LABORATORY TESTING OF PATIENT SAMPLES IN TRANSFUSION MEDICINE

RECOMMENDATIONS issued by the Swiss Transfusion Medicine Association (SVTM) and Swiss Transfusion SRC (B-CH)
for healthcare professionals, laboratories and medical institutions on immunohaematological and molecular testing of patient blood samples

Content changes in the current version 6, valid from 01.02.2023

Nomenclature: both notations (RH1 and RH/KEL1) are shown side by side only once in the text, thereafter only the ISBT nomenclature is used.

Updating the used abbreviations

5.6.1 Written information on accompanying documents: Date and signature/initials of the employee who **released the pRBC**. Before: who performed the tests.

6.2 Release/validation of the results: Each laboratory **establishes** its medical validation policy to ensure that sensitive results are not **withheld that could compromise** patient safety. Before: Each laboratory establishes its medical validation policy to ensure that sensitive results are not retained to the detriment of patient safety.

7.1.4 Foetal RHD determination from maternal blood: Note completed: The analysis was not validated for twin pregnancies.

7.2.3 Direct anti-human globulin test: completed: If no antibodies are detectable in the mother's ABS (and no antibodies with specificity anti-A/-B are present in the infant's eluate), a compatibility test with serum/plasma of the mother and the infant's or paternal erythrocytes could be considered (attention ABO incompatibility!). If HDN is suspected due to ABO incompatibility between mother and child, the eluate should be prepared with at least one A or B test cell.

7.4.2 Transfusions in premature infants, neonates and children until the end of the fourth month [13; 14]: If further transfusions are given, the XM is done with antigen-negative pRBC and serum/plasma from the mother as long as the child **has reached the end of the 4. month of life**. **Maternal serum with alloantibodies can be frozen to perform XM with antigen-negative pRBC if needed**.

9.5 Autoimmunohaemolytic anaemia: in an emergency situation, in which there is no time to wait for the laboratory results, the transfusing doctor must be informed about the risk, **see also chapter 9.3. If known**, the pRBC should be chosen in accordance with the RH/KEL1 phenotype and, if **appropriate**, the extended phenotype.

9.7 Transfusion of irradiated pRBC: completed: With the consent of the attending physician, these time limits may be deviated from in exceptional cases. Such exceptional cases are situations in which the benefit of the deviation outweighs the potential risk of transfusion delay. Such exceptional cases must be well documented.

9.9 Procedure and selection of blood products for patients being treated with monoclonal antibodies**:** **In the presence of a negative ABS, by appropriate method (e.g., tube or DTT, trypsin, or alternative procedure to inhibit interference),** pRBC (compatible with ABO/RH1 / RH/KEL1 / KEL3 (Kpa)) can be released by T&S. Before: If a negative ABS using the tube method or a DTT panel is available, pRBC (compatible with ABO/RH1 / RH/KEL1 / KEL3 (Kpa)) can be released by T&S.

9.10.2 Allogeneic stem cell transplantation (from a donor): competed: In case of a positive DAT after an ABO incompatible HSCT, an additional A or B test cell must be prepared with the eluate.

9.11 Sickle cell disease (new chapter): This clinical situation can affect all patients with phenotypes homozygote HbSS, compound heterozygote HbS-β thalassaemia (HbS-β+ or HbS-β°thalassaemia), HbSC, HbS OArab, HbS Lepore, HbSD and HbSE. Transfusions may be necessary depending on the form and clinical characteristics. The supply of blood for these patients presents an immunohaematological challenge for three reasons:

- There is considerable genetic diversity between the patients (African origin) and the donor population.
- Alloimmunization and severe immune haemolytic reactions can occur more frequently.
- *RHD* and *RHCE* variants are more frequent than in the Caucasian population [31; 32].

The recommendations are therefore as follows:

- Previous results of pre-transfusion tests and a transfusion history must be obtained to organize the patient care as well as possible.
- If no previous phenotype/genotype data are available, the following tests should be performed:
- Extended phenotyping: RH1, RH2, RH3, RH4, RH5, KEL1, KEL2, JK1, JK2, FY1, FY2, MNS1, MNS2, MNS3 and MNS4 (RhD, C, E, c, e, K, k, Jk^a, Jk^b, Fy^a, Fy^b, M, N, S and s) if there has been no transfusion within the past four months.
- Extended genotyping: KEL*01.01, KEL*02, JK*01, JK*02, FY*01, FY*02, FY*02.N.01, GYPA*01, GYPA*02, GYPB*03 and GYPB*04
- Genotyping can optionally be extended with the alleles *DO*01*, *DO*02*, *KEL*02.03*, *KEL*02* (c.841C, c.1790T), *KEL*02.06* (Do^a, Do^b, Kp^a, Kp^b, Js^a and Js^b).
- In addition, the most frequent and most relevant variants of the *RHD* and *RHCE* genes should be investigated. Extended genotyping should also be carried out if the extended phenotype is already known.
- Where a patient has been transfused > 12 units pRBC without forming alloantibodies or autoantibodies, forgoing in-depth testing of the RHD and RHCE genes can be considered [33].
- The antibody testing should be carried out using the enzyme technique (e.g. papain) in addition to IAT. In particular, in the event of the occurrence of a vaso-occlusive crisis after transfusions, an inadequate increase in haemoglobin or a suspected transfusion reaction, alloimmunization should be excluded as the cause. This can be done with additional tests such as elution despite negative DAT or compatibility testing with eluate.
- Certain alloantibodies, which in most cases play a minor role in transfusion medicine (see Table 8.1.3.2), should be generously taken into account in sickle cell patients (e.g. LE1 in papain), even when they can no longer be detected.
- The following antigens should be taken into account as a preventive measure in every RBC transfusion: RH1, RH2, RH3, RH4, RH5, KEL1, KEL2, JK1, JK2, FY1, FY2, MNS3 and MNS4 (RhD, C, E, c, e, K, k, Jk^a, Jk^b, Fy^a, Fy^b, S, s). If this is not possible, the prescribing doctor must be informed of the risk of possible immunization.
- Detection of an initial irregular antibody or autoantibody should be considered a warning sign: The patient may be a responder and could be at risk of forming further alloantibodies, which could result in a transfusion shortage.
- Release of pRBC using the T&S procedure is explicitly advised against. Compatibility for all pRBC to be transfused is recommended, even if no irregular antibodies are present. This ensures that the risk of a transfusion reaction by an anti-private antibody is minimized.
- Since certain antibodies rapidly fall back below the limit of detection, antibody testing should be carried out again 10 to 21 days after each transfusion.
- Every vaso-occlusive crisis that occurs within 21 days of a transfusion should be considered as potential alloimmunization, which must be actively investigated [34].

10.2.2 Immunohaematological investigations: completed: In case of ABO incompatibility, e.g. PC or after administration of IVIG, the eluate should additionally be prepared with an A or B test cell.

Updating the bibliography, other resources and members of the responsible expert group.

Abbreviations used

ABS	antibody screen
AG	antigen
AIHA	autoimmune haemolytic anaemia
B-CH	Swiss Transfusion SRC
BG	blood group
CMV	cytomegalusvirus
COMAL	criteria for the operation of medical analytical laboratories
DAT	direct anti-human globulin test (formerly direct Coombs test)
DTT Panel	test erythrocytes treated with Dithiotreitol
EDTA	whole blood anticoagulated with ethylenediaminetetraacetic acid
EFI	European Federation for Immunogenetics
EQC	external quality control
FFP	fresh frozen plasma
HDN	haemolytic disease of the newborn
IAT	indirect anti-human globulin test (formerly indirect Coombs test)
IgG/A/M	class G/A/M immunoglobulins
IQC	internal quality control
IVIG	Intravenous Immunoglobulins
LDH	lactatdehydrogenase
LISS	low ionic strength solution, describes a solution with a lower ionic concentration than NaCl solution
MDAT	monospecific DAT
MPLO	Medicinal Products Licensing Ordinance
n/a	not applicable
NaCl	sodium chloride
PC	platelet concentrate
PCR	polymerase chain reaction
pRBC	packed red blood cells / erythrocyte concentrate / blood unit
QC	quality control
QUALAB	Swiss Association for Quality Development in the Medical Laboratory (previously: Swiss Commission for Quality Assurance in Medical Laboratories)
RBC	red blood cells
RHD*06	RHD variant *RHD*06* (RHD*DVI)
RHIG	RH immunoglobulin prophylaxis
RH/KEL1-phenotype	RH2 (C), RH3 (E), RH4 (c), RH5 (e) and KEL1 (IK)
SVTM	Swiss Transfusion Medicine Association
T&S	type and screen (blood group determination and antibody screen)
TPA	Therapeutic Products Act

TPO	Therapeutic Products Ordinance
WP	week of pregnancy
XM	Compatibility testing (crossmatch)

