Clostridia (multi-component) rabbit antiserum (for vaccines for veterinary use) BRP batch 1 consists of 1 ml of freeze-dried rabbit serum and is presented in vials. The BRP has been prepared by blending sera obtained by using the immunisation schedule prescribed in the Ph. Eur. monographs Clostridium novyi vaccine for veterinary use, Clostridium perfringens vaccine for veterinary use, Clostridium septicum vaccine for veterinary use and tetanus vaccine for veterinary use (0362, 0363, 0364 and 0697). The titres have been assigned using a toxin neutralisation test in mice. The BRP has an assigned activity of:

- C. perfringens beta antitoxin 10.5 IU per vial
- C. perfringens epsilon antitoxin 11.0 IU per vial
- C. septicum antitoxin 7.5 IU per vial
- C. novyi type B antitoxin 11.0 IU per vial
- C. tetani antitoxin 8.0 IU per vial

The BRP is for use in the context of serological potency assays of Clostridia vaccines for veterinary use by immunochromal methods. The BRP is intended to serve as a primary reference material. It is therefore advised that laboratories establish their own internal reference material by direct comparative assay with the BRP using their own validated immunochromal assay.

The BRP may also be used, in place of the International Standard (equine) antitoxins to establish the activity of an internal reference material using toxin neutralisation.

**STORAGE**

Keep vials unopened at – 20 °C. Do not store at lower temperatures to avoid deterioration of the rubber stoppers.
USE

- Allow the vial and content to reach room temperature.
- Tap vial gently to collect material at the bottom.
- Using an appropriate syringe reconstitute the reference preparation by injecting 1.0 ml of sterile distilled water through the rubber cap. The vial should be gently shaken to ensure that all of the powder dissolves and the dissolved preparation withdrawn using a syringe and needle and placed in a suitable sterile container. To ensure that all the BRP is removed from the vial, 1.0 ml of sterile 0.85% saline solution (or other suitable diluent - see below) should be injected through the cap, mixed, withdrawn and added to the BRP. The vial should be washed in this way at least three times. The reconstituted BRP plus the washings from the vial should then be made up with 0.85 % saline (or other suitable diluent) to a volume suitable for use. This volume will be dependent on the precise nature of the assay to be used but should be designed such that the material can be dispensed into volumes suitable for a single assay. In general it will be appropriate to make the volume up to 10 ml. The prepared solution should then be aseptically dispensed into suitable containers in a volume appropriate to the assay technique. For most assays dispensing in 1.0 ml aliquots will be suitable. Aliquots should then be stored at or below –20 °C. It is recommended that the maximum storage period for the reconstituted material does not exceed 6 months. However, a shorter storage period is advised if the material is stored at a significantly lower concentration than indicated above.

SELECTION OF DILUENT

Initial reconstitution of the BRP should always be made using sterile distilled water. Subsequent washing of the vial and dilution of the material may be made using the diluent normally used for dilution of test samples in the assay method. Suitable diluents include, but are not restricted to peptone water, borate buffered saline, phosphate buffered saline and 0.85% saline.

ADVICE TO LABORATORIES

1). Establishment of in-house reference materials:
The intra-laboratory residual standard deviation of the assigned values of the BRP is approximately 10 % in respect of each component. It is therefore recommended that, when establishing an in-house reference material for routine use in potency tests, laboratories conduct a sufficient number of valid, direct comparative assays between the BRP and their own in-house reference material to permit calculation of an assigned value for the in-house reference material which has a comparable residual standard deviation (i.e. approximately 10 %).

2). Monitoring of reference activity:
The stability of the BRP will be monitored by EDQM. However, stability of in-house reference materials, which may not be lyophilised and may be stored under a variety of conditions should be monitored by the laboratory responsible for establishing those materials.
The most appropriate method will depend upon the precise nature of the immunochemical assay used but it is recommended that stability of these materials be evaluated by means of trend analysis of the results obtained in each assay performed. It is suggested that a rolling mean of these values be calculated using the results obtained in the most recent ten assays. If assays are performed only infrequently it may be more appropriate to establish the rolling mean on the basis of all the results obtained over a defined time period. Any change of more than 10% in the value of the rolling mean should be considered as indicative of a change in the activity of the material and indicate the need to either replace the in-house reference material or reassess its assigned value by direct comparison with the BRP.

3). **Evidence of assay validity:**
The BRP is intended to facilitate the use of immunochemical assay methods in place of toxin neutralisation. However, the relevant Ph. Eur. monographs specify the use of “validated immunochemical methods”. There are numerous aspects to the demonstration of validity of an assay technique which are beyond the scope of this document. However, for the use of the BRP to be valid in any specific technique, it is important that the range of activities over which the response given by the BRP is log-linear is clearly defined and that calculations are based on results obtained only within that range. Similarly, for any in-house reference material calibrated against the BRP, the log-linear range of responses should be defined and the Standard Operating Procedure (SOP) for the assay should ensure that only results falling within that range are used in the calculation of final potency results.

**CAUTION**

Clostridia (multi-component) rabbit antiserum (for vaccines for veterinary use) BRP batch 1 is not appropriate for administration to humans and/or to animals. This preparation must be handled according to the appropriate QA system for biological testing laboratories. Please refer to the corresponding safety data sheet, which can be downloaded from the internet web site of the EDQM ([http://www.edqm.eu](http://www.edqm.eu)) or delivered upon request.

**LITERATURE**