Human vaccines in the European Pharmacopoeia

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Ph. Eur. texts applicable to human vaccines
General chapters of the Ph. Eur. supporting human vaccine monographs

- 2.5 Assays
- 2.6 Biological tests
- 2.7 Biological assays
- 5.2 General texts on biological products

5.2.1 Terminology used in monographs on biological products

- Seed-lot system (MSL/WSL)
- Cell bank system (MCB/WCB)
- Production using cell culture (single/pooled harvest)
- Vaccine final bulk/final lot
- Combined vaccines
5.2.3 Cell substrates for the production of vaccines for human use

Testing requirements for cell seeds, MCB, WCB

Testing methods:

- Identity and purity
- Extraneous agents
- Tumorigenicity
5.2.3 Cell substrates for the production of vaccines for human use

- **Recent update** (*Supplement 9.0*)
- In depth revision to **harmonise with WHO recommendations** (WHO TRS 978 Annex 3 “Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterisation of cell banks”)
- Extraneous agents: greater flexibility; R/A to establish the testing strategy; molecular methods now considered
- Tumorigenicity: deletion of the tests for *in vitro* tumorigenicity
Tests for extraneous agents in viral vaccines for human use (2.6.16)

- Applied to starting materials and substrates used for production and control of viral vaccines
- Panel of *in vivo* and *in vitro* methods
  - Cell culture methods
  - *In vivo* tests using adult mice, suckling mice (IC) and guinea-pigs
- **Drawbacks:**
  - Use of live animals
  - Animals cannot detect all possible agents
  - Feedback from users: time consuming, costly, not sensitive enough
Tests for extraneous agents in viral vaccines for human use (2.6.16)

Revision (Supplement 9.3)

- Revision to introduce risk assessment for selection of suitable tests, allow for the use of molecular methods

- Testing strategy (package of suitable tests) to be built on R/A

- List of tests must be adapted depending on the extraneous agents that have the potential to contaminate the product
Tests for extraneous agents in viral vaccines for human use (2.6.16)

Revision (Supplement 9.3)

✓ Redundant tests on adult mice and guinea pigs deleted
✓ Tests on suckling mice and control eggs used only if R/A indicates that tests provide risk mitigation
✓ Molecular methods for specific extraneous agents
✓ Test for viruses using broad molecular methods

→ Revision takes into account article ‘Systemic evaluation of in vitro and in vivo adventitious virus assays for the detection of viral contamination of cell banks and biological products’ by R. Sheets et al., Vaccine 32 (24) (2014)
General monograph
Vaccines for human use (0153)
Vaccines for human use (0153)

• Applies to all vaccines, including those for which there is no specific monograph

• Essential requirements which supplement and expand on requirements contained in the specific monographs
0153 General provisions

- Consistency of production process: batches must be comparable to batches of proven safety and efficacy

- Omission of tests is possible when consistency is demonstrated
  - validation
  - agreement by the competent authority
Consistency of production is an important feature of vaccine production. Monographs on vaccines for human use give limits for various tests carried out during production and on the final lot. These limits may be in the form of maximum values, minimum values, or minimum and maximum tolerances around a given value. While compliance with these limits is required, it is not necessarily sufficient to ensure consistency of production for a given vaccine. For relevant tests, the manufacturer must therefore define for each product a suitable action or release limit or limits to be applied in view of the results found for batches tested clinically and those used to demonstrate consistency of production. These limits may subsequently be refined on a statistical basis in light of production data.
Use of animals

• “In accordance with European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986), tests must be carried out in such a way as to use the minimum number of animals and to cause the least pain, suffering, distress or lasting harm”

→ General monograph vaccines for human use
Use of animals

- Specific monographs encourage alternative “3Rs” methods, humane endpoints
- The detailed protocol of a validated method may be provided as an example, where available
Specific vaccine monographs
Specific vaccine monographs

- Single type vaccines
- Combined vaccines

→ A combined vaccine must also comply with the specific monographs for each valence of the vaccine
Specific vaccine monographs

No duplication of the requirements of the general monograph
DEFINITION

- Defines the scope of the monograph and its applicability to products on the market
- If a new vaccine is developed against the same disease, a new monograph is developed or the existing monograph is revised to include the new type of vaccine in its definition
PRODUCTION

- Substrate for propagation
- Seed lots
- Propagation and harvest of the virus or Culture and harvest of bacteria
- Purification and Inactivation for inactivated vaccines
- Final bulk vaccine
- Final lot
IDENTIFICATION TESTS ASSAY

• IDENTIFICATION: how to identify the product
• TESTS: series of batch tests with limits. Product should comply throughout its shelf life
• ASSAY: potency test (in specific monograph or in separate chapter)

• STORAGE & LABELLING: given for information
Vaccines for human use
Recent developments
General chapter 2.7.14: Assay of hepatitis A vaccine

• The chapter has been revised following BSP study: Validation of a new ELISA method for in vitro potency testing of hepatitis A

New structure of the chapter:

• “The assay of Hepatitis A is carried out either in vitro, by an immunochemical determination of antigen content (method A), or in vivo, by comparing in given conditions its capacity to induce specific antibodies in mice with the same capacity of a reference preparation (method B)”

• METHOD A. IN VITRO ASSAY
• METHOD B. IN VIVO ASSAY

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Carrier proteins for the production of conjugated polysaccharide vaccines for human use (5.2.11) (Supplement 8.3)

- Harmonisation of quality profile
- Applied to all carrier proteins currently authorised and used
- Revision needed whenever new carrier protein is used
Carrier proteins for the production of conjugated polysaccharide vaccines for human use (5.2.11)

• Description of relevant tests required for all carrier proteins;
• Description of characteristics for each type of carrier protein (diphtheria and tetanus toxoids, CRM 197, OMP and recombinant protein D)
• Production process for each type of carrier protein currently used in vaccines: production, concentration, purification, inactivation/treatment;
Substitution of in vivo methods by in vitro methods for the quality control of vaccines

(New chapter 5.2.14, Supplement 9.3)

• Guidance to facilitate the transition from in vivo to in vitro methods
• Alternative approaches for replacement when direct head-to-head comparison is not possible
• Recommendations on substitution for a potency test, safety test with examples
3Rs for human vaccines

8 February 2017, EDQM Strasbourg, France

Dr. Catherine Milne
The European Directorate for the Quality of Medicines & HealthCare, Department of Biological Standardisation, OMCL Network & HealthCare
(EDQM - DBO)
Outline

- What are the 3Rs?
- Legal Context in Europe/EU
- Animal use in the field of pharmaceuticals
- Quality Control of vaccines
- 3R examples
- Vaccine field ripe for change
- 3Rs in the European Pharmacopoeia
- EDQM Biological Standardisation Programme
- Partners
- Future
What are the 3Rs?