Table of contents

1 Introduction and scope ... 9
1.1 General transfusion requirements [2] ... 9
2 Quality assurance system and documentation [4] ... 11
2.1 General quality requirements ... 11
2.2 Requirement for the electronic release of pRBC ... 11
2.3 Recording and storage obligation ... 11
3 Reagents, equipment and quality controls ... 12
3.1 Reagents ... 12
3.1.1 General ... 12
3.1.2 Cell wash solutions ... 12
3.1.3 Test cells ... 12
3.1.4 Test sera ... 12
3.2 Equipment ... 13
3.3 Quality controls ... 13
3.3.1 Internal quality controls ... 13
3.3.2 External quality controls ... 14
4 Pre-analytics ... 15
4.1 Sampling and identification ... 15
4.2 Pre-transfusion requirements ... 15
4.2.1 Blood group ABO/RH1 (RhD) ... 15
4.2.2 Antibody testing ... 16
4.3 Validity of sample material and results of investigations ... 16
5 Immunohaematological investigation [6] ... 17
5.1 ABO and RH1 blood group determination ... 17
5.1.1 Full blood group determination ABO/RH1 ... 17
5.1.2 Result and interpretation of ABO blood group determination ... 17
5.1.3 Result and interpretation of RH1 antigen determination ... 17
5.1.4 AB/RH1 antigen check ... 18
5.1.5 Result and interpretation of the AB/RH1 antigen check ... 18
5.2 RH/KEL1 and extended phenotype ... 18
5.2.1 Determination of RH/KEL1 and extended phenotype ... 18
5.2.2 Result and interpretation of the RH/KEL1 phenotype and other blood group antigens 19
5.3 Antibody screen and antibody identification ... 19
5.3.1 General ... 19
5.3.2 Methods for the antibody screen and identification ... 19
5.3.3 Result of the antibody screen ... 19
5.3.4 Antibody identification ... 19

5.4 Direct anti-human globulin test and elution ... 20
5.4.1 Direct anti-human globulin test ... 20
5.4.2 Elution ... 20
5.5 Pre-transfusion compatibility testing ... 23
5.5.1 Release of pRBC for transfusion ... 23
5.5.2 Release by T&S ... 23
5.5.3 Release by XM ... 23
5.6 Written information, issue of pRBC ... 24
5.6.1 Written information on accompanying documents ... 24
5.6.2 Issue of released pRBC ... 24
5.7 Post-transfusion immunohaematological control ... 24
6 Post-analysis ... 25
6.1 Data entry of results ... 25
6.2 Release/validation of the results ... 25
6.3 Communication of results ... 25
6.3.1 Report ... 25
6.3.2 Blood group card ... 25
7 Pregnancy and paediatrics [8; 9; 10] ... 27
7.1 Immunohaematological management during pregnancy ... 27
7.1.1 Prenatal check between 8th and 16th WP [8; 9; 23] ... 27
7.1.2 Pregnancy follow-up in the 28th week [8; 23] ... 27
7.1.3 Pregnant women with RH1 variants ... 27
7.1.4 Foetal *RHD* determination from maternal blood ... 27
7.1.5 RH immunoglobulin prophylaxis ... 27
7.1.6 Alloantibodies during pregnancy ... 28
7.2 Testing of neonates and children under four months of age ... 28
7.2.1 Blood samples ... 28
7.2.2 Determination of AB and RH1 antigen ... 28
7.2.3 Direct anti-human globulin test ... 29
7.2.4 Pre-transfusion testing [13; 14] ... 29
7.2.5 Results ... 29
7.3 Testing of children over four months of age ... 29
7.4 Transfusions in children ... 29
7.4.1 Intrauterine transfusions ... 29
7.4.2 Transfusions in premature infants, neonates and children until the end of the fourth month [13; 14] ... 30
7.4.3 Transfusions in children (fifth to twelfth months) ... 30
7.4.4 Exchange transfusions, see § 9.2. ... 30
8 Selection of blood groups for labile blood products ... 31
8.1 Selection of blood groups for packed red blood cells ... 31
8.1.1 Selection of the ABO blood group ... 31
8.1.2 Selection of the RH1 antigen ... 31
8.1.3 Selection of other blood group antigens ... 32
8.2 Selection of ABO blood group for fresh frozen plasma ... 34
8.3 Selection of ABO/RH1 in platelet concentrates ... 34

8.4 Selection of ABO/RH1 in specific situations ... 35

9 Procedure and selection of blood products in specific clinical situations ... 36

9.1 Autologous transfusion ... 36

9.2 Exchange transfusions ... 36

9.3 Emergency transfusion ... 36

9.3.1 Selection of ABO and RH1 blood groups in emergency transfusions ... 36
9.3.2 Other pre-transfusion tests ... 36

9.4 Massive transfusions ... 36

9.4.1 General ... 36
9.4.2 Selection of ABO/RH1 blood groups in massive transfusions ... 37

9.5 Autoimmunohaemolytic anaemia ... 37

9.6 Chronic transfusion requirement ... 37

9.7 Transfusion of irradiated pRBC ... 38

9.8 Procedure and selection of blood products if allergic/anaphylactic transfusion reactions occur and in IgA-deficient patients ... 38

9.9 Procedure and selection of blood products for patients being treated with monoclonal antibodies ... 38

9.10 Transplantation ... 39

9.10.1 Organ transplants ... 39
9.10.2 Allogeneic stem cell transplantation (from a donor) ... 39

9.11 Sickle cell disease ... 39

10 Adverse transfusion reactions ... 41

10.1 General ... 41

10.2 Investigation of suspected haemolytic transfusion reactions ... 41

10.2.1 Material ... 41
10.2.2 Immunohaematological investigations ... 41
10.2.3 Further investigations ... 42

10.3 Reporting system ... 42

11 Standards for molecular blood group typing ... 43

Editorial

This document was composed in collaboration with the Swiss Transfusion Medicine Association (SVTM) and Swiss Transfusion SRC (B-CH SRK) and revised according to the current state of science and technology.

It provides guidelines for good laboratory practice in immunohaematology and also supports decision-taking in specific clinical situations. When dealing with cases not described here, it is recommended to consult reference documents and/or the doctor responsible for the transfusion.

The Therapeutic Products Act requires the establishment of a quality assurance system that conforms to the current state of medical science and technology not only for the manufacture but also for the use of labile blood products (TPA art. 34 para 2 lit. b, VAM art. 65, para. 4).

Swissmedic was involved in the consultation process for the revised version and supports the document. These recommendations describe methods suitable for verifying the compatibility of labile blood products with the recipients. Further minimum requirements in terms of pre-analytics, the ordering and selection of suitable blood components and documentation of the process steps with the aim of ensuring safety in transfusion medicine be defined. These recommendations must be taken into account in the context of pre-transfusion testing and for all processes leading to the dispatch of a product for blood transfusion.

A procedure that deviates from these recommendations may be used if, on the basis of current scientific findings, it can reliably be assumed that the procedure will achieve an outcome that is at least equivalent to the quality and safety objectives underlying the recommendations. These recommendations will also be used as a reference during inspections. Additionally, they will be taken into account when evaluating whether an institution performing transfusions has a quality assurance system that is adequate for the use of labile blood products.

As the competent authority, we would like to thank all the organisations and individuals involved.

SWISSMEDIC, Haemovigilance

These recommendations were drawn up by the “Immunohaematology” expert group.

1 Introduction and scope

The use of labile blood products (transfusion) is a complex therapeutic activity demanding professional skills of the staff involved in pre-transfusion testing and blood transfusions. Persons handling labile blood products, in particular, shoulder a great responsibility preventing serious adverse events. Although there are no statutory requirements governing pre-transfusion testing, the Therapeutic Products Ordinance (art. 65, para. 4) [1] requires institutions that use labile blood products to establish a quality assurance system for their use that is in keeping with the current state of knowledge and to designate a person who is responsible for haemovigilance. Evidently, the laboratory must comply with the recognised standards for quality assurance systems (COMAL) (ISO 15189 and/or 17025 should be sought).

These recommendations apply to laboratories performing immunohaematological testing for users of labile blood products. They describe the procedure as well as the scope and nature of tests and their interpretation. Furthermore, they define the administrative steps involved in the identification of samples and blood products, the recording and transfer of the results and the minimum quality requirements.

The laboratory staff, under the responsibility of the management, advises the responsible doctor on the performance of immunohaematological testing and on the choice of blood products in order to ensure a competent transfusion. Laboratory management and nursing staff ensure that the blood products comply with the requirements stipulated in the doctor's prescription (see Guidelines for quality assurance in transfusions issued by the Swiss Working Group on Quality Assurance in the Use of Blood Products [22]).

Information is provided on the following topics:

- Immunohaematological testing
- Information on the transfusion of blood products
- Information on quality management
- Haemovigilance for recipients

As of 2022, the nomenclature of the blood group systems in this document will be adapted to the ISBT terminology in order to comply with the internationally used notation [25, 26]. To simplify the legibility and use of the new nomenclature, a table – which is by no means complete – showing the traditional notation and the ISBT terminology has been compiled (see Addendum 1). The ABO blood group system is an exception.

To simplify legibility, the conventional male form of personal substantives and pronouns will be used in this document.

