

## Vaccine for humans prepared in human diploid cells

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An inactivated rabies vaccine for human use was first prepared in cell culture in 1964 (1). In 1966 it was shown that the human diploid cell (HDC) strain WI-38 was a suitable substrate for the propagation of the Pitman-Moore (PM) strain of fixed rabies virus (2). The original procedure for the production of this vaccine was described in the previous edition (3). Since 1967, research and development have been carried out on this vaccine at the Mérieux Institute. The vaccine was first licensed for use in France in 1974 (4) and commercial production started in 1978.

### Preparation of the vaccine

#### *Cell cultures*

The virus is now cultivated in MRC-5 human diploid cells (5), which are propagated and controlled according to the recommendations published by WHO (6–8) and the regulations of national authorities.

#### *Seed lot of virus*

The working seed lot is prepared from the PM strain and frozen in aliquots at  $-70^{\circ}\text{C}$ . Each aliquot is used to produce a single batch of vaccine. Infectious titrations of the virus are performed by intracerebral inoculation of adult mice. When kept at  $-70^{\circ}\text{C}$ , the virus suspension is perfectly stable for at least 11 years. The infectivity titre of the virus in the seed lot should not be lower than  $10^{6.7}$  LD<sub>50</sub> per ml.

#### *Infection of cultures and propagation of virus*

Every batch of vaccine is produced starting from a working cell bank, consisting of a single ampoule of the cell seed at the sixteenth population doubling level (PDL). The cells are expanded by serial subculture up to the 29.5th PDL (see Annex).

#### *Infection of cells in suspension*

1. Inoculate freshly trypsinized cells at the twenty-seventh PDL in suspension with the seed virus at an input multiplicity of 1 LD<sub>50</sub> per 25–50 cells and stir with a magnetic stirrer for 15 minutes at  $37^{\circ}\text{C}$ .

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2. Seed the cells in culture flasks and add culture medium. The culture medium consists of the standard Eagle's basal medium (EBM) (see Chapter 18, Annex) containing 2.5 g of sodium bicarbonate ( $\text{NaHCO}_3$ ) and 5 mg of neomycin sulfate per litre and supplemented with 10% fetal calf serum. It should be gassed with carbon dioxide ( $\text{CO}_2$ ) before use.
3. Incubate at 37 °C for 3–4 days until a complete monolayer is formed. The cells should be at the 28.5th PDL. Remove samples for testing for bacterial contaminants and adventitious agents.
4. Wash the cells with phosphate-buffered saline (PBS).
5. Trypsinize the cells and replant in equal portions in two new culture flasks.
6. Incubate at 37 °C for 3–4 days in culture medium (see step 2).
7. Discard the supernatant containing fetal calf serum and wash the cells three times with a protein-free medium such as EBM to remove any remaining bovine proteins.
8. Replace the culture medium by virus propagation medium, consisting of EBM containing 4 g of sodium bicarbonate and 5 mg of neomycin sulfate per litre and supplemented with 0.3% human albumin. It should be gassed with  $\text{CO}_2$  before use.
9. Incubate the culture flasks for 3–4 days at 35 °C. Remove samples for testing for bacterial contaminants and adventitious agents.
10. Harvest the culture supernatants three times at intervals of 3–4 days.

#### ***Clarification of infectious medium***

The harvested virus is clarified by filtration through a 0.8- $\mu\text{m}$  membrane. Virus samples are pooled to constitute the bulk virus suspension.

#### ***Concentration and purification of the virus***

The bulk virus suspension is concentrated and purified by ultrafiltration through a membrane with a relative molecular mass cut-off of 10 000, followed by filtration through a 0.45- $\mu\text{m}$  membrane.

#### **Control tests**

##### ***Testing of the cells***

The cell seed should be approved by and registered with the national control authority and should comply with the requirements for rabies vaccine for human use published by WHO (6, 8) for freedom from extraneous agents, lack of tumorigenicity, normal karyology throughout approximately the first two-thirds of its normal life-span, and identity.

##### ***Testing of the virus strain***

The virus strain to be propagated in diploid cells should be a fixed strain and should be identified by historical records. The strain should have a short, stable and reproducible incubation time when administered intracerebrally to suitable

animals and should not form Negri bodies. In addition, it should be shown, by tests in animals and cell cultures, to be free from extraneous agents.

### **Tests on the cell culture**

#### *Absence of extraneous agents*

At least 10% of the cell suspension at the twenty-sixth PDL 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 2 weeks 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 in a control culture of any extraneous agent, the virus grown in the corresponding inoculated cultures should not be used for vaccine production.

#### *Other tests*

At the time of the harvest of the production cultures, the supernatants of the control cultures should be collected, pooled and tested for mycoplasmas and for sterility. A small quantity of cells should be tested for normal karyology.

### **Tests on inactivated virus**

#### *Test for absence of live virus*

This test is carried out on the inactivated virus 10, 12, 14 and 26.5 hours after the addition of  $\beta$ -propiolactone. A sample (0.03 ml) of the virus suspension is inoculated intracerebrally into a group of 20 adult mice, which are observed for 21 days. The test is satisfactory if none of the mice show any signs of rabies.

### **Tests on bulk vaccine**

#### *Test for absence of live virus*

At least 25 ml of the bulk vaccine is inoculated into human diploid cell cultures, which are observed for 21 days. At day 14 and 21, supernatant samples are removed and inoculated intracerebrally into a group of 20 adult mice, which are observed for 21 days. The test is satisfactory if none of the mice show any signs of rabies.

#### *Test for glycoprotein content*

The glycoprotein content of the bulk vaccine is determined by *in vitro* tests such as single radial immunodiffusion, radioimmunoassay (9) and the antibody-binding test (10) (see Chapters 40 and 43).

#### *Other tests*

Tests for sterility and for freedom from mycoplasmas are performed.

### **Tests on final vaccine**

#### *Potency test*

The potency of each final lot of vaccine is determined by the NIH test (9) (see also

Chapter 37). The potency should be no less than 2.5 IU per human dose and should be measured after 1 month at 4 °C and 37 °C.

#### *Test for glycoprotein content*

The glycoprotein content of each final lot of vaccine is determined by *in vitro* tests (see above).

#### *Other tests*

Each final lot of vaccine is tested for appearance, residual moisture content, total protein content, sterility and safety.

### **Conclusion**

Since 1974, more than 10 million doses of this vaccine have been used in humans, the majority for post-exposure treatment. It has been shown to be safe and effective when used properly.

### **References**

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## LABORATORY TECHNIQUES IN RABIES

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### Annex

#### Flow chart for the production of HDC vaccine using the MRC5 cell strain

Day	Passage or population doubling level (PDL)	Step	Container
0	16		One thawed ampoule of seed virus
6 or 7	18		One 75-ml culture flask
10 or 11	20		Two 75-ml culture flasks
13 or 14	22		Culture flasks up to 1.2 litres
16 or 17	24		Culture flasks up to 4.8 litres
21 or 22	26		Culture flasks up to 19.2 litres
24 or 25	27	Inoculation of the seed virus	Culture flasks up to 38.4 litres
27 or 28	28.5		Culture flasks up to 115.2 litres
30 or 31	29.5	First 2 rinsings	Culture flasks up to 230.4 litres
31 or 32	29.5	3rd rinsing	
34 or 35	29.5	1st harvest	
38 or 39	29.5	2nd harvest	
42 or 43	29.5	3rd harvest	
43	29.5	Concentration of the virus	
44	29.5	Inactivation with BPL	
45	29.5	Inactivation with BPL	
		Control tests	
		Distribution of the final vaccine into ampoules	
		Lyophilization	