Russell and Burch (1959*) did an ethical review of current experimental practice and proposed that improvements toward humane animal use were possible based on:

**Refinement** e.g. humane endpoints

**Reduction** e.g. historic data used to rationalise (single dilution) or remove test

**Replacement** e.g. *in vitro* test or remove and replace by monitoring different parameters

Basic principles remain today though solutions and approaches have evolved

Applicable to all sectors of experimental use of animals

Legal Context in Europe/EU

Long time objective to reduce, refine and replace the use of animals for the quality control of medicines (3RS)

European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (COE, 1986)

Legal obligations in the EU

- Directive 2010/63/EU, in force from 01/1/2013
  - Fully applicable to regulatory testing
  - Sets out clear rules for use of animals for experimental purposes
  - **Methods using the least number of animals and the lowest amount of suffering should be favoured** (Articles 4 and 13)
  - **An animal method shall not be used if a non-animal method is officially recognised and fits the purpose** (Article 13)
  - Ethical committee and other animal welfare measures
Animal use in the field of Pharmaceuticals

Pharmaceutical industry has relied on the use of various animal tests in the development and quality control of medicines

Quality control – heavy reliance on animals from the start

e.g. Paul Ehrlich in the 1890’s

• Production of diphtheria anti-serum highly variable

• Used diphtheria toxin, calibrated against a reference sera, mixed with the test sera and injected into guinea pigs to check the potency of diphtheria anti-serum. End point = death

Tests developed with scientific rigour using the techniques and understanding of the time

Allowed safe and efficacious medicines to reach the patients, provided confidence and helped to save lives
Quality Control of Vaccine

Vaccine field no exception: animals tests historically used for Quality Control.

• Tests for inactivation
• Tests for neurovirulence
• Tests for extraneous agents
• Test for residual toxicity or reversion to toxicity
• *In vivo* potency tests
• Etc.

In many cases the test has been a requirement for batch release – repeated again and again > Large number of animals

GOAL to remove or replace animal tests as far as possible while still ensuring the quality and safety of the product
3R Examples

Rabies vaccine potency assay (inactivated vaccine, human and vet)

Classic test
- Many mice
- Highly variable results
- ½ of mice die of rabies

Refinement: Humane end-points (euthanize animals before suffering from rabies)

Refinement/reduction: Serological assay, sera collected from mice after inoculation with vaccine. Antibody level in sera measured in vitro.

Reduction: Single dilution assay – only 1 dilution tested. Based on historical data with multi-dilution. Compare to reference if activity is greater the vaccine passes.

Replacement: Measure the antigen content in the vaccine directly. Use specific antibodies directed against functional epitopes in an ELISA (completely in vitro)

* ELISA= enzyme-linked immunosorbent assay

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3R Example: Single dilution assay

**Multiple Dilutions Assay**
- 3 or 4 Dilutions / reference
- 3 or 4 Dilutions / tested vaccine
- 12, 15 or 16 animals/dilution
- Challenge Dose Control: each test
  - 5 animals
- Toxin activity Control: each test
  - 5 animals & 3 dilutions
- Calculations
  - ED50 & LD50 determination
- Results
  - Potency in IU/Dose

**Diphtheria Tetanus Serology Assays**

**One Dilution Assay**
- 1 Dilution / reference
- 1 Dilution / tested vaccine
- 12, 15 or 16 animals/dilution
- Challenge Dose Control: 2 times/year
  - 5 animals
- Toxin activity Control: 2 times/year
  - 5 animals & 3 dilutions
- Calculations
  - Fisher’s probability test
- Results
  - PASS / FAIL

Validation from multiple dilution assay (historical data)

Number of animals used/ tested vaccine : 143

Number of animals used/ tested vaccine : 30

Decrease of 80%
Vaccine Field Ripe For Change

**Better understanding of the product**
- Composition (what should be there, what shouldn’t?)
- What is the mode of action and what elements are important to provide the effect?

**Better understanding of the production process**
- What parameters are necessary and sufficient to produce a batch of the same quality, safety and efficacy as a batch proven in the clinic and how can those parameters be monitored?

**Demonstrated consistency of production**
- Confidence that the same procedure will provide the same result each time (demonstrated consistency can lead to suppression of tests in some cases)

**Possibility to find or generate the necessary tools**
- *In vitro* assays require specific reagents e.g. monoclonal antibodies, specific cell lines, specialised reaction substrates, appropriate reference material etc.

**Adequate validation**
- Scientifically based decisions: proof of assay suitability, repeatability, reproducibility, sensitivity – recognised limitations of the ‘gold standard’ *in vivo* tests

**APPLICABLE FOR EXISTING PRODUCTS: PRIMORDIAL FOR NEW ONES**
3R Possibilities in European Pharmacopoeia

From open doors to concrete leaps.....

Introduction to the European Pharmacopoeia

- Includes a statement on commitment to the application of the convention and encouragement to apply the 3Rs where possible

- Refers to the convention
- Promotes minimal animal use
- Promotes principles of consistency
- Allows alternatives with approval of the authority (same pass fail result as Ph. Eur.)

- Consistency concept – demonstrate consistency with proven batches (tools/limits needed)
- Test omission possible with proof of consistency and agreement with authorities
- Animal tests – minimum number, least pain and suffering, replacement where possible

Dedicated web page

https://www.edqm.eu/en/alternatives-animal-testing

(detailed presentation E. Charton main session 7/02/2017)
General Chapters and Individual Monographs

Many examples of specific 3R improvements in general chapters and monographs e.g.

**NEW CHAPTER 5.2.14** in supplement 9.3 (in force 01/01/2018)

**Substitution of *in vivo* methods by *in vitro* methods for the quality control of vaccines**

**Existing Problem**
Traditionally validation of *in vitro* alternatives has required a ‘head to head’ comparison to the *existing in vivo* method. The existing *in vivo* method may be highly variable and may not have been validated according to current practice thus rendering direct comparison difficult to impossible.

