

Production of human rabies immunoglobulin¹

P. Fournier² & R. K. Sikes³

Introduction

The combination of local treatment of the wound, passive immunization with rabies immunoglobulins and vaccination is recommended for all severe (category III) exposures to rabies (1). The use of homologous immunoglobulins for human post-exposure treatment virtually eliminates the risk of anaphylaxis and serum sickness associated with heterologous serum products. In 1965, approximately 16% of persons treated with antirabies serum of equine origin were reported to have developed serum sickness; among persons over 15 years of age, the incidence was 46% (2). In the past few years, purified equine immunoglobulins have become available, and in recent studies, the incidence of serum sickness among recipients was reported to be < 1–6.2% (3–6).

To avoid such reactions, human rabies immunoglobulin (HRIG) preparations have been developed and used for post-exposure treatment in most industrialized countries. HRIG is well tolerated, but it is expensive and available in only limited quantities. Since 1975, this product has been administered to more than 250 000 people in the United States and no cases of serum sickness have been reported.

Formula

HRIG for intramuscular administration is a 10–18% solution of immunoglobulin in 0.3 mol/l glycine, and preserved with a 1:10 000 thiomersal⁴ solution. The rabies virus-neutralizing antibody content is standardized to contain 150 International Units (IU) per ml. It is supplied in 2-ml (300 IU) and 10-ml (1500 IU) vials for paediatric and adult use respectively.

Source and shipment of blood

Donors of plasma for the production of HRIG should have demonstrated high levels of virus-neutralizing antibody (at least 5 IU/ml) following pre-exposure or post-exposure immunization with a potent cell-culture rabies vaccine, preferably human diploid cell (HDC) vaccine. One or more booster immunizations with HDC vaccine should be given 1–2 weeks before the first collection of blood. In order to prepare HRIG of sufficient potency, and assuming a 15–20-fold concentration of antibody during preparation, it is necessary to start with a rabies immune plasma

¹ Based on the chapter by R.K. Sikes in the previous edition.

² Former Production Manager, Sera and Vaccines, Pasteur-Mérieux Institute, Marcy l'Etoile, France.

³ Former Veterinary Director, Office of Veterinary Public Health Services, Centers for Disease Control and Prevention, Atlanta, GA, USA.

⁴ Also known as thimerosal and mercurthiolate.

pool containing 15 IU per ml (as measured by the RFFIT). Studies have shown that, following a booster dose of antirabies vaccine, about 10% of people who have received pre-exposure prophylaxis and about 40% of those with a history of post-exposure treatment will develop antibody titres of sufficient levels.

The collecting centre may obtain a unit of plasma by plasmapheresis or by separation of a unit of whole blood aseptically. The plasma is shipped refrigerated to the laboratory and is stored frozen until it is ready to be pooled for fractionation.

Reagents

The preparation and storage of the reagents required are described in the Annex.

Technique

The immunoglobulins are separated from the plasma by the cold ethanol fractionation technique described below (7).

Precipitation of fraction I

1. Pool the plasma; pour individual units through cheesecloth into a tared vat large enough to contain 1.2 litres per litre of plasma.
2. Determine the pH; if necessary, adjust to 7.0–7.4 with acetate buffer (80-fold concentrate) or 0.5 mol/l sodium phosphate solution.
3. Freeze three 15-ml samples of pooled plasma.
4. Determine the weight of plasma.
5. Keeping the plasma at 0 °C, add 163 g of cold 95% ethanol per kg of plasma. Adjust the rate so that the addition of ethanol takes about 1 hour. Reduce the temperature to –2 °C during the addition. Stir for 30 minutes after the addition of ethanol is completed.
6. Centrifuge at 10 000 *g* for 30 minutes, collecting the centrifugate in a tared vat large enough to contain 1.7 litres per litre of original plasma. Maintain the temperature at –2 °C to –3 °C during centrifugation. Freeze a 15-ml sample of supernatant I (SI) and weigh it. Keep the sample at –2 °C, and begin precipitation of fraction II–III.

