

Guidelines for the Blood Transfusion Services

11.1: Guidelines for reagent manufacture

<http://www.transfusionguidelines.org/red-book/chapter-11-reagent-manufacture/11-1-guidelines-for-reagent-manufacture>

11.1: Guidelines for reagent manufacture

11.1.1: Introduction

All reagents used to determine the group of human red cells and to detect red cell antibodies must comply with Medical Devices Regulations 2002 (SI 2002 No 618, as amended) (UK MDR 2002) and all associated standards.

General guidelines for reagent manufacture are presented in this section. In other sections additional guidelines are given for particular reagents.

Where specific reference to British Standard European Standard (BS EN) documents is given this is the most recent version. It is intended that these guidelines refer to the current requirements contained in the applicable documents so the phrase 'and subsequent revisions' should be assumed whenever a specific reference is given.

11.1.2: Reference preparations

Reference preparations for use with these guidelines can be found on the National Institute for Biological Standards and Control (NIBSC) website at www.nibsc.org.

See section 11.3 for further information.

11.1.3: Definitions

Antibody identification is a test or combination of tests designed to determine the specificity of atypical antibodies.

Antibody screening is a test or combination of tests designed to detect atypical antibodies.

A **batch** of reagent is a defined quantity of material or of bulk, intermediate or finished product that is intended or purported to be uniform in character and quality, and which has been produced during a defined cycle of manufacture. A batch may be divided into sub-batches. A batch is sometimes described as a 'lot'.

A **batch of tests** is defined as a number of tests set up at the same time, under the same conditions and processed in a similar manner.

A **blood grouping kit** comprises a set of blood grouping components (reagents or materials), packaged together, intended by the manufacturer to be used together for determining one or more blood groups.

A **blood grouping reagent** is a reagent, used alone or in combination with other materials, intended by the manufacturer for the determination of a blood group of an individual.

- A blood grouping reagent recommended by the manufacturer for the detection of A (i.e. subgroups A₁ and A₂) A_x and B should be named **anti-A,B blood grouping reagent**.

A **blood grouping system** is an *in vitro* diagnostic medical device intended by the manufacturer to be used for determining one or more blood groups.

Clinically important or clinically significant antibody is a red cell antibody which will produce significantly accelerated red cell destruction when combined *in vivo* with its corresponding antigen.

Expiry date is the date beyond which performance of the reagent cannot be assured and is based upon the stability of the reagent.

Fresh serum for complement activity stored in the liquid state should be used within 8 hours of donation. When used after storage at –70°C or below, the 8-hour liquid storage period refers to the time both before and after frozen storage. Unless validated, the maximum period of frozen storage shall be 6 months at this temperature.

An **immediate container** is a medium adequate to protect the content(s) from contamination and/or physical damage. For example, a sealed vial, ampoule or bottle, a foiled pouch or a sealed plastic bag.

Atypical blood group antibodies are those of non-ABO specificity.

The **manufacturer** is a natural or legal person who manufactures or fully refurbishes a device or has a device designed, manufactured or fully refurbished, and markets that device under its name or trademark.

The name for a blood grouping reagent derived from monoclonal materials should include the word **monoclonal**.

A **monospecific blood grouping reagent** is one containing an antibody or blend of antibodies specific for one antigen, e.g. anti-A, anti-IgG.

A **polyspecific blood grouping reagent** is one containing a blend of antibodies specific for more than one antigen.

Polyspecific anti-human globulin reagent should be the name for a reagent which contains anti-human IgG and anti-human complement (C3d) activity, and is recommended by the manufacturer for use in both the direct and indirect anti-human globulin techniques, i.e. for the detection of red cell bound human IgG, and C3 complement in the form EiC3b and EC3d irrespective of the presence of other human immunoglobulin or human complement specificities.

Potency titre is a term used to describe the highest dilution of a reagent that effects a grade 2 endpoint reaction in tube or a grade 1 endpoint in column agglutination technologies.

Prozone is the term used to denote the absence or weakening of agglutination with excess of antibody.

A **reagent control** is a reagent made to the same formulation as a blood grouping reagent but without the specific blood group antibody reactivity. If the reagent control contains serum or plasma, the reagent control should be shown to be free from specific blood group antibody reactivity.

A **reference preparation** is prepared nationally or locally and contains a known or agreed concentration of the activity being measured. It should be assayed to establish the sensitivity or calibration of a test procedure or reagent.

Sensitivity in relation to these guidelines is a term defining the limit of detectable specific reactions using reagents or test systems. These guidelines specify levels of sensitivity that should be achieved.