**Aim of Chapter**
- Facilitate the transition from *in vivo* to *in vitro* methods

**Content**
- Highlight on scientific value of non-animal methods
- Guidance on how to validate alternative *in vitro* methods in scenarios where a direct head-to-head comparison to an existing *in vivo* method is not possible.
- Specific recommendations on the substitution of *in vivo* potency and safety tests are provided together with examples.

The general chapter is a non-mandatory text that provides an additional tool in the continuing efforts of the Ph. Eur. Commission to further reduce animal testing.
General Chapters and Individual Monographs (2)

Adopted – Ph Eur methods are ‘officially recognised’ in the context of Directive 2010/63/EU

- Individual monographs reorganised to favour the *in vitro* option (method A) and or open doors to ‘allow’ alternatives even if one is not described in detail (e.g. rabies vaccine)

- **Hepatitis A vaccine (2.7.14) Hepatitis B (rDNA) vaccine (2.7.15)** - introduction of an *in vitro* assay to replace the vaccination challenge (*in vivo*) assay

- **D,T,P containing vaccines** – detailed description of serology assay given preference and possibility to combine evaluation of multiple components (D,T,P) in 1 assay

- **Tests for extraneous agents in viral vaccines for human use (2.6.16) and Cell Substrates for the production of vaccines for Human use (5.2.3)** – update in supplement 9.3 (in force 01/01/2018)
  - 2.6.16 rewritten with emphasis on risk assessment and considers use of molecular biology methods, (2.6.16, 5.2.3) deletion of tests on adult mice and guinea pigs, tests on suckling mice and control eggs only if justified by risk assessment, sub-blind passage deleted in the test on suckling mice

- **Monocyte Activation Test (2.6.30)** – update in supplement 9.2 (in force 01/07/2017) revised to make the MAT, an alternative to Rabbit Pyrogen Test and Endotoxin test, more widely usable. Detects or quantifies endotoxin and non-endotoxin pyrogens.

Ongoing

- **Residual pertussis toxin (2.6.33)** – *in vitro* assay to replace HIST sensitisation test in mice. Possibility to waive testing

- **Proposal to delete abnormal toxicity test completely from the Ph Eur** (all individual monographs and chapter 2.6.9)
EDQM Biological Standardisation Programme (BSP)

**Sponsors:** Council of Europe (EDQM) and the EU Commission.

**Secretariat:** European Directorate for the Quality of Medicines & HealthCare (EDQM), DBO.

**Steering Committee (SC):** Ph. Eur. group chairs (15, 15V, 6, 6B) + EMA, WHO and co-opted experts – determine priorities and programme.

**Scope - BiologicaIs**

Biotech products, Human/Vet sera and vaccines, blood products, allergens (gene/cell therapy)

**Aim**

- Establishment of Ph. Eur. working standards (BRPs) & reagents and method development/standardisation (for biologicals – vet and human),
- **Application of 3R concept (refine, reduce, replace) with focus on fostering implementation and regulatory acceptance,**
- International harmonisation (ICH, VICH): collaboration with WHO, FDA, Japan....
- Collaborate and avoid overlap with other programmes (eg. EPAA, ECVAM etc.)

**Designed to take promising methods to the implementation and regulatory acceptance stage including publication in Pharmeuropa Bio & Scientific Notes** (presentation E. Terao main session 7/02/2017)
EDQM BSP 3R: Projects

Successfully Completed 3R Projects for Human Vaccines/Sera

BSP016/107: Hepatitis A vaccine, \textit{in vitro} assay (Replacement)
BSP021: Inactivated Polio vaccine, serological assay (Refinement / Reduction)
BSP019/035: Tetanus vaccine, serological assay (Refinement / Reduction)
BSP034/036: Diphtheria vaccine, serological assay (Refinement / Reduction)
BSP079: Human Tetanus Immunoglobulin, \textit{in vitro} assays (Replacement)
BSP083: Acellular Pertussis vaccine, serological assay (Refinement / Reduction)
BSP114: Acellular Pertussis vaccine, HIST alternative (Replacement) *

All included (or in progress*) in relevant Ph. Eur. monographs/method chapter
EDQM BSP 3R: Projects (2)

Examples of New/Ongoing 3R Projects for Human Vaccines/Sera

BSP105: Whole cell pertussis vaccine, serology assay (Refinement/Reduction)
BSP113: Tetanus/Diphtheria Vaccine \textit{in vitro} assay (Replacement)
BSP136: Tetanus Toxoid BINACLE assay (Replacement)
BSP148: Rabies vaccine \textit{in vitro} potency assay (Replacement)

NEW PROJECT PROPOSALS WELCOME

Goal to include them in relevant Ph. Eur. monographs/method chapter

Challenges for 3R method implementation

Practical/Scientific Issues

- Reagents and Reference material
  - Specificity, long term availability (preferably commercially) especially for *in vitro* assays
- Availability of appropriate samples (failed/borderline)
  - Makes it difficult to show the assay can detect unsuitable batches
- Highly variable *in vivo* assay (e.g. NIH assay for rabies vaccine)
  - Validation challenge – direct correlation difficult or impossible

Availability of Resources

- Time, funding, expertise: a challenge for all partners

Administrative/Regulatory Issues

- Implementation of alternative methods
  - Administrative hurdles can be significant
- Appropriate time for introduction of alternatives
  - Validation required is time consuming
- Lack of global harmonisation
  - Global market can mean multiple requirements for the same product. No incentive to implement an alternative in one region if the other regions still require the *in vivo* test and won’t accept the 3R method.
Many Partners for 3R Improvement

Other European organisations/initiatives e.g.

- OMCLs, Manufacturers, Academia
- EURL ECVAM (EU Reference Laboratory for alternatives to animal testing)
- EMA; CVMP/CHMP ad hoc Joint expert group on 3Rs
- EPAA (The European Partnership for Alternative Approaches to Animal Testing)
- VAC2VAC (Private/public project funded by Innovative Medicines Initiative)

Many other organisations at the international level

Goal is to work together to harmonise the application and facilitate change on a global level
European Medicines Agency JEG 3Rs

The joint *adhoc* CVMP/CHMP expert group on the application of the 3Rs (JEG 3Rs) was established to improve and foster the application of 3Rs in the regulatory testing of medicinal products throughout their lifecycle – new mode of action as of 2017 and new name (Joint CHMP/CVMP Working Group on the Application of the 3Rs in Regulatory Testing of Medicinal Products (J3RsWG))

- Provides information and advice on 3Rs to stakeholders
- Considers how progress on 3Rs issues can most usefully be used to influence development of regulatory guidance at an international level through ICH, VICH etc.
- Contributes to the development of guidelines and reflection papers in which 3Rs issues are applicable in collaboration with relevant Working Parties
Guidance for individual laboratories for transfer of quality control methods validated in collaborative trials with a view to implementing 3Rs EMA/CHMP/CVMP/JEG-3Rs/94436/2014

...the principle of the 3Rs ... needs to be considered when selecting approaches for validating quality control tests in laboratories for regulatory testing of human and veterinary medicinal products.