Precipitation of fraction II–III (P2)

1. For every kg of supernatant I, add 552 g of 95% ethanol to which has been added 1.33 ml of acetate buffer, 80-fold concentrate. Adjust the rate so that the addition of the ethanol takes about 1 hour. Cool to –9 °C during the addition.
2. Stir at –9 °C for 30 minutes after the addition of ethanol is completed.
3. Centrifuge at 10 000 *g* for 30 minutes, collecting the centrifugate in a tared bowl. Maintain the temperature at –6 °C to –9 °C during centrifugation.
4. Determine the weight of the precipitate, which is fraction II–III (P2). Freeze in the bowl at –20 °C, and hold for precipitation of fraction II–IIIw.

Precipitation of fraction II–IIIw (P2w)

1. Remove frozen P2 from the centrifuge bowl and rapidly homogenize to a

- uniform suspension in a mixture of water and crushed ice. Use 2 g of water-and-ice mixture per gram of P2. Avoid excessive foaming.
- For every gram of P2 immediately add a mixture containing 0.107 ml of 0.5 mol/l sodium phosphate, dibasic (Na_2HPO_4) in 2.89 g of water and crushed ice.
 - Stir in the cold until all the solid is in suspension.
 - Pour into a vat containing 20 g of cold water per gram of P2, and stir at 1 °C for 30 minutes or until no ice remains in the mixture. The vat should be large enough to contain 4 litres per 100 g of P2.
 - Remove 5 ml of the mixture from the vat, add 5 ml of 0.15 mol/l sodium chloride (NaCl) solution, and determine the pH; if necessary, adjust the pH of the mixture to 7.0–7.4 by adding acetate buffer (80-fold concentrate) or 0.5 mol/l sodium phosphate, dibasic, solution.
 - Freeze a 15-ml sample.
 - Add 14.1 g of 95% ethanol per gram of P2; adjust the rate so that the addition of the ethanol requires about 1 hour. Cool to -6°C during this step. Stir at -6°C for 2–4 hours after the addition of ethanol is completed.
 - Centrifuge at 10 000 g for 30 minutes and collect the centrifugate in a tared bowl. Maintain the temperature at -5°C to -7°C during centrifugation.
 - Freeze a 15-ml sample of supernatant II–IIIw (S2w).
 - Determine the weight of precipitate, which is fraction II–IIIw (P2w). Freeze in the bowl at -20°C and hold for precipitation of fraction III.

Precipitation of fraction III

- Remove the frozen P2w from the bowl, and rapidly homogenize to a uniform suspension in a mixture of crushed ice and water. Use 2 g of water-and-ice mixture per gram of P2w. Avoid excessive foaming.
- Immediately add 2 ml of cold 0.175 mol/l sodium acetate solution per gram of P2w, and stir in the cold until all the solid is in suspension.
- Adjust the pH to 5.2 ± 0.1 by adding acetate buffer (80-fold concentrate), diluted 1:25 with cold water, and then add more cold water until the total amount added is 1 ml per gram of P2w.
- Stir in the cold for 1 hour or until no ice remains in the mixture. Determine the pH and adjust, if necessary, to the range 5.1–5.3.
- Pour into a vat containing 13.5 g of cold water per gram of P2w. The vat should be large enough to contain 2.9 litres per 100 g of P2w.
- Add 8.1 g of 95% ethanol per gram of P2w; adjust the rate so that the addition of ethanol requires about 1 hour. Cool to -6°C during this step. Stir at -6°C for 30 minutes after the addition of ethanol is completed.
- Centrifuge at 10000 g for 30 minutes, collecting the centrifugate in a cold pressure tank and filter into a tared vat. The vat should be large enough to contain 3.1 litres per 100 g of P2w. Use a 1.2- μm epoxy reinforced glass filter over a 0.8- μm membrane. Maintain the temperature at -5°C to -6°C during centrifugation and filtration.
- Freeze a 15-ml sample of supernatant III (S3).
- Determine the weight of S3, maintain at -6°C , and proceed with the precipitation of fraction II.