1.1 General transfusion requirements [2]

Labile blood products must be used in accordance with the current state of knowledge. Requirements concerning the following points must be observed:

- Pre- and post-analytics
- Pre-transfusion immunohaematological testing
- Issuing labile blood products
- Full traceability of samples, analyses, labile blood products (supplied and returned, link between product and recipient)
- Important information (transfusion recommendations, transfusion-related events and transfused products) should be entered in the patient's electronic medical record in the responsibility of the prescriber.

The various aspects of the transfusion process must be regulated in internal regulations (in the hospital, doctor's practice and testing laboratory). Indications and rules for applying individual blood products are the responsibility of the doctor performing the transfusion. Every institution transfusing labile blood products is required to nominate a quality manager in accordance with the current legal provisions [3; 23].

2 Quality assurance system and documentation [4]

2.1 General quality requirements

The laboratory tests, quality controls and laboratory documents must comply with the requirements stipulated in the quality assurance system.

- Laboratory management is responsible:
  - for ensuring that detailed operating procedures for the tests performed in the laboratory are available to all staff members and that these are followed
  - for the training/qualification of all members of staff
  - for the qualification and servicing of the equipment
  - for the qualification of consumables
  - for compliance with the requirements relating to the premises
  - for documentation and change management
- Laboratory documentation includes:
  - Results and interpretation of pre-transfusion testing
  - Date and signature/initials of the employee who performed the tests (or electronic alternative)
  - List of labile blood products delivered (product specifications and collection numbers)

2.2 Requirement for the electronic release of pRBC

If release is performed electronically, the following conditions must be fulfilled:

- The system must comply with the recognised standards and be qualified.
- A manual backup system must be available in case the system cannot be used.
- These preconditions must be recorded in writing (e.g. documented in an SOP).

If there are discrepancies in the blood group and/or antibody determinations, electronic release may not be performed until these have been resolved.

2.3 Recording and storage obligation

Since 2020, in accordance with art. 39 and 40 of the Therapeutic Products Act (TPA), it is mandatory to store records and all important documents for a period of 30 years [4].

In accordance with the Swissmedic guidelines on Inspections of blood banks (§ 5.4.6 “Documentation”) from 17.01.2020, the following requirements should be complied [24]:

- Guarantee of traceability from the donor (via donation number) to the patient and vice versa over a period of 30 years (preferably by the issuing authority, not only in the patient dossier, this requires feedback to the issuing authority on the transfusion that has been carried out)
- Specification documents (work instructions, SOPs) for all procedures
- Results and interpretation of compatibility testing
- Traceability of the materials used (incl. lot number) and test procedures
- Recalls and look backs performed
- Use of IT systems (laboratory systems, patient systems)

3 Reagents, equipment and quality controls

3.1 Reagents

3.1.1 General

- The laboratory reagents used should be CE marked.
- Non-CE-marked products or reagents produced in-house must be validated in accordance with the current reference standards before use.
- If no information about quality standards is present, it is recommended that a certificate of analysis be obtained from the manufacturer.
- The reagents must be used according to the manufacturer's instructions (package insert). Any deviations from these instructions must be validated and documented.

3.1.2 Cell wash solutions

- Buffered NaCl solutions with a pH between 7.0 and 7.5 are used to wash pRBC.

3.1.3 Test cells

For reverse typing in ABO blood group determination:

- pRBC from groups A_1, B and O are used for reverse typing (isoagglutinins) in ABO blood group determination. The use of test cells from group A_2 is optional.

For antibody screen (ABS) and for antibody identification:

- The test cells from group O used for ABS and antibody identification must have the following antigens: RH1 (RhD), RH2 (C), RH3 (E), RH4 (c), RH5 (e), RH8 (C^w), KEL1 (K), KEL2 (k), KEL3 (Kp^a) JK1 (Jk^a), JK2 (Jk^b), FY1 (Fy^a), FY2 (Fy^b), MNS1 (M), MNS2 (N), MNS3 (S), MNS4 (s), LE1 (Le^a) LE2, (Le^b) P1PK1 (P1), and if possible LU1 (Lu^a).

The antigens RH2 (C), RH3 (E), RH4 (c), RH5 (e), JK1 (Jk^a), JK2 (Jk^b), FY1 (Fy^a), FY2 (Fy^b), MNS3 (S) and MNS4 (s) must be expressed homozygously on at least one test cell. Commercial test cells used for ABS must be negative for the antigens MNS9 (Vw), MNS11 (Mg) and DI3 (Wr^a).

- If alloantibodies are present, the presence of further antibodies is excluded using test cells with the same criteria as the test cells used for antibody screening. If an anti-RH1 (anti-D) is identified, the heterozygous presence of antigen RH2 (C) and RH3 (E) is sufficient to exclude them.
- Test cells must not be mixed together.

3.1.4 Test sera

For determination of the ABO blood group antigens and the RH1 antigen:

- Monoclonal anti-A and anti-B test sera are recommended for ABO antigen determination. The use of an anti-AB test serum is optional. Monoclonal anti-B test sera must not determine an acquired B antigen.
- For Rh1 antigen determination, two monoclonal anti-RH1 test sera that originate from different clones should be used. At least one anti-RH1 reagent must not determine the *RHD*06 (RHD*DVI)* variant. For neonates: see § 7.2.

For determination of the RH/KEL1 phenotype and other blood group antigens:

- Monoclonal test sera should be used where commercially available (see also § 5.2).

3.2 Equipment

Laboratory equipment must be qualified. The laboratory equipment used for immunohaematological testing must be maintained regularly. The laboratory equipment must be monitored in compliance with the internal quality assurance system and the findings must be recorded and archived in accordance with the current requirements (see § 2.3).

Thermally controlled equipment used for blood products (refrigeration units, freezer units, platelet shakers, FFP thawing equipment) must be operated according to the requirements issued by Swissmedic or cantonal authorities.

3.3 Quality controls

3.3.1 Internal quality controls

The IQC must comply with at least the following requirements:

- Check of test cells
  - For reverse typing in ABO determination
    - Once a day or at least when the test is performed
    - Controlling of test cells is done using sera/plasma with known anti-A and anti-B antibodies.
  - For ABS
    - Once a day or at least when the test is performed
    - The test cells for the ABS should be tested using a weak anti-RH1 (maximum identification limit ≤10 ng anti-RH1 / ml) [5].
- Testing test sera
  - For AB/RH1 antigen determination
    - Once a day or at least when the test is performed
    - Controlling of test sera is done with RBC with known AB/RH1 antigens.
  - For the RH/KEL1 phenotype
    - Once a day or at least when the test is performed
    - Controlling of test sera is done with RBC with known heterozygous RH2 (C), RH3 (E), RH4 (c), RH5 (e) and KEL1 (K) antigens.
  - For determination of other blood group antigens
    - Once a day or at least when the test is performed
    - One positive, if possible heterozygous, and one negative control per antigen tested must also be set up.
- Verification of the result of the antigen determination carried out by IAT
  - A DAT must be set up in parallel using the same test system to exclude false positive reactions in the IAT.
- Verification of the techniques for the DAT and IAT (tube method)
  - Every negative result must be checked using a Coombs control reagent.
- DAT testing

- There are currently no suitable commercial tests (e.g. test cells with small amounts of IgG or C3d).

- Checking compatibility testing
  - Once a day or at least when the test is performed
  - Compatibility testing should be checked using RH1-positive and RH1-negative donor RBC and a serum with a known weak anti-RH1 (maximum identification limit ≤10 ng anti-RH1 / ml) [5].

- Checking molecular genetic test methods
  - Checking is determined by the test method (CE kit or in-house; see § 11).

- Checking all techniques
  - If analyses are performed using several methods/techniques, each one should be checked separately.

3.3.2 External quality controls

Laboratories that perform immunohaematological work are obligated to take part four times a year in EQC for immunohaematology organised by a recognised inter-laboratory testing laboratory (see QUALAB); this must cover all analyses for which an EQC is available.

Laboratories that perform molecular genetic testing are obligated to take part in corresponding EQC twice a year (see § 11).

4 Pre-analytics

4.1 Sampling and identification

- A native blood sample (without separating agent) and/or an EDTA blood sample should always be requested for an immunohaematological workup.
- Blood samples for immunohaematological testing should, if possible, not be taken from a venous access for drugs, infusions or transfusions (risk of dilution). If this is not possible, care must be taken to ensure that a large enough volume of blood is discarded before sampling to ensure that the sample is not diluted.
- The person taking the sample must ensure that the patient's identity was checked unequivocally beforehand by the competent administration (hospital, doctor's practice, etc.).
- The person taking the sample checks the correct identity in a suitable manner (signature/initials on the order form and/or tube, reading into an electronic recording system, etc.). The laboratory must be able to verify this information.
- All sample tubes must be labelled in a way that they can be assigned unequivocally to the patient:
    - Surname, first name, full date of birth, or
    - Unique patient identification number
- The date and time at which the sample was taken must be documented for each tube (tube and/or order form and/or laboratory information system).
- If blood samples are not labelled correctly but still assignable, it is the responsibility of the person in charge of the laboratory to decide whether testing can be carried out. Aberrations must be documented.
- Pre-transfusion testing must not be carried out with blood samples that are not labelled or not assignable.
- Every laboratory manager must draw up an emergency plan to ensure that samples can be assigned to patients securely if the computer system is not operating.