Collaborative studies between laboratories may be carried out to introduce new 3Rs methods for regulatory purposes where animal tests have been used traditionally. This guidance aims to facilitate transfer of the new methods validated in such trials with a view to implementing 3Rs for testing in a product specific context in laboratories originally involved in the collaborative trial or in new laboratories.

Supporting data can come from a number of sources, including accumulation of product data, published data from individual laboratories, and published study reports from collaborative trials. A laboratory’s own data from participation in a given collaborative study can also be used to support final product specific validation for regulatory acceptance.

Six different scenarios as guidance depending on level of involvement in the collaborative study.

Public consultation: 29/07/2016 – 31/01/2017
Other relevant papers/guidelines/activities

• **Guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement) testing approaches** (EMA/CHMP/CVMP/JEG-3Rs/450091/2012) – adopted December 2016 – available on EMA website
  
  • Highlights possibility to get regulatory approval for 3R methods that have not undergone formally recognised validation (e.g. in Ph. Eur.) via case by case assessment in marketing authorisation application and possibilities for specific EMA procedures for advice and/or ‘safe harbour’ submission of data.

• **Reflection paper providing an overview of the current regulatory testing requirements for veterinary medicinal products and opportunities for implementation of the 3Rs** (EMA/CHMP/CVMP/JEG-3Rs/164002/2016) – public consultation ended 31/10/2016

• **Review of existing animal testing requirements in EMA guidelines** (EMA/CHMP/CVMP/JEG-3Rs/677407/2015) - 3Rs – public consultation ended 31/10/2016

• **Review of CAP dossiers for 3R possibilities and contact with MAHs**

• **Communication of 3R issues and adopted practices on EMA JEG webpage including Recommendation highlighting the need to ensure compliance with 3R methods described in the Ph. Eur.** [http://www.ema.europa.eu/ema/index.jsp?curl=pages/contacts/CVMP/people_listing_000094.jsp&mid=W0Ob01ac05803a9d6d](http://www.ema.europa.eu/ema/index.jsp?curl=pages/contacts/CVMP/people_listing_000094.jsp&mid=W0Ob01ac05803a9d6d)
**Other Perspectives and Future Challenges**

**EPAA: Collaboration between the European Commission, European trade associations, and companies with the involvement of regulatory authorities and public laboratories**

- **15-16 September 2015 - EPAA workshop: Modern science for better quality control of medicinal products**
  - Extensive review of animal testing requirements in the global regulatory environment with a goal to harmonise globally.
  - First target: encourage deletion of *in vivo* ATT/GST/TABST from national/jurisdictional and legal requirements as well as international guidance (Ph. Eur. Monographs, WHO recommendations, OIE guidelines)

- EPAA method development initiatives: rabies vaccine for human use; development of a common *in vitro* assay – successfully taken forward to EDQM BSP programme

**European Project VAC2VAC: public/private collaboration**

- Vaccine consistency approach may replace *in vivo* potency or specific testing for quality control.
- Based on the characterization of the vaccine during the production and the comparison between new batches and reference batches (of proven potency and safety) = a control strategy - not necessarily a 1/1 replacement
- Demonstrate proof of concept of the consistency approach for batch release of established vaccines.
- Proven concepts to be taken forward to large scale validation e.g. BSP
Considerable progress has been made

........but still room for more improvements with many opportunities, in particular for new products, to be animal-test free

We look forward to continued advances in future.

THANK YOU FOR YOUR ATTENTION
“A journey inside the blood product monographs”

EDQM TRAINING SESSION: STRASBOURG
THE EUROPEAN PHARMACOPOEIA 9TH EDITION

Sébastien Jouette, PhD
Scientific Officer, European Pharmacopoeia Department
Outline

• Key figures
• Monographs and chapters (overview, structure and technical guide)
• General provisions
• Immunoglobulins
• Coagulation factors
• General chapters/methods
Outline

• Key figures
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Key Figures

Group 6B: Human blood and blood products since 1970

26 experts from 18 countries

31 monographs

21 general chapters/methods

8 Texts under revision
Outline

- Key figures
- Monographs and chapters (overview, structure and technical guide)
- General provisions
- Immunoglobulins
- Coagulation factors
- General chapters/methods
# HUMAN PLASMA-DERIVED PRODUCT MONOGRAPHS

## Anticoagulants and preservative solutions for human blood (0209)

### Containers
- Sterile plastic containers for human blood and blood components (3.2.3)
- Glass container 3.2.1
- Empty sterile containers of plasticised poly(vinyl chloride) for human blood and blood components (3.2.4)
- Sterile containers of plasticised poly(vinyl chloride) for human blood containing anticoagulant solution (3.2.5)

### Materials for containers for human blood and blood components (3.1.1)

## Human plasma for fractionation (0853)

### Human normal Immunoglobulin for intramuscular administration (0338)
- Human plasma (pooled and treated for virus inactivation) (1646)
- Human normal Immunoglobulin for intramuscular administration (0338)
- Human anti-D immunoglobulin (0557)
- Human Hepatitis B immunoglobulin (0722)
- Human Hepatitis A immunoglobulin (0769)
- Human varicella immunoglobulin (0724)
- Human rabies immunoglobulin (0723)
- Human rubella immunoglobulin (0617)
- Human tetanus immunoglobulin (0398)
- Human measles immunoglobulin (0397)

### Human normal Immunoglobulin for subcutaneous administration (2788)
- Human normal Immunoglobulin for intravenous administration (0918)
- Human plasma for fractionation (0853)
- Human Coagulation factors
  - Human coagulation factor VII (1224)
  - Human coagulation factor VIII (0275)
  - Human coagulation factor IX (1223)
  - Human coagulation factor XI (1644)