Shelf life is the period until expiry date.

Specificity in relation to these guidelines is a term defining the ability of a reagent or test system to react selectively. In particular terms, it represents the absence of unwanted or false-positive reactions.

Test monitors are a series of samples included as part of each batch of tests, which provide part of the release algorithm for a batch of tests.

Validation is the confirmation, through the provision of objective evidence, that the requirements for a specific or intended use have been fulfilled. Validation of a manufacturing method is to ensure that the product will be of the quality required for its intended use and that tests used in monitoring will accurately reflect the quality of the product.

Verification is the confirmation, through the provision of objective evidence, that specific requirements have been fulfilled.

Undiluted in these guidelines means the reagent as intended for use by the manufacturer. This term includes a diluted reagent if the reagent is supplied in a form requiring dilution by the user prior to use, as specified in the manufacturer's 'instructions for use'.

An **unequivocal** reaction in a test system is a reaction that is unambiguous. In the manual tube test, this is defined as a reaction of grade 3 or greater. In column tests this is defined as a 1+ or greater reaction.

11.1.4: General manufacturing considerations

11.1.4.1: Good manufacturing practice

Reagents for blood group serology must be manufactured in accordance with the Medical Devices Regulation 2002 (SI 2002 No 618) (UK MDR 2002) and all associated documents.

Guidance on the principles of good manufacturing practice can be obtained from *Rules and Guidance for Pharmaceutical Manufacturers and Distributors*.²

- The method of manufacture should result in a product within an immediate container that is homogeneous and free of properties which adversely affect its intended use throughout its recommended shelf life. The reagent should have no precipitate, particles or fibrin gel.
- Each batch or sub-batch should be specifically identified by a distinctive combination of numbers and /or letters (batch reference) which permits its history to be traced.
- Reagents should be produced by a validated process that is shown to be suitable for the intended purpose, including any methods for preserving red cells prior to their preparation as reagent red cells.
- The manufacturer should monitor the batch-to-batch performance of the blood grouping reagent (e.g. by the reaction against some internal reference material) in order to provide consistency of performance. This is particularly important when the blood grouping reagent is provided as a test system, kit or kit component, when the performance may be dependent on the characteristics of other system variables or kit components.

11.1.4.2: Risk management

Risk management should be performed in accordance with:

- BS EN ISO 14971:2019 Medical Devices – Application of Risk Management to Medical Devices.

- BS EN 13641:2002 Elimination or Reduction of Risk of Infection Related to *in vitro* Diagnostic Reagents.

11.1.4.3: Performance evaluation

Performance evaluation should be undertaken in accordance with:

- BS EN ISO 23640 *In vitro* diagnostic medical devices, Evaluation of stability of *in vitro* diagnostic reagents.
- Reagents must also comply with the Common Technical Specifications for *In Vitro* Diagnostic Medical Devices (where they exist).

11.1.4.4: Stability data

Stability testing should be performed in accordance with:

- BS EN ISO 23640 *In vitro* diagnostic medical devices, Evaluation of stability of *in vitro* diagnostic reagents.

11.1.4.5: Date of manufacture

- For blood grouping reagents the date of manufacture is the date of commencement of the last potency test on the batch or sub-batch that indicates attainment of the required specification.
- For reagent red cells the date of manufacture is the date of collection from the donor. Where reagent red cells are prepared from more than one donor, the date of collection of the oldest donation should be recorded as the date of manufacture.
- Where a freezing process is used to preserve red cells before their preparation for issue as reagent red cells, the date of manufacture is the date of recovery from the frozen state.

11.1.4.6: Colour coding of reagents

No colouring agent should be added to reagents for blood group serology except that:

- Polyspecific anti-human globulin reagents may be coloured green, anti-A may be coloured blue, anti-B may be coloured yellow.
- The colorant should not interfere with the observation of the test result.
- 'Bespoke' antisera for use on automation may be coloured providing the information contained in the barcode on each bottle contains sufficient identifiers (specificity and lot number) to provide assurance that the intended test has been performed. The colours used for other specificities should not be coloured blue or yellow to avoid confusion with those for anti-A and anti-B reagents.

11.1.4.7: Freedom from microbial contaminants

- Reagents should be prepared using validated processes to produce a final product free from microbial contaminants that adversely affect the unopened product during storage at the recommended temperature. The manufacturer should routinely monitor the efficacy of the process used in the manufacture of the reagent.

