the date of release. It should be kept between 2 and 8 °C.

***Table 27.1 Potency of the PCEC vaccine after storage at different temperatures***

| Temperature (°C) | Period of storage (in months) | Number of vaccine lots tested | Change in potency (%) |
|---|---|---|---|
| 2–8 | 57 | 2 | – 4.8 |
| 20 | 12 | 5 | – 6.2 |
| 31 (with 75% relative humidity; tropical conditions) | 24 | 5 | – 11.4 |
| 37 | 6 | 4 | – 35.7 |
| 55 | 24 | 4 | – 26.6 |

## Laboratory tests

Numerous laboratory tests were carried out on the vaccine before clinical trials in humans were begun. They included:

1. Tests for freedom from residual chick-embryo cell protein:
   —agar gel precipitation;
   —immunoprecipitation and SDS–PAGE;
   —studies in guinea-pigs (immunization with the vaccine, followed by intravenous administration of chick-embryo cell protein).
2. Innocuity tests in monkeys and dogs.
3. Tests for the absence of pyrogens in rabbits.
4. Studies on the induction of neutralizing antibodies in sera from mice and monkeys immunized with the vaccine.
5. Post-exposure efficacy studies with the vaccine in mice and guinea-pigs compared with HDC vaccine.
6. Potency tests, including the NIH test and *in vitro* tests such as the antibody-binding test.
7. Stability tests following storage at high temperatures.

## References

1. Barth R, Jaeger O. Untersuchungen mit einigen Tollwutvirusstämmen in verschiedenen Gewebekultursystemen. [Studies with several rabies virus strains in various tissue-culture systems.] *Zentralblatt für Veterinärmedizin, Reihe B*, 1970, 17: 363–380.

2. Hilfenhaus J et al. Large-scale purification of animal viruses in the RK-model zonal ultracentrifuge. *Journal of biological standardization*, 1976, 4: 263–271.

3. Barth R et al. The antibody-binding test: a useful method for quantitative determination of inactivated rabies antigen. *Journal of biological standardization*, 1981, 9: 81–89.

4. Barth R et al. A new inactivated tissue-culture rabies vaccine for use in man. Evaluation of PCEC vaccine by laboratory test. *Journal of biological standardization*, 1984, 12: 29–46.

5. Barth R et al. Purified chick-embryo cell (PCEC) rabies vaccine for human use—laboratory data. In: Kuwert E et al., eds. *Rabies in the tropics*. Berlin, Springer-Verlag, 1985: 117–124.

6. Bijok U et al. Clinical trials in healthy volunteers with the new purified chick-embryo cell rabies vaccine for man. In: Kuwert E et al., eds. *Rabies in the tropics*. Berlin, Springer-Verlag, 1985: 125–132.

7. Bijok U. Purified chick-embryo cell (PCEC) rabies vaccine: a review of the clinical development 1982–1984. In: Vodopija I et al., eds. *Improvements in rabies post-exposure treatment*. Zagreb, Zagreb Institute of Public Health, 1985: 103–111.

8. Vodopija I, Smerdel S, Bijok U. PCEC rabies vaccine and HRIG in post-exposure protection against rabies. In: Kuwert E et al., eds. *Rabies in the tropics*. Berlin, Springer-Verlag, 1985: 133–137.

9. Petrović M, Petrović M, Damjanović J. Laboratory examination of PCEC rabies vaccine produced by the Behring Institute, Marburg, FRG. In: Vodopija I et al., eds. *Improvements in rabies post-exposure treatment*. Zagreb, Zagreb Institute of Public Health, 1985: 63–69.

10. Sehgal S. Report of the trials of PCEC (purified chick-embryo cell) rabies vaccine in India. In: Vodopija I et al., eds. *Improvements in rabies post-exposure treatment*. Zagreb, Zagreb Institute of Public Health, 1985: 71–75.

11. Ljubičić M et al. Experience with PCEC rabies vaccine in healthy adults. In: Vodopija I et al., eds. *Improvements in rabies post-exposure treatment*. Zagreb, Zagreb Institute of Public Health, 1985: 77–84.

12. Wasi C et al. Immunogenicity and reactogenicity of the new tissue culture rabies vaccine for human use (purified chick-embryo cell culture). In: Vodopija I et al., eds. *Improvements in rabies post-exposure treatment*. Zagreb, Zagreb Institute of Public Health, 1985: 85–94.

13. Ljubičić M et al. Efficacy of PCEC vaccine in post-exposure rabies prophylaxis. In: Vodopija I et al., eds. *Improvements in rabies post-exposure treatment*. Zagreb, Zagreb Institute of Public Health, 1985: 95–101.

14. Requirements for rabies vaccine for human use. *WHO Expert Committee on Biological Standardization. Thirty-first report*. Geneva, World Health Organization, 1981 (WHO Technical Report Series, No. 658), Annex 2.

15. Requirements for rabies vaccine for human use (amendment 1992). *WHO Expert Committee on Biological Standardization. Forty-third report*. Geneva, World Health Organization, 1994 (WHO Technical Report Series, No. 840), Annex 4.

16. *Acceptability of cell substrates for production of biologicals. Report of a WHO Study Group*. Geneva, World Health Organization, 1987 (WHO Technical Report Series, No. 747).

17. *The International Pharmacopoeia*, 3rd ed. Geneva, World Health Organization, 1979 (Volume 1: general methods of analysis); 1981 (Volume 2: quality specifications); 1988 (Volume 3: quality specifications); 1994 (Volume 4: tests, methods, and general requirements; quality specifications for pharmaceutical substances, excipients, and dosage forms).

18. Barth R, Diderrich G, Weinmann E. NIH test, a problematic method for testing potency of inactivated rabies vaccine. *Vaccine*, 1988, 6: 369–377.

19. Barth R et al. Purified chick-embryo cell (PCEC) rabies vaccine: its potency performance in different test systems and in humans. *Vaccine*, 1990, 8: 41–48.

20. Barth R. The NIH test and its problems. In: Thraenhart O et al., eds. *Progress in rabies control. (Proceedings of the Second International IMVI Essen/WHO Symposium on "New Developments in Rabies Control", Essen, 5–7 July 1988 and Report of the WHO Consultation on Rabies, Essen, 8 July 1988.)* Royal Tunbridge Wells, Wells Medical, 1988: 281–287.