### Other fractionated products
- Human albumin solution (0255)
- Human fibrinogen (0024)
- Fibrin sealant kit (0903)
- Human antithrombin III concentrate (0878)
- Human prothrombin complex (0554)
- Human von Willebrand factor (2298)
- Human α-1-proteinase inhibitor (2387)
- Human C1-esterase inhibitor (2818)

## General Chapters/methods
- Prekallikrein activator (2.6.15.)
- Test for anticomplementary activity of immunoglobulin (2.6.17.)
- Anti-A and anti-B haemagglutinins (2.6.20.)
- Activated coagulation factors (2.6.22.)
- Nucleic acid amplification techniques (2.6.21.)
- Test for anti-D antibodies in human immunoglobulin (2.6.26.)
- Assay of human coagulation factor VIII (2.7.4.)
- Test for Fc function of immunoglobulin (2.7.9.)
- Assay of human coagulation factor VII (2.7.10.)
- Assay of human coagulation factor IX (2.7.11.)
- Assay of heparin in coagulation factors (2.7.12.)
- Assay of human anti-D immunoglobulin (2.7.13.)
- Assay of human antithrombin III (2.7.17.)
- Assay of human coagulation factor II (2.7.18.)
- Assay of human coagulation factor X (2.7.19.)
- Assay of human von Willebrand factor (2.7.21.)
- Assay of human coagulation factor XI (2.7.22.)
- Assay of human plasmin inhibitor (2.7.25.)
- Assay of human protein C (2.7.30.)
- Assay of human protein S (2.7.31.)
- Assay of human alpha-1-proteinase inhibitor (2.7.32.)
- Assay of human C1-esterase inhibitor (2.7.34.)
In general, labelling of medicines is subject to supranational and national regulation and to international agreements. The statements under the heading Labelling are not therefore comprehensive and, moreover, for the purposes of the Pharmacopoeia only those statements that are necessary to demonstrate compliance or non-compliance with the monograph are mandatory. Any other labelling statements are included as recommendations. When the term ‘label’ is used in the Pharmacopoeia, the labelling statements may appear on the container, the package, a leaflet accompanying the package, or a certificate of analysis accompanying the article, as decided by the competent authority.
Technical guide

• Technical guide for the elaboration and use of monographs on human plasma-derived products (available on EDQM website)

Outline

• Key figures
• Monographs and chapters (overview, structure and technical guide)
• General provisions
• Immunoglobulins
• Coagulation factors
• General chapters/methods
General provisions applicable to blood product monographs (1/4)

- To comply with the requirements of Human plasma for fractionation (0853) (DEFINITION)

  - is obtained from plasma that complies with the requirements of the monograph Human plasma for fractionation (0853).

- Specification for Potency indicated in DEFINITION (or in a specific section i.e. Ig)

  - The potency of the preparation, reconstituted as stated on the label, is not less than 20 IU of von Willebrand factor per millilitre.
Potency (Biological activity)

- ICH Q6B states: “Potency is the quantitative measure of the biological activity”

“The results of biological assays should be expressed in units of activity calibrated against an international or national reference standard, when available and appropriate for the assay utilized. Where no such reference standard exists, a characterized in-house reference material should be established and assay results of production lots reported as in-house units.”
General provisions applicable to blood product monographs (2/4)

- **Use of excipients** (e.g. heparin) described in DEFINITION
  
  The preparation may contain excipients such as stabilisers, heparin and antithrombin.

- **No use of Antibiotic/Microbial preservatives**, statement in PRODUCTION
  
  No antimicrobial preservative or antibiotic is added.

  Unless otherwise mentioned in DEFINITION

  e.g: Human normal immunoglobulin for intramuscular administration (0338)

  Multidose preparations contain an antimicrobial preservative.

- **IDENTIFICATION**: Assay (except Immunoglobulins)
TESTS: General tests to be performed

pH: “physiological range” by default: pH 6.5 - 7.5

Osmolality: minimum 240mosmol/kg (average serum osmolality 240-340mosmol/kg)

Total protein: Specification limit depending on product (not always provided in the monograph)

Sterility: Complies with the test for sterility (2.6.1)

Water: Specification limit as approved by competent authority
 TESTS: General tests to be performed

Tests for pyrogenicity (1/2)

- In accordance with *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986)* and the directive 2010/63/EU

- Bacterial endotoxin test was introduced in blood products monographs

- BET: covers only endotoxins from gram–

- See EMA guideline

“Guideline on the replacement of rabbit pyrogen testing by an alternative test for plasma derived medicinal products” (EMA/CHMP/BWP/452081/2007)
Tests for pyrogenicity (2/2)

Typical wording: **Pyrogens or Bacterial endotoxins**

Complies with Bacterial endotoxins chapter (2.6.14) or where not possible Pyrogens test* (2.6.8)

*Rabbit pyrogen test

Use of other **in-vitro test** (e.g. MAT) encouraged

**Calculation of Endotoxins limit:** 5.1.10 considering:

K: threshold pyrogenic dose of endotoxin per kilogram of body mass (see table 5.1.10-1 for values)

M: maximum recommended bolus dose of product per kilogram of body mass

**Pyrogens** (2.6.8) or **Bacterial endotoxins** (2.6.14). It complies with the test for pyrogens or, preferably and where justified and authorised, with a validated *in vitro* test such as the bacterial endotoxin test.

For the pyrogen test, inject per kilogram of the rabbit’s mass a volume equivalent to 0.5 g of immunoglobulin, but not more than 10 mL per kilogram of the rabbit’s mass.