4.2 Pre-transfusion requirements

4.2.1 Blood group ABO/RH1 (RhD)

pRBC may only be transfused if at least two documented valid ABO/RH1 blood group determinations are available (type). If the ABO/RH1 blood group has never been determined, a full blood group determination should be performed on two blood samples obtained independently of each other with independent patient identification in each case in order to identify any mix-ups.

- If only one valid blood group determination is available (internal/external), a second full blood group determination must be performed. Foreign documents must be clearly legible and validated by the person responsible for the laboratory.
- For planned interventions it is recommended that the first blood sample should be taken before the patient is admitted to hospital, for example (blood group determined with simultaneous ABS if appropriate); the second sample should be taken when the patient is admitted, for example (blood group determination, possibly ABS).
- If two documented full blood group determinations (see § 5.1) or a valid blood group card with two entries are available, it is sufficient to check AB/RH1 antigens.
    - A "bedside test" cannot replace regular blood group determination. Deviations from the above procedure (for example in emergency transfusions) are the

responsibility of the doctor performing the transfusion and must be documented (see also § 9.3).

- The transfusion of FFP is governed by the same rules as for pRBC.
- A single determination is sufficient for the transfusion of PC.

4.2.2 Antibody testing

- If a valid ABS (screen) or antibody identification is available:
  - Immunohaematological tests can be performed during the validity period of the sample (max. 96 hours).

4.3 Validity of sample material and results of investigations

- The blood sample used for pre-transfusion investigations must have been obtained no longer than 96 hours before the start of the transfusion.
- After the expiration of the validity, a reasonable effort must be made to investigate the presence of potentially newly formed antibodies. Minimum requirement: Exclusion with test cells being homozygous for: RH (Rh), FY (Fy), JK (Jk), MNS3 (S) and MNS4 (s) or ensure antigen-compatible transfusion.
- The retained samples can usually be stored between 2 and 8°C for 7 days. If the serum is kept longer than 7 days it must be frozen.
- A blood sample from the patient and a sample of the pRBC supplied (e.g. segment or blood bag) must be retained in the laboratory for at least 7 days.
- For patients who have not received a transfusion in the past four months and those who are not pregnant, the validity of a negative ABS can be extended to 21 days. In this case:
  - the ABS must be performed by the laboratory, or at least under the responsibility, of the laboratory at the hospital in which the patient receives the transfusion;
  - at the latest by the time the first request for blood is sent, the transfusion laboratory must have received a document initialled by the responsible doctor, confirming that the patient has not received a transfusion in the meantime (since the sample was taken) and that the patient is not pregnant. If this confirmation has not been received, the result of the ABS is only valid for 96 hours, i.e. an extension of its validity to 21 days is not in conformity with the recommendations (see also § 5.5).

5 Immunohaematological investigation [6]

This chapter deals solely with serological methods; molecular diagnostics are covered in § 11.

5.1 ABO and RH1 blood group determination

5.1.1 Full blood group determination ABO/RH1

Full ABO/RH1 blood group determination comprises:

- AB antigen determination of the patient's RBC and reverse typing using the patient's serum/plasma
- RH1 antigen determination

Manual determination:

- AB antigen determination, reverse typing and RH1 antigen determination should be performed by two different members of staff. If the determination is done by only one person, the antigen determination must be checked using the same sample in a second test (new suspension).

Automated determination:

- Automated determination comprises determination using an automatic analyser and electronic data transfer to a laboratory information system.
- If AB/RH1 antigen determination and reverse typing (full blood group determination) are performed using an automatic analyser, a single test is sufficient.

5.1.2 Result and interpretation of ABO blood group determination

- The results of blood group determination and their interpretation are shown in Table 5.1.2. The blood groups must be documented in the simple form "O", "A", "B" or "AB".
- If deviating or questionable results are obtained, the blood group must not be interpreted. Further investigations must be performed (see § 11).
- If the first blood group determination is performed by molecular genetics, the second blood group determination may be done by serology. The serological result should be consistent with the first determination.

Table 5.1.2 Test results and interpretation of ABO blood group determination

Agglutination of patient's pRBC with test serum			**Agglutination of patient's serum/ plasma with test cells**				**Interpretation**
Anti-A	Anti-B	Anti-AB*	A_1	A_2*	B	O	Blood group
–	–	–	+	+	+	–	O
+	–	+	–	–	+	–	A
–	+	+	+	+	–	–	B
+	+	+	–	–	–	–	AB

***** optional

5.1.3 Result and interpretation of RH1 antigen determination

- The results of RH1 determination and their interpretation are shown in Table 5.1.3.
- If deviating or questionable results are obtained, the RH1 antigen must not be interpreted. Further investigations must be performed (see § 11).
- If the first RH1 determination is performed by molecular genetics, the second RH1 determination may be done by serology. The serological result should be consistent with the first determination.

- If the alleles *RHD*01W.1 (RHD*weak D type 1), RHD*01W.2 (RHD*weak D type 2), RHD*01W.3 (RHD*weak D type 3)* and *RHD*09.04 (RHD*weak D type 4.1, RHD*DAR4)* are identified using a molecular genetic method, the patient is considered to be RH1 positive; all other RH1 variants are considered to be RH1 negative. In the absence of clear evidence, we recommend that patients with *RHD*09.03.01 (RHD*weak D type 4.0, RHD*DAR3.1)* should be considered RH1 negative until further notice.

Table 5.1.3 Test results and interpretation of RH1 determination

Agglutination of patient's RBC by			**Interpretation of RH1**
First anti-RH1 test serum	Second anti-RH1 test serum	control serum	
positive	positive	negative	positive
negative	negative	negative	negative
weak positive	weak positive	negative	RH:W1/RH:P1* (weak D / RhD partial)
XX**	XX**	negative	RH:W1/RH:P1*
neg./pos.	neg./pos.	positive	not determinable, investigate

* Transfusion recommendations and during pregnancy: see § 7.1 and 8.1.2. Around 80% of RH:W1 (weak D) cases are *RHD*01W.1, RHD*01W.2* or *RHD*01W.3*

** discrepant results between the two antisera used

5.1.4 AB/RH1 antigen check

In order to check the AB/RH1 antigens it is sufficient to determine them using an anti-A, anti-B and anti-RH1 test serum.

5.1.5 Result and interpretation of the AB/RH1 antigen check

- The results must be consistent with the documented full blood group determination.
- If the results of the AB/RH1 antigen check are discrepant or questionable, a full blood group determination of AB and RH1 must be done with a new blood sample.

Important note: All possible errors must be taken into consideration, especially previous or current mix-ups of tubes and/or patients. In such cases several patients may be affected at the same time, and the investigations must be performed as a matter of urgency and the issue of further blood products that may potentially be involved must be postponed.

- If the patient is known to be RH:W1 (weak D) (investigated), a serologically negative result in the tube test is not a contradiction. If RH1 negative status was documented in the past (before 2012; non-differentiated RH:W1/RH:P1 [weak D / partial D]), a positive RH1 result does not represent a deviating result.

5.2 RH/KEL1 and extended phenotype

5.2.1 Determination of RH/KEL1 and extended phenotype

- Determination of the RH/KEL1 phenotype comprises the antigens RH2 (C), RH3 (E), RH4 (c), RH5 (e) and KEL1 (K).
- The extended phenotype comprises at least the following blood group antigens: JK1 (Jk^a), JK2 (JK^b), FY1 (Fy^a), FY2 (Fy^b), MNS3 (S) and MNS4 (s).

The minimum requirement is determination using the corresponding test sera and one method.

5.2.2 Result and interpretation of the RH/KEL1 phenotype and other blood group antigens

- The results must be clearly positive or negative.
- If deviating or questionable results are obtained, the blood group antigens must not be interpreted. Further investigations must be performed (see § 11).
- Molecular genetic determination of the major blood group antigens should be considered for patients who have received a transfusion in the last four months (see § 11).

5.3 Antibody screen and antibody identification

5.3.1 General

- Possible erythrocyte alloantibodies (or autoantibodies) that are present are identified in serum/plasma or eluate by means of ABS.
- If the ABS is positive, identification of the erythrocyte alloantibodies (or autoantibodies) is performed.
- The investigation of erythrocyte alloantibodies must at least capture warm reacting alloantibodies of IgG type.

5.3.2 Methods for the antibody screen and identification

- The selected method must be equivalent to the tube method in the two-stage IAT with monovalent or polyvalent anti-human globulin serum.
- The patient's serum/plasma or eluate is tested using test cells of group O with known antigen profiles at 37°C (see also § 3.1.3).
- Sensitivity and specificity must be at least equivalent to an identification limit of ≤10 ng (0.05 IU) anti-RH1 / ml.
- Additional methods, e.g. enzyme testing, are not mandatory.
- It is advisable for the laboratory that investigates the alloantibodies to perform at least an AB/RH1 check using the same sample tube.