- A preservative may be included in the reagent to minimise the effects of contamination during use if the preservative has been shown not to adversely affect the product during storage or use.
- Other than reagent red cells, all reagents for blood group serology recommended by the manufacturer for storage in the liquid state, should be filtered through a sterile filter of pore size not exceeding 0.22 µm. All reagents should be dispensed into the immediate container under aseptic conditions.
- Tests for contamination do not give absolute assurance of freedom from microbial contaminants. Bactericidal agents in common use for blood grouping reagents do not guarantee the absence of microbial agents after opening of the container.

11.1.4.8: Retained samples

- A minimum of 1% or three immediate containers, whichever is less, of each batch of reagents other than reagent red cells should be retained and stored as recommended by the manufacturer to enable analysis of reported defects. Such samples should be retained for at least 6 months beyond the expiry date.
- A minimum of two final containers of each batch of reagent red cells should be retained and stored as recommended by the manufacturer to enable analysis of reported defects. Such samples should be retained for at least 10 days beyond the expiry date.

11.1.4.9: Tests required

The manufacturer should test, as described in these guidelines, each lot of a reagent obtained from the immediate container to be supplied for use (see section 11.2.1).

11.1.4.10: Human source material

Existing procedures in the UK Blood Transfusion Services for consent to donate are sufficient to allow cellular and plasma materials collected as part of the donation process to be used as reagents without further explicit consent.

Samples/donations that are obtained specifically for reagent purposes will require additional consenting of the donor, and must have appropriate ethical approval. Donor materials that are obtained and retained for genomic or nucleic acid testing must comply with the regulations laid down by The Human Tissue Act 2004 (except Scotland).³

Residual samples retained from patient testing laboratories may be used without further explicit consent, if anonymised.⁴ Additional samples taken from patients specifically for reagent use will require ethical approval and explicit consent. All patient samples acquired and retained must comply with the regulations laid down by the Human Tissue Act (2004).

Each individual donation or sample of human material in a reagent for blood group serology shall be tested and found negative for mandatory microbiological tests required by the UK Blood Transfusion Services for blood donations (see Chapter 9). A statement is required in the 'instructions for use' to this effect.

In case there is a need to perform retrospective microbiological testing on material used to prepare *in vitro* diagnostic devices, an archive sample collected at the same time and from the same donor(s) used to prepare the device should be taken and stored for up to six months.

11.1.4.11: Label requirements

The label must conform to the requirements of:

- BS EN 18113:2011 Information Supplied by the Manufacturer with *in vitro* Diagnostic Reagents for Professional Use.
- The label fixed to the immediate container of a reagent should leave uncovered sufficient area of the full length or circumference of the container to allow ready visual inspection of the contents.
- The specificity of the reagent for blood group serology should be of a print size which is clearly legible. The print size of other information on the label should not exceed that used for the specificity of the reagent.
- The typeface used should clearly differentiate between antigens and related antibody specificities represented by upper and lower-case characters, e.g. C/c, S/s and K/k.
- For products needing to be prepared in the final form by the user following the instructions of the manufacturer and to be retained in the manufacturer's immediate container, a space should be available on the container label for the user to write the expiry date of the prepared product when stored as recommended by the manufacturer.
- The main panel of labels of enzyme-treated reagent red cells may be coloured pink in order to be distinguishable from non-enzyme-treated reagent red cells. Pantone colour reference 223 is recommended.

For other reagents, any colour appearing on the main panel of the label should comply with Food and Drug Administration regulations (21 CFR 660.28) as shown in Table 11.1.

Table 11.1 Label colour coding

Specificity	Colour	Code
anti-A	Blue	305C
anti-B	Yellow	102C
anti-C	Pink	204C
anti-D	Grey	429C
anti-E	Brown	465C
anti-CDE	Orange	151C
anti-c	Lavender	529C
anti-e	Green	577C

11.1.4.12: Instructions for use (package insert)

The instructions for use must conform to the requirements of BS EN 18113:2011 Information Supplied by the Manufacturer with *in vitro* Diagnostic Reagents for Professional Use.

In addition:

- For blood grouping reagents containing monoclonal antibodies, the identity of the cell line(s) from which the monoclonal antibodies have been derived.
- For reagent red cells for antibody screening and for antibody identification, the 'antigen profile' of the component cell samples should have the lot number and expiry date of the reagent to which it refers.
- A statement that loss of reactivity may occur during the stated shelf life of the red cells and that since this loss is partly determined by characteristics of individual blood donations or donors, which cannot be predicted or controlled, the conditions of storage and use recommended by the manufacturer should be rigidly applied.
- For enzyme-treated reagent red cells, information should be given concerning those antigens which are rendered inactive or less active by the enzyme treatment used.