Where the bacterial endotoxin test is used, the preparation to be examined contains less than 0.5 IU of endotoxin per millilitre for solutions with a protein content not greater than 50 g/L, and less than 1.0 IU of endotoxin per millilitre for solutions with a protein content greater than 50 g/L but not greater than 100 g/L.
Outline

• Key figures
• Monographs and chapters (overview, structure and technical guide)
• General provisions
• Immunoglobulins
• Coagulation factors
• General chapters/methods
Human Immunoglobulins

✓ Since 2014, 3 overarching monographs depending on the route of administration

Human normal immunoglobulin for:

- Intramuscular administration (0338)
- Subcutaneous administration (2788)
- Intravenous administration (0918)

✓ Specific Ig to comply with 0338, 0918 or 2788:

It complies with the monograph *Human normal immunoglobulin (0338)*, except for the minimum number of donors and the minimum total protein content.
Practical example:

Ex: Human anti-D immunoglobulin products for intravenous administration

1. To comply with its specific monograph 1527

And

2. To comply with the overarching monograph (IVg, 0918)
(All tests mentioned in it to be performed unless otherwise indicated)
Immunoglobulins: PRODUCTION section

Specific provisions for manufacturing process:

- Removal/inactivation of infectious agents
- No adverse effects of residual inactivation agents (when used)
- Removal of Procoagulant agents: e.g. activated coagulation factors

Revision ongoing: add a test to quantify them

PRODUCTION

The method of preparation includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses, it shall have been shown that any residues present in the final product have no adverse effects on the patients treated with the immunoglobulin. The method of preparation also includes a step or steps that have been shown to remove thrombosis-generating agents. Emphasis is given to the identification of activated coagulation factors and their zymogens and process steps that may cause their activation. Consideration is also to be given to other procoagulant agents that could be introduced by the manufacturing process.

The product shall have been shown, by suitable tests in animals and evaluation during clinical trials, to be well tolerated when administered intravenously.

Patient Safety
Immunoglobulins: PRODUCTION section

No exhaustive list of methods or specifications

Suitability of production process established by competent authorities i.e. by examination of data and/or inspection

“does not exhibit” = acceptable safety level defined by the competent authority

Human normal immunoglobulin for intravenous administration is prepared from pooled material from not fewer than 1000 donors by a method that has been shown to yield a product that:

- does not transmit infection;
- at an immunoglobulin concentration of 50 g/L, contains antibodies for at least 2 of which (1 viral and 1 bacterial) an International Standard or Reference Preparation is available, the concentration of such antibodies being at least 3 times that in the initial pooled material;
- has a defined distribution of immunoglobulin G subclasses;
- complies with the test for Fc function of immunoglobulin (2.7.9);
- does not exhibit thrombogenic (procoagulant) activity.
Immunoglobulins: TESTS & POTENCY

• All Ig (IM, SC, IVI) to comply respectively with all tests mentioned in overarching monograph 0338, 2788 or 0918 except:

  Additional Potency test for specific Ig

  Other specific exceptions, e.g. Hep B surface antigens may be skipped in Human anti-D immunoglobulin
Outline

• Key figures
• Monographs and chapters (overview, structure and technical guide)
• General provisions
• Immunoglobulins
• Coagulation factors
• General chapters/methods
Coagulation factors: PRODUCTION SECTION

- General provisions for manufacturing process:
  - Maintain functional integrity
  - Removal/inactivation of infectious agents
  - No adverse effects of residual inactivation agents (when used)
  - Minimise activation of other coagulation factors (where relevant)

- Consistency of the method of production:
  
  \[\text{\textit{e.g.}} \ FVIII: \text{ check activities of FII, IX, X and FVIIa} \]
Coagulation factors: POTENCY, TESTS and ASSAY

• No overarching monograph
• Specific potency for each factor
• Other factors considered as impurity

Additional tests/assay can be prescribed:

FVII: Test for other coagulation factors (II, IX, X) to show consistent manufacture/removal with respect to these factors

FVIII: No additional test for coagulation factors but assay for VWF*

*intended use for treatment VW's disease

• Additional typical tests:
  Anti-A & Anti-B haemagglutinins (2.6.20)
  Activated coagulation factors (2.6.22)
  Heparin (2.7.12)
Outline

• Key figures
• Monographs and chapters (overview, structure and technical guide)
• General provisions
• Immunoglobulins
• Coagulation factors
• General chapters/methods
### Human plasma for fractionation (0853)

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human plasma (pooled and treated for virus inactivation)</td>
<td>1646</td>
</tr>
<tr>
<td>Human normal immunoglobulin for intramuscular administration</td>
<td>0338</td>
</tr>
<tr>
<td>Human anti-D immunoglobulin</td>
<td>0557</td>
</tr>
<tr>
<td>Human Hepatitis B immunoglobulin</td>
<td>0722</td>
</tr>
<tr>
<td>Human Hepatitis A immunoglobulin</td>
<td>0769</td>
</tr>
<tr>
<td>Human varicella immunoglobulin</td>
<td>0724</td>
</tr>
<tr>
<td>Human rabies immunoglobulin</td>
<td>0723</td>
</tr>
<tr>
<td>Human rubella immunoglobulin</td>
<td>0617</td>
</tr>
<tr>
<td>Human tetanus immunoglobulin</td>
<td>0398</td>
</tr>
<tr>
<td>Human measles immunoglobulin</td>
<td>0397</td>
</tr>
<tr>
<td>Human normal Immunoglobulin for subcutaneous administration</td>
<td>2788</td>
</tr>
<tr>
<td>Human Hepatitis B immunoglobulin</td>
<td>0722</td>
</tr>
<tr>
<td>Human normal immunoglobulin for intravenous administration</td>
<td>0918</td>
</tr>
<tr>
<td>Human anti-D immunoglobulin for intravenous administration</td>
<td>1527</td>
</tr>
<tr>
<td>Human varicella immunoglobulin for intravenous administration</td>
<td>1528</td>
</tr>
<tr>
<td>Human Hepatitis B immunoglobulin for intravenous administration</td>
<td>1016</td>
</tr>
</tbody>
</table>

### Human Coagulation factors

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human coagulation factor VII</td>
<td>1224</td>
</tr>
<tr>
<td>Human coagulation factor VIII</td>
<td>0275</td>
</tr>
<tr>
<td>Human coagulation factor IX</td>
<td>1223</td>
</tr>
<tr>
<td>Human coagulation factor XI</td>
<td>1644</td>
</tr>
</tbody>
</table>

### Other fractionated products

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human albumin</td>
<td>0255</td>
</tr>
<tr>
<td>Human fibrinogen</td>
<td>0024</td>
</tr>
<tr>
<td>Fibrin sealant kit</td>
<td>0903</td>
</tr>
<tr>
<td>Human antithrombin III concentrate</td>
<td>0878</td>
</tr>
<tr>
<td>Human prothrombin complex</td>
<td>0554</td>
</tr>
<tr>
<td>Human von Willebrand factor</td>
<td>2298</td>
</tr>
<tr>
<td>Human α-1-proteinase inhibitor</td>
<td>2387</td>
</tr>
<tr>
<td>Human C1-esterase inhibitor</td>
<td>2818</td>
</tr>
</tbody>
</table>

### General Chapters/methods

<table>
<thead>
<tr>
<th>Section Title</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prekallikrein activator</td>
<td>2.6.15.</td>
</tr>
<tr>
<td>Test for anticomplementary activity of immunoglobulin</td>
<td>2.6.17.</td>
</tr>
<tr>
<td>Anti-A and anti-B haemagglutinins</td>
<td>2.6.20.</td>
</tr>
<tr>
<td>Activated coagulation factors</td>
<td>2.6.22.</td>
</tr>
<tr>
<td>Nucleic acid amplification techniques</td>
<td>2.6.21.</td>
</tr>
<tr>
<td>Test for anti-D antibodies in human immunoglobulin</td>
<td>2.6.26.</td>
</tr>
<tr>
<td><strong>Assay of human coagulation factor VIII</strong></td>
<td>2.7.4.</td>
</tr>
<tr>
<td>Test for Fc function of immunoglobulin</td>
<td>2.7.9.</td>
</tr>
<tr>
<td>Assay of human coagulation factor VII</td>
<td>2.7.10.</td>
</tr>
<tr>
<td>Assay of human coagulation factor IX</td>
<td>2.7.11.</td>
</tr>
<tr>
<td>Assay of heparin in coagulation factors</td>
<td>2.7.12.</td>
</tr>
<tr>
<td>Assay of human anti-D immunoglobulin</td>
<td>2.7.13.</td>
</tr>
<tr>
<td>Assay of human antithrombin III</td>
<td>2.7.17.</td>
</tr>
<tr>
<td>Assay of human coagulation factor II</td>
<td>2.7.18.</td>
</tr>
<tr>
<td>Assay of human coagulation factor X</td>
<td>2.7.19.</td>
</tr>
<tr>
<td>Assay of human von Willebrand factor</td>
<td>2.7.21.</td>
</tr>
<tr>
<td>Assay of human coagulation factor XI</td>
<td>2.7.22.</td>
</tr>
<tr>
<td>Assay of human plasmin inhibitor</td>
<td>2.7.25.</td>
</tr>
<tr>
<td>Assay of human protein C</td>
<td>2.7.30.</td>
</tr>
<tr>
<td>Assay of human protein S</td>
<td>2.7.31.</td>
</tr>
<tr>
<td>Assay of human alpha-1-proteinase inhibitor</td>
<td>2.7.32.</td>
</tr>
<tr>
<td>Assay of human C1-esterase inhibitor</td>
<td>2.7.34.</td>
</tr>
</tbody>
</table>
Example: 2.7.4 Assay of human coagulation factor VIII

- Description of the Chromogenic assay + BRP
- Reagents
- Assay procedure

This is an example of a chromogenic assay
Different chromogenic kits may be used for this purpose

- Additional information in Knowledge Database
Check Knowledge Database: 2.7.4 Assay of human coagulation factor VIII

View HISTORY
The use of reference standards for plasma-derived products

Eriko Terao, PhD
Scientific Officer
Council of Europe

European Directorate for the Quality of Medicines & HealthCare (EDQM)
Department of Biological Standardisation, OMCL Network & Healthcare (DBO)
Outlines

1. Reference standards in Ph. Eur. monographs & texts
   CRS, BRP and BRR

2. Reference Standards: what are they made of?

3. Example: a CRS and a BRP for a coagulation factor (FIX)

4. Example: BRPs for system suitability (virus RNA/DNA)

5. Example: a panel of BRPs (anti-A, anti-B immunoglobulins)
Reference standards in Ph. Eur. monographs & texts (1)

Physico-chemical tests → Chemical Reference Substances (CRS)

Biological assays → Biological Reference Preparations (BRP)

Biological Reference Reagents (BRR)
### Reference standards in Ph. Eur. monographs & texts (2)

<table>
<thead>
<tr>
<th>Physico-chemical tests</th>
<th>Chemical Reference Substances (CRS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• content in mg/vial</td>
</tr>
<tr>
<td></td>
<td>• chromatogram(s)/spectrum</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Identification Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular size distribution</td>
</tr>
<tr>
<td>Related proteins</td>
</tr>
<tr>
<td>Impurities</td>
</tr>
<tr>
<td>assay for protein</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Glycan analysis (2.2.25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gamma-carboxyglutamic acid (Gla) content</td>
</tr>
<tr>
<td>peptide mapping (2.2.55.)</td>
</tr>
<tr>
<td>PAGE (2.2.31.)</td>
</tr>
<tr>
<td>liquid chromatography (2.2.29.)</td>
</tr>
<tr>
<td>SEC (2.2.30.)</td>
</tr>
</tbody>
</table>

→ as reference solution for comparison of profile, mobility,...

→ for system suitability
Reference standards in Ph. Eur. monographs & texts (3)

Biological assays / tests → Biological Reference Preparation (BRP)
• content in IU, titre,.../ampoule or vial
• in weight

Tests
• anticomplementary activity (2.6.17)
• prekallikrein activator activity (2.6.15)
• anti-A, anti-B haemagglutinin (2.6.20)
• anti-D antibodies (2.6.26)
• immunochemical detection of antigens (2.7.1)
• bacterial endotoxins (2.6.14)
• pyrogens (2.6.8)
• nucleic acid detection (2.6.21)

contaminants
•...

Assays
•...

Potency
•...

→ as reference solution for the determination of a biological activity
  (in IU, as a titre,...)

→ for system suitability
Reference standards in Ph. Eur. monographs & texts (4)

Biological assays → Biological Reference Reagents (BRR)

critical reagents for an assay/test

primary antibodies
detection antibodies
# Reference standards: what are they made of?