5.3.3 Result of the antibody screen

- If the ABS is negative, no further investigations are required.
- If the ABS is positive, the reason for the positive result must be investigated (alloantibodies, autoantibodies, anti-CD38, LISS, etc.).

5.3.4 Antibody identification

- If possible, alloantibodies should be confirmed with at least two, preferably three antigen-positive and three antigen-negative test cells.
- If possible, an identified alloantibody (caution: previous transfusions) should be rendered plausible by the absence of the corresponding antigen on the patient's RBC.
- Identified alloantibodies must be taken into account according to their relevance for transfusion medicine (see § 8.1.3.2) [6].
- See § 5.5. 1 (compatibility testing) and § 8.1.3.2 (minimum requirements for the selection of RBC if antibodies are present) for known but no longer detectable antibodies.
- Alloantibodies with the specificities anti-A1, -H1 (H), -H1(I1) (H[I]), -P1PK1 (P1), -LE1 (Le^a), -LE2 (Le^b), -MNS1 (M) and -MNS2 (N) are normally not relevant provided that they are only cold- or enzyme-active (negative results in the agglutination test at 37°C or if the result is negative in IAT) (see § 8.1.3.2).

- The enzyme test is an additional method that is used predominantly by reference laboratories. Occasionally this may result in the identification of anti-RH3 (anti-E) or anti-RH8 (anti-C^w) "enzyme-only" antibodies. In such cases RH/KEL1-compatible pRBC (RH8 [C^w] not tested) can be released by T&S.
- In patients who have previously received a transfusion and whose results in IAT are unclear, elution may be considered even if the DAT is negative.
- For patients with free autoantibodies see § 9.5.
- For patients on anti-CD38 therapy see § 9.9.

5.4 Direct anti-human globulin test and elution

5.4.1 Direct anti-human globulin test

The DAT is used for the detection of antibodies and complement factors which have bound in vivo to the own RBC and/or transfused pRBC. The DAT should preferably be performed using a column agglutination test.

The indications for a polyspecific DAT are shown in Figure 5.4.1.

- If the DAT is negative with no signs of haemolysis, no further steps are required.
- If the DAT is negative with signs of haemolysis (e.g. LDH, total bilirubin and haptoglobin), see § 5.4.2.
- If a DAT is positive but not indicated, no further investigations are required. This also applies if the transfusion history is unknown. The doctor in charge is responsible for informing the laboratory about a prior transfusion in the past 14 days.
- If the result is positive, a monospecific DAT (IgG/C3d) should be performed (see Figure 5.4.2). An extended monospecific DAT (IgM/IgA) is recommended if signs of haemolysis are present. If signs of haemolysis are present and C3d alone is detected for the first time in the monospecific DAT, cold agglutinins, drug-induced antibodies or delayed haemolytic transfusion reactions due to alloantibodies should be considered for differential diagnosis.

5.4.2 Elution

Elution is used to demonstrate the presence of and to identify alloantibodies and/or autoantibodies attached to RBC.

- The indications for performing an elution are shown in Figure 5.4.2.
- If clinically relevant alloantibodies are detected in the eluate, these must be taken into account (XM and AG neg.), otherwise pRBC can be released by T&S.
- If autoantibodies are present in the eluate, see § 9.5. Reasons for a negative eluate in a positive DAT include, for example, use of certain medications, a large number of diseases and antibodies that may be destroyed by the elution technic.
- Two unpublished studies show that alloantibodies attached to RBC are most likely to have a DAT strength of < up to 2+.
- A significant increase is considered to exist if the reaction strength is ≥1+.
- Elution is always performed for every transfusion reaction with signs of haemolysis – irrespective of whether the polyspecific DAT is positive or negative. If there are signs of haemolysis, elution is also always performed if the DAT is negative.

Figure 5.4.1

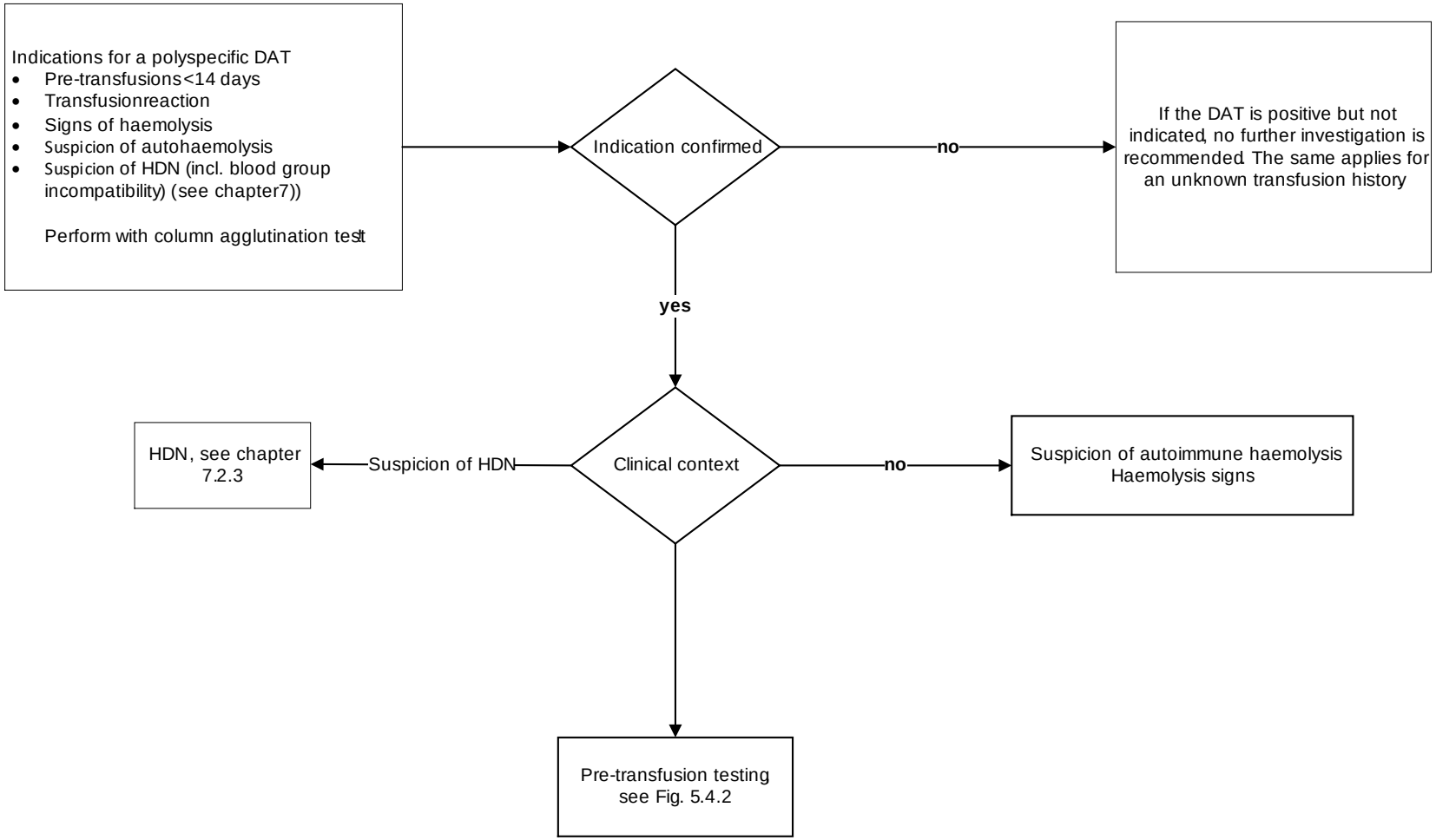
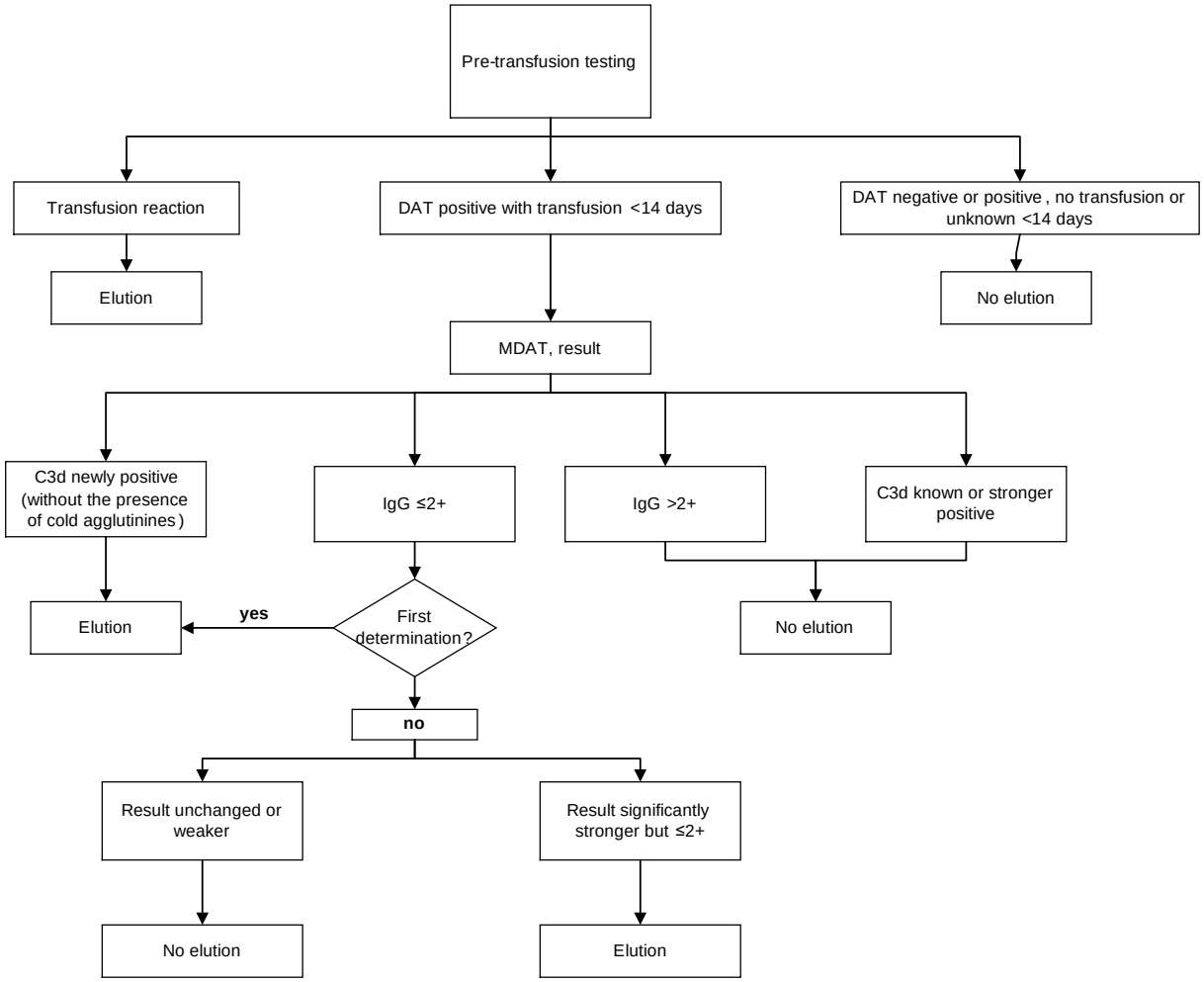