Precipitation of fraction II

1. Stir S3 vigorously at -6°C , and for every kg of this solution slowly add 2 g of sodium bicarbonate (NaHCO_3).
2. Add 2 ml of the vat mixture to 8 ml of 0.15 mol/l sodium chloride (NaCl) solution, and determine the pH; if necessary, add additional sodium bicarbonate to adjust the pH to 7.2–7.6.
3. Add 94.7 g of 95% ethanol per kg of solution. Adjust the rate so that the addition of ethanol requires about 1 hour. Cool to -9°C during this step.
4. Centrifuge at 10 000 *g* for 30 minutes, collecting the centrifugate in a tared bowl. Maintain the temperature at -6°C to -9°C during centrifugation.
5. Determine the weight of fraction II, freeze in the bowl at -20°C , and hold for lyophilization.

Fraction II lyophilization and final preparation

1. Remove the frozen fraction II from the bowl, and rapidly homogenize to a uniform suspension in cold 0.3 mol/l glycine, using 1 ml per gram of frozen paste.
2. Add 4 g of cold water¹ per gram of paste; stir for 2–4 hours in the cold, and leave to stand overnight at 0°C without stirring.
3. Decant the supernatant, lyophilize, and determine the weight of the lyophilized product.
4. For preparation of the final solution of the product, the quantities are calculated as follows:
 - (a) Dry weight of powder = weight of lyophilized powder (g) \times 0.98.
 - (b) Volume of powder (ml) = $a \times 0.75$.
 - (c) Volume of water to be added (ml)
= ml of glycine added before lyophilization – b .
 - (d) Dry weight of globulin (g)
= $a - (0.0225 \times \text{ml of glycine added before lyophilization})$.
 - (e) Final volume (ml) = $d/0.17$.
 - (f) Volume of 0.3 mol/l glycine to be added (ml) = $e - (b - c)$.
 - (g) Volume of 10% thiomersal to be added (ml) = $e \times 10^{-3}$.
5. Put the required volume of water¹ and 0.3 mol/l glycine (see step 4) in a beaker with a magnetic stirring bar, and add the lyophilized globulin. Stir until the globulin is completely dissolved; avoid excessive foaming.
6. Add the required volume of 10% thiomersal (see step 4). A second person should check this calculation and observe the addition. Determine the pH and adjust, if necessary, to 6.8 with acetate buffer, 80-fold concentrate.
7. Filter the globulin solution through depth filters down to $1.2 \mu\text{m}$ and then through membrane filters down to $0.45 \mu\text{m}$.
8. In the sterile room, sterilize the globulin solution by filtration through a $0.2\text{-}\mu\text{m}$ filter into the sterilized bulk product container.
9. Remove a 2-ml sample from the bulk container for sterility testing with liquid thioglycolate medium.

¹ Use Water for Injection, see reference 8.

- Place the bulk container material in a refrigerator at 1–3°C, and remove samples for testing (see below). When all the tests are complete, distribute into final containers.

Testing

Tests for safety and sterility, pyrogenic substances, heat stability, pH and turbidity are needed to ensure that the product satisfies the minimum requirements recommended by the WHO Expert Committee on Biological Standardization (9). For potency requirements, see Chapter 47, page 420.

Disadvantages

HRIG is expensive, because of the cost of HDC vaccine and the time required for rabies virus-neutralizing antibodies to be produced in donors following immunization. Since the manufacturing process requires a supply of donors whose antibody titres are at least 15 IU per ml, the product is also limited in terms of quantity. In spite of these disadvantages, the Immunization Practices Advisory Committee (ACIP) of the United States recommends that HRIG should be used in preference to ERIG (10).

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Annex

Preparation of reagents

All reagents must fulfil the specifications described in *The international pharmacopoeia*.¹

Distilled water

Water is collected from the still directly into a pressure-filtration tank and filtered through a sterile 2- μ m membrane into a sterile dispensing vessel. Except when pyrogen-free water for injection¹ is required, this water is used in all procedures and for making ice and reagents.

95% (v/v) ethanol

95% ethanol is filtered through a 0.2- μ m membrane (not sterile) into dispensing vessels and stored under refrigeration (cold room).

Acetate buffer, 80-fold concentrate

Sodium acetate	108.9 g
Glacial acetic acid	240.2 g
Distilled water to make	1000 ml

When this concentrate is diluted with 80 parts of water, the pH of the buffer should be 3.98–4.02.

¹ See reference 8.