**Materials**
- plasma
- plasma-derived products (immunoglobulins, albumin,...)

<table>
<thead>
<tr>
<th>✓ Unprocessed compliant product</th>
<th>✓ Unprocessed non-compliant product</th>
<th>✓ Processed product</th>
</tr>
</thead>
<tbody>
<tr>
<td>• normal lots/batches</td>
<td>• polymers, aggregates,...</td>
<td>• forced degradation</td>
</tr>
<tr>
<td></td>
<td>• unusual level of a contaminant</td>
<td>• spiking with purified contaminant</td>
</tr>
<tr>
<td></td>
<td>• potency issue</td>
<td>• tailor-made plasma/Ig purification</td>
</tr>
<tr>
<td></td>
<td>• stability issue</td>
<td>• ...</td>
</tr>
<tr>
<td></td>
<td>• adverse effect of unidentified cause</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• ...</td>
<td></td>
</tr>
</tbody>
</table>
Example: a CRS and a BRP for a coagulation factor (FIX) – (1)

<table>
<thead>
<tr>
<th>Human coagulation Factor IX (monograph 1223)</th>
<th>Human coagulation Factor IX (rDNA) conc. solution (monograph 2522)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ASSAY</strong></td>
<td></td>
</tr>
<tr>
<td>Human coagulation factor IX (2.7.11.)</td>
<td>✔</td>
</tr>
<tr>
<td><em>Human coag. Factor IX concentrate BRP</em></td>
<td>✔</td>
</tr>
</tbody>
</table>

**PRODUCTION / IDENTIFICATION / TESTS**

- glycan analysis (2.2.25)
- peptide mapping (2.2.55.)
- PAGE (2.2.31.)
- liquid chromatography (2.2.29.)
- Size Exclusion Chromatography (2.2.30.)

*Human coag. Factor IX (rDNA) CRS*
**Example: a CRS and a BRP for a coagulation factor (FIX) – (2)**

<table>
<thead>
<tr>
<th></th>
<th>Human coagulation Factor IX concentrate BRP</th>
<th>Human coagulation Factor IX (rDNA) CRS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Catalogue code</strong></td>
<td>H0920500</td>
<td>Y0001659</td>
</tr>
<tr>
<td><strong>Unit quantity</strong></td>
<td>ca. 30 mg</td>
<td>10 mg</td>
</tr>
<tr>
<td><strong>Intended use</strong></td>
<td>assay of coagulation factor IX (2.7.11.)</td>
<td>monograph 2522</td>
</tr>
<tr>
<td><strong>Declared potency</strong></td>
<td>10.5 IU/ampoule</td>
<td>&quot;as is&quot; content of 0.426 mg/vial</td>
</tr>
<tr>
<td><strong>Other information in leaflet</strong></td>
<td>Literature</td>
<td>chromatograms</td>
</tr>
<tr>
<td></td>
<td>BSP study report publication</td>
<td></td>
</tr>
<tr>
<td><strong>Batch Validity Statement</strong></td>
<td>batch 3</td>
<td>batch 1</td>
</tr>
</tbody>
</table>
Example: BRPs for system suitability (virus RNA/DNA for NAT testing)

- Hepatitis A virus RNA for NAT testing BRP 4x10^4 IU/vial
- Hepatitis C virus RNA for NAT testing BRP 500 IU/vial
- Hepatitis E virus RNA for NAT testing BRP 2.1x10^4 IU/vial
- B19 virus DNA for NAT testing BRP 10^{5.80} IU/vial

Monograph 1646
Human plasma (pooled and treated for virus inactivation)

→ to be used as positive controls in the NAT test
Anti-A, anti-B immunoglobulins: a panel of BRPs for the control of contaminant levels

- Immunoglobulin (anti-A, anti-B antibodies) Positive control BRP
- Immunoglobulin (anti-A, anti-B antibodies) Negative control BRP
- Immunoglobulin for anti-A, anti-B antibodies Limit test BRP

- Human normal immunoglobulin for IV administration (0918)

- 2.6.20. Anti-A and anti-B haemagglutinins
  B. Direct method

- Collaborative study (BSP089) - Vox Sang 2009
- Pharmeuropa Bio Sci Notes 2010
Anti-A, anti-B immunoglobulins: a panel of BRPs for the control of contaminant levels

- Immunoglobulin (anti-A, anti-B antibodies) Positive control BRP
- Immunoglobulin (anti-A, anti-B antibodies) Negative control BRP
- Immunoglobulin for anti-A, anti-B antibodies Limit test BRP

→ EDQM website
→ Knowledge database

Practical information

<table>
<thead>
<tr>
<th>Test(s)</th>
<th>Brand Name/Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preservative solution</td>
<td>Alsever's solutions and modified Alsever's solutions, which are commercially available, can be used as preservative solutions for the storage of red cells</td>
</tr>
<tr>
<td>Microtitre plates</td>
<td>V-bottomed polystyrene microtitre plates from Greiner catalogue N° 651101 have been found suitable</td>
</tr>
</tbody>
</table>
Anti-A, anti-B immunoglobulins: a panel of BRPs for the control of contaminant levels

- Immunoglobulin (anti-A, anti-B antibodies) Positive control BRP
- Immunoglobulin (anti-A, anti-B antibodies) Negative control BRP
- Immunoglobulin for anti-A, anti-B antibodies Limit test BRP

→ EDQM website
→ Knowledge database

Additional information

The interpretation of intermediate results between clear buttons and streams can be difficult. The above pictures show examples of how such results are interpreted (+ is a positive result; – is a negative result).

Figure 2.6.20.-1. – Examples of interpretation of results
Anti-A, anti-B immunoglobulins: a panel of BRPs for the control of contaminant levels

- Immunoglobulin (anti-A, anti-B antibodies) Negative control BRP $<2$
- Immunoglobulin (anti-A, anti-B antibodies) Positive control BRP 32

a. tilt the plate at an angle of 70° until all negatives have streamed

b. record the endpoint titre
   i.e., the reciprocal of the highest dilution that gives rise to a positive result

N.B.: the experimental setup should be optimised:
- if the negative control BRP does not show a titre $<2$,
- if the Positive control BRP does not show a titre of 32 (around 1 dilution step)

→ BRP used as negative and positive controls
→ BRP used as guide for operators: system suitability
Anti-A, anti-B immunoglobulins: a panel of BRPs for the control of contaminant levels

- Immunoglobulin (anti-A, anti-B antibodies) Positive control BRP 32
- Immunoglobulin for anti-A, anti-B antibodies Limit test BRP 64

c. if the sample shows a titre similar to the Positive control,
d. then, re-test the sample using the Limit test BRP:
   compare the sample to the Limit test BRP
   if similar or above: non-compliant product

→ the Limit BRP is used to set a specification limit
   at which the tested product is non-compliant
• 3 types of reference standard: CRS, BRP, BRR
• RS used for physico-chemical characterisation
• RS used for potency determination (also of contaminants)
• RS used for system suitability
• RS used to set a limit
• leaflets, Batch Validity Statements, literature
• knowledge database