Indications for a polyspecific DAT
• Pre-transfusions<14 days
• Transfusionreaction
• Signs of haemolysis
• Suspicion of autohaemolysis
• Suspicion of HDN (incl. blood group incompatibility) (see chapter7))
Perform with column agglutination test
Indication confirmed
no
If the DAT is positive but not indicated, no further investigation is recommended. The same applies for an unknown transfusion history
yes
Clinical context
Suspicion of HDN
HDN, see chapter 7.2.3
no
Suspicion of autoimmune haemolysis
Haemolysis signs
Pre-transfusion testing
see Fig. 5.4.2

Figure 5.4.2


Pre-transfusion testing
Transfusion reaction
DAT positive with transfusion <14 days
DAT negative or positive , no transfusion or unknown <14 days
Elution
No elution
MDAT, result
C3d newly positive (without the presence of cold agglutinines)
IgG ≤2+
IgG >2+
C3d known or stronger positive
Elution
yes
First determination?
No elution
no
Result unchanged or weaker
Result significantly stronger but ≤2+
No elution
Elution

5.5 Pre-transfusion compatibility testing

The compatibility of the patient's sample and blood products can be ensured by T&S (standard method) or XM.

- The A, B and RH1 antigens in the pRBC must be checked.
- The blood group of the patient and the pRBC must be compatible (see § 8.1).
- If antigen specificities are taken into account as a preventive measure, these do not necessarily have to be tested on the pRBC.
- If clinically relevant alloantibodies are currently detectable or known, the pRBC must be tested for each corresponding antigen and a XM must be performed (see Table 8.1.3.2).
- If low-frequency ("private") antibodies are present, the pRBC can be released by a negative XM.
- If the results of antibody identification are doubtful or unclear, a XM must be performed.
- If anti-RH1 is due to RHIG prophylaxis and other clinically relevant antibodies have been excluded, pRBC can be released by T&S.
- If an anti-RH3 (anti-E) or anti-RH8 (Anti-C^w) "enzyme only" antibody is present, RH/KEL1-compatible pRBC can be released by T&S.

5.5.1 Release of pRBC for transfusion

In this context, release means the provision of a blood product that fulfils the immunohaematological compatibility criteria for a specific patient.

5.5.2 Release by T&S

- Conditions for release by T&S:
  - Determination of the ABO blood group and the RH1 antigen in the patient's sample (type)
  - A negative, valid antibody screen must be available (screen)
  - AB/RH1 antigen check of the pRBC
  - Verification and documentation of the compatibility of the patient's AB/RH1 with the AB/RH1 of the pRBC

5.5.3 Release by XM

- Conditions for release by XM:
  - Determination of the ABO blood group and the RH1 antigen in the patient's sample
  - A valid antibody screen or antibody identification must be available
  - XM of patient serum/plasma with each pRBC using IAT
  - AB/RH1 antigen check of the pRBC and check of antigen negativity if alloantibodies are present
  - Compatibility verification:
    - of the patient's AB/RH1 with the AB/RH1 of the pRBC
    - of any alloantibodies that the patient may have with the correspondingly antigen-negative pRBC
- If the XM is positive and no reason for this can be found, further investigations must be performed before products are transfused. If further investigations produce no result, the prescribing doctor must be informed about possible risks and precautions.

5.6 Written information, issue of pRBC

5.6.1 Written information on accompanying documents

- If pRBC are released for a specific patient, at least the following must be present:
  - Recipient's surname, first name and full date of birth
  - Recipient's ABO blood group and RH1 antigen
  - Product identification number, ABO blood group and RH1 antigen of the pRBC
  - Expiration date (within the 96-hour period of validity)
  - Date and signature/initials of the employee who released the pRBC

5.6.2 Issue of released pRBC

In this context, issue means the delivery of blood products that fulfil the release criteria.

- Documentation of the date and signature/initials of the employee who issued the pRBC.
- When the 96-hour rule is applied, the released pRBC (T&S and XM) must be transfused within 96 hours (see § 4.2.2) after blood sampling. The transfusion must have started within 96 hours. After this time has elapsed, a newly drawn patient blood sample must be obtained to repeat pre-transfusion testing before further transfusions can be performed.

5.7 Post-transfusion immunohaematological control

Following homologous transfusions of pRBC, it is recommended to check for the possible formation of alloantibodies. Since certain antibodies are not detectable until several weeks later, and others can rapidly fall below the limit of detection, this follow-up should preferably be done 6 to 12 weeks after the transfusion.

6 Post-analysis

6.1 Data entry of results

- Manual data entry
  - Data entry should be verified, documented and initialled by a second person.
- Electronic data transfer
  - Correct data transfer must be verified beforehand by validation.

6.2 Release/validation of the results

Final results, whether determined manually or automatically, cannot be released until they have been validated.

Release means the validation and communication of the results to the prescriber (client).

- The results are validated by the laboratory manager (manual or electronic signature). The delegation of this responsibility must be described in documented internal guidelines.
- Each laboratory establishes its medical validation policy to ensure that sensitive results are not withheld that could compromise patient safety.

6.3 Communication of results

The use of the international nomenclature (ISBT) is to be aimed for in the long term.

6.3.1 Report

The analytical report must contain the following:

- Name and address of the laboratory
- Sample number
- Patient's surname, first name and date of birth
- Date of sample withdrawal
- Date of analyses
- Results of the analyses
- Antibodies that are no longer detectable must also be mentioned in the document
- Interpretation and evaluation of the analyses
- Date and signature/initials of the person responsible for validation (or electronic alternative) or their deputy
- The methods used should preferably be specified

6.3.2 Blood group card

- Minimum requirements for the blood group card:
  - Surname, first name, full date of birth
  - ABO blood group and RH1, including information on any RH1 variants
  - Date and signature/initials (or electronic alternative)
  - Identified erythrocyte alloantibodies
  - The blood group card is not valid until the second blood group determination becomes available (see § 4.2.1). This information must be printed clearly on the blood group card.
- Extended requirements for the blood group card:

- RH/KEL1 phenotype and other blood group antigens if the data are available and if possible with computer system
- Reference to transfusion recommendations as required

- The laboratory manager, their deputy or a person trained for this purpose releases (resident physician, biomedical analyst, etc.) the blood group card with their signature.

7 Pregnancy and paediatrics [8; 9; 10]

7.1 Immunohaematological management during pregnancy

7.1.1 Prenatal check between 8th and 16th WP [8; 9; 23]

- ABO determination
- RH1 antigen determination
- RH/KEL1 phenotype determination
- ABS

7.1.2 Pregnancy follow-up in the 28th week [8; 23]

Another antibody screen is performed in the 28th week of pregnancy, although the evidence for RH1-positive pregnant women tends to be scant in the literature. The blood sample should be taken from RH1-negative pregnant women prior to RHIG prophylaxis.

7.1.3 Pregnant women with RH1 variants

For patients with a serologically weak RH1 antigen (see § 5.1.3), a determination by molecular biology of the exact genotype should be performed (see § 11).

7.1.4 Foetal *RHD* determination from maternal blood

Foetal *RHD* genotyping from maternal blood is recommended for RH1-negative pregnant women from the 18th week of pregnancy [23]. This test is used to decide whether RHIG prophylaxis is indicated. The pre-analytical conditions must be observed strictly for this test (it is vital to contact the responsible laboratory beforehand) (see § 11).

Note: If the pregnant woman has an RH1 variant, foetal *RHD* determination is not possible. The analysis was not validated for twin pregnancies.

7.1.5 RH immunoglobulin prophylaxis

- RHIG prophylaxis is recommended for RH1-negative pregnant women.
- *RHD*01W.1 (RHD*weak D type 1), RHD*01W.2, (RHD*weak D type 2), RHD*01W.3 (RHD*weak D type 3)* and *RHD*09.04 (RHD*weak D type 4.1)* are considered to be RH1 positive and do not require RHIG prophylaxis.
- All other RH1 variants are considered to be RH1 negative and RHIG prophylaxis is recommended (see Table 7.1.5).
- In the absence of clear evidence, we recommend that patients with *RHD*09.03.01 (RHD*weak D type 4.0, RHD*DAR3.1)* should be considered RH1 negative until further notice.

Injection of RHIG prophylaxis is intended to avoid maternal immunisation against the RH1 of the foetus. RHIG prophylaxis is recommended around the 28th week of pregnancy for an *RHD*-positive or undetermined foetus and if complications have arisen during pregnancy (please refer to Expert Letter No. 68 for more details [23]).

Post-partum RHIG prophylaxis should be administered within 72 hours of the birth of an RH1-positive child [23].

Table 7.1.5 RHIG prophylaxis and RH1 variants

RH1 phenotype	Genotype	RHIG prophylaxis during pregnancy
RH:–1 (RhD negative)	n/a	yes, if the result of foetal *RHD* determination is positive or unknown
RH:W1/RH:P1 (weak D / RhD partial)	unknown	yes, until the result of PCR is available
RH:W1/RH:P1	*RHD*01W.1/.2/.3 (RHD*weak D type 1/2/3)* or *RHD*09.04 (RHD*weak D type 4.1)*	no
RH:W1/RH:P1	no *RHD*01W.1/.2/.3 (RHD*weak D type 1/2/3)* or *RHD*09.04 (RHD*weak D type 4.1)*	yes

7.1.6 Alloantibodies during pregnancy

- If the ABS is positive, the alloantibodies must be identified (see § 5.3).
- If alloantibodies relevant for the pregnancy are present, it is recommended to test the child's father for the corresponding antigen.
- If alloantibodies relevant for pregnancy are present, regular antibody determination during pregnancy is recommended.
- The titration should always be performed using the same method and, if possible, in the same laboratory and set-up as the retention sample (sample stored when the previous investigation was done). It is recommended to state the titre as a whole number (e.g. titre 2, 4, 8, etc.).
- The samples should be kept frozen until the end of the pregnancy (retain samples).
- Each anti-RH1 detected must be considered in the clinical context since the analysis cannot distinguish between a passive and an active immunisation.

7.2 Testing of neonates and children under four months of age

7.2.1 Blood samples

- The following samples can be used to determine blood groups and for DAT:
  - Umbilical cord blood
  - Capillary/venous blood
- If the results obtained from umbilical cord blood are unclear, the RBC should be washed with buffered NaCl solution or the determination should be repeated with capillary or venous blood. If the results are still unclear, the sample should be sent to a specialised laboratory.

7.2.2 Determination of AB and RH1 antigen

- Determination of the ABO blood group/RH1 is done using RBC. No reverse typing is performed.
- The first ABO and RH1 determination are each performed with two different test sera (in a double-set-up with at least one different clone each). If the results are weak positive, a DAT must be done to exclude false positive results.
- One of the two RH1 test sera used must identify the *RHD*06 (RHD*DVI)* variant.
- Umbilical cord blood may only be used for the first blood group determination. The results must be clear.
- A blood group card is not issued.

7.2.3 Direct anti-human globulin test

DAT must be performed if HDN is suspected or before transfusion. If the DAT is ≥2+ positive and/or signs of haemolysis are present, elution should be performed to identify the alloantibodies involved.

- If no antibodies are detectable in the mother's ABS (and no antibodies with specificity anti-A/-B are present in the infant's eluate), a compatibility test with serum/plasma of the mother and the infant's or paternal erythrocytes could be considered (attention ABO incompatibility!).
- If HDN is suspected due to ABO incompatibility between mother and child, the eluate should be prepared with at least one A or B test cell.

7.2.4 Pre-transfusion testing [13; 14]

- Testing is performed with the mother's blood and the child's blood:
  - Mother's blood ABO/RH1 and ABS
  - Child's blood: ABO/RH1 and DAT
  - If the mother's blood is not available and the DAT is positive, an elution, or ideally an ABS, could additionally and exceptionally be performed with the child's blood

7.2.5 Results

- Detection of anti-RH1 in the child must be interpreted in the clinical context (passive or active immunisation of the mother).
- The AB antigen determination may show a weakened result.
- A strong prevalence of maternal antibodies on the neonate's RBC may lead to a false negative antigen determination. This must be checked by a DAT and the result must be validated in the clinical situation for plausibility.
- Serological determination or extended phenotype of ABO/RH1 in premature infants or neonates who have received intrauterine transfusions may produce false results

7.3 Testing of children over four months of age

- The immunohaematological analyses and interpretation of the results are identical to those employed for adults.
- A blood group card can be issued:
  - if a full AB/RH1 antigen determination and reverse typing have been performed and the interpretation of the results conforms with the table in table 5.1.1;
  - if the isoagglutinin determination or full ABO blood group determination is not possible, PCR may be performed (transfusion: see § 7.4.3, PCR testing: see § 11).

7.4 Transfusions in children

7.4.1 Intrauterine transfusions

Immunohaematological testing and the provision of blood for intrauterine transfusions should be performed by a specialised laboratory.

The following rules normally apply to transfusions of pRBC:

- pRBC of blood group O are administered.

- The RH1 and RH/KEL1 phenotype must be compatible with the mother's blood. Other maternal antigens should also be taken into account (JK1 [Jk^a], JK2 [Jk^b], FY1 [Fy^a], FY2 [Fy^b], MNS3 [S], MNS4 [s]).
- XM-negative pRBC that are compatible with the alloantibodies in the mother's blood must be transfused.
- Concentrated (haematocrit 70–85%) and irradiated pRBC must be used for intrauterine transfusions.
- The storage time of the pRBC should be as short as possible (ideally not more than 5 days).

7.4.2 Transfusions in premature infants, neonates and children until the end of the fourth month [13; 14]

The following rules apply:

- pRBC should be compatible with the ABO blood group of the mother and that of the child.
- An AB/RH1 antigen check should be performed using a second sample before the first transfusion is given. This ensures that the transfusions are identical for blood group and RH1. If not, BG O pRBC must be given.
- If the mother has no anti-RH1, pRBC compatible with the child's RH1 are transfused.
- If the mother's ABS and the neonate's DAT are negative, pRBC can be transfused by T&S. In such cases, T&S can be extended to the end of the 4. month of the child's life without the need for further pre-transfusion testing.
- If the mother's ABS and/or the neonate's DAT are positive, the following procedure is adopted after the antibodies have been identified:
    - For the first transfusion, a XM is performed with antigen-negative pRBC and serum/plasma from the mother.
    - If further transfusions are given, the XM is done with antigen-negative pRBC and serum/plasma from the mother as long as the child has reached the end of the 4. month of life. Maternal serum with alloantibodies can be frozen to perform XM with antigen-negative pRBC if needed. Alternatively XM can be done with serum/plasma from the child.
- If the child's positive DAT and/or the mother's positive ABS can be unequivocally attributed to RHIG prophylaxis (passive immunisation), no further T&S is required until the child has reached the end of the 4. month of life (see bullet point 4). Other maternal alloantibodies must be excluded during differentiation.
- Whether or not irradiation is indicated and the age of the pRBC depend on the child's age and the clinical context. The decision rests with the responsible doctor [5; 14].
- The storage time should be as short as possible; ideally the pRBC should not be more than 5 days old. If the pRBC to be transfused are more than 5 days old, the clinical situation should be discussed with the responsible doctor to avoid complications such as hyperkalaemia.
- Blood group AB must be selected for transfusions of FFP.

7.4.3 Transfusions in children (fifth to twelfth months)

The following rules apply:

- Should it be impossible to carry out a full ABO blood group determination in children over the age of four months because isoagglutinins are not yet present, ABO- and RH1-identical pRBC and BG AB plasma can continue to be transfused. ABO PCR may be considered (see § 11).

7.4.4 Exchange transfusions, see § 9.2.

8 Selection of blood groups for labile blood products

8.1 Selection of blood groups for packed red blood cells

8.1.1 Selection of the ABO blood group

- If possible, the ABO blood group of the pRBC to be transfused must be identical to the patient's blood group.
- Transfusion of products that are not ABO-identical must be avoided unless there is a good medical and/or logistical reason. If an exception is made this needs to be clearly stated.
- If no ABO-identical pRBC are available, or if alloantibodies are present, ABO-compatible products may be transfused.
- After non-ABO-identical pRBC have been transfused, the current state of medical science and technology requires the products to be switched to the patient's own ABO blood group as soon as this is medically and logistically justifiable. For massive transfusions, see § 9.4.

Table 8.1.1 ABO compatibility rules

Patient's blood group	Blood group of pRBC
O	O
A	A and O
B	B and O
AB	AB, A, B and O

8.1.2 Selection of the RH1 antigen

- For recipients with a normal RH1 antigen status (positive or negative):
  - The RH1 antigen of the pRBC should be identical with the recipient's RH1 antigen, particularly for women under 50 years of age. If no RH1-identical pRBC are available, RH1-negative pRBC may be administered to a RH1-positive recipient. However, this must remain the exception. If an exception is made this needs to be clearly stated.
  - RH1-positive pRBC may be administered to RH1-negative recipients in certain situations (see § 9.4.2).
- For recipients with serologically weak RH1:
  - Not investigated by molecular biology:
    - Men and women over 50 can be administered RH1)-positive pRBC as long as no anti-RH1 has been determined.
    - Girls and women under 50 must be administered RH1-negative pRBC.
  - Investigated by molecular biology:
    - If the alleles *RHD*01W.1 (RHD*weak D type 1), RHD*01W.2 (RHD*weak D type 2), RHD*01W.3 (RHD*weak D type 3)* or *RHD*09.04 (RHD*weak D type 4.1)* are present, RH1-positive pRBC should be transfused; this also applies to women under 50.
    - All other RH1 variants should be given RH1-negative pRBC.
    - In the absence of clear evidence, we recommend that patients with *RHD*09.03.01 (RHD*weak D type 4.0, RHD*DAR3.1)* should be considered RH1 negative until further notice.

Table 8.1.2 Selection of the RH1 antigen

RH1 phenotype	Genotype	Transfusions, woman <50	Transfusions, woman ≥50 or man
RH:–1	n/a	RH1 neg.	RH1 neg.
RH:W1/RH:P1	Unknown	RH1 neg., until the PCR result is available	RH1 pos.*, until the PCR result is available
RH:W1/RH:P1	*RHD*01W.1/.2/.3, (RHD*weak D type 1/2/3)* or *RHD*09.04 (RHD*weak D type 4.1)*	RH1 pos.	RH1 pos.
RH:W1/RH:P1	no *RHD*01W.1/.2/.3, (RHD*weak D type 1/2/3)* or *RHD*09.04 (RHD*weak D type 4.1)*	RH1 neg.	RH1 neg.

* If unequivocally RH:P1 (RhD partial), transfuse RH1 neg.

8.1.3 Selection of other blood group antigens

8.1.3.1 Alloantibodies present

- If transfusion-relevant alloantibodies are present, the pRBC must be checked for the corresponding antigen(s) and must be negative. This also applies to known clinically relevant antibodies that are no longer detectable.
- Once the first alloantibody has occurred, it is recommended to perform extended antigen typing (KEL1 [K], KEL2 [k], JK1 [Jk^a], JK2 [Jk^b], FY1 [Fy^a], FY2 [Fy^b], MNS3 [S] and MNS4 [s]) and to transfuse suitably compatible products whenever possible in order to avoid further immunisation. Corresponding genotyping is recommended in patients who have recently received a transfusion (see § 11).

8.1.3.2 Minimum requirements for the selection of pRBC if antibodies are present

- If the antibody does not appear in the following table, it is recommended to contact the reference laboratory.

Antibody	Environment				
	NaCl	Enzyme only	ID/IAT	Antibodies no longer detectable	Phenotype RH/KEL1 compatible
ABO					
Anti-A1	T&S	T&S	Ag neg. & XM neg.	T&S	♀ <50 years
RH					
RHIG prophylaxis	n/a	T&S	T&S	T&S	♀ <50 years
Other anti-RH antibodies**	AG neg. & XM neg.	AG neg. & XM neg.	AG neg. & XM neg.	AG neg. & XM neg.	Yes
KEL					
All KEL (Kell) antibodies	AG neg. & XM neg.	AG neg. & XM neg.	AG neg. & XM neg.	AG neg. & XM neg.	Yes
JK					
All JK (Kidd) antibodies	AG neg. & XM neg.	AG neg. & XM neg.	AG neg. & XM neg.	AG neg. & XM neg.	Yes
FY					
All FY (Duffy) antibodies	AG neg. & XM neg.	n/a	AG neg. & XM neg.	AG neg. & XM neg.	Yes
MNS					
Anti-MNS1 (anti-M), anti-MNS2 (anti-N)	T&S	n/a	AG neg. & XM neg.	T&S	♀ <50 years
Anti-MNS3 (anti-S), anti-MNS4 (anti-s), anti-MNS5 (anti-U)	AG neg. & XM neg.	AG neg. & XM neg.	AG neg. & XM neg.	AG neg. & XM neg.	Yes
LE					
Anti-LE1 (anti-Le^a), anti-LE2 (anti-Le^b)	T&S	T&S	XM neg.	T&S	♀ <50 years
P1PK					
Anti-P1PK1 (anti-P1)	T&S	T&S	XM neg.	T&S	♀ <50 years
LU					
Anti-LU1 (anti-Lu^a)	T&S	n/a	XM neg.	T&S	♀ <50 years
Anti-LU2 (anti-Lu^b)	AG neg. & XM neg.	n/a	AG neg. & XM neg.	AG neg. & XM neg.	Yes
DI					
Anti-DI3 (anti-Wr^a)	T&S	T&S	XM neg. / Ag neg., T&S	T&S	♀ <50 years
CO					
Anti-CO1 (anti-Co^a)	AG neg. & XM neg.	AG neg. & XM neg.	AG neg. & XM neg.	AG neg. & XM neg.	Yes
Anti-CO2 (anti-Co^b)	AG neg. & XM neg.	AG neg. & XM neg.	AG neg. & XM neg.	AG neg. & XM neg.	Yes
YT					
Anti-YT1 (anti-Yt^a)	T&S	n/a	Ag neg. & XM neg.	AG neg. & XM neg.	Yes
Anti-YT2 (anti-Yt^b)	T&S	n/a	XM neg.	T&S	♀ <50 years
Other antibodies					
Anti-HLA	n/a	n/a	T&S	T&S	♀ <50 years
Anti-HTLA	n/a	n/a	T&S	T&S	♀ <50 years
Anti-H1I1 (anti-HI)	T&S	T&S	Ag neg. & XM neg.*	T&S	♀ <50 years
Anti-I1 (anti-I)	T&S	T&S	T&S	T&S	♀ <50 years
Auto-antibodies in IAT	n/a	n/a	T&S	T&S	Yes
Antibodies against the stabilizing solution	T&S	T&S	T&S	T&S	♀ <50 years

* ABO-identical blood

** Anti-RH3 (anti-E) and anti-RH8 (anti-C^w) enzyme-only antibodies: see § 5.3.4 and § 5.5

Abbreviations

- Ag neg. and XM neg.: antigen-negative pRBC corresponding to the antibody with negative XM
- XM neg.: transfusion of pRBC with negative XM without confirmation of the antigen negativity
- T&S: transfusion of pRBC by T&S
- ♀ <50 years old: women from birth to the age of 50

8.1.3.3 Further indications for the selection of phenotyped/genotyped pRBC

- It is recommended to transfuse pRBC with a compatible RH/KEL1 phenotype in the following situations:
  - Transfusions for girls and women under 50 years of age.
  - Where erythrocyte autoimmunisation is present. If the phenotype cannot be determined serologically, RH/KEL1 genotyping must be considered (see § 11), for free autoantibodies see § 9.5.
  - Where there is a chronic need for transfusion (e.g. haemoglobinopathies such as sickle cell anaemia or thalassaemia, etc.) it is advisable to select pRBC which are compatible in terms of RH/KEL1 phenotype and, if possible, JK1 (Jk^a), JK2 (Jk^b), FY1 (Fy^a), FY2 (Fy^b), MNS3 (S) and MNS4 (s) as well.

Note

- For prophylactic antigen-compatible transfusion, it is not necessary to perform a serological check of the reported antigen profile.
- However, preventive consideration of antigens must not put patients with irregular antibodies at a disadvantage. This means that RH4-(c)- or RH5-(e)-negative blood cannot be used unreservedly for preventive antigen-compatible transfusions.
- Preventive treatment with RH/KEL1 antigens is not urgently recommended for female recipients under four months of age since the risk of alloimmunisation is thought to be very low according to literature [20].

8.2 Selection of ABO blood group for fresh frozen plasma

The following recommendations apply to adults and children from five months.

- If possible, the ABO blood group of the FFP must be identical to the patient's blood group.
- The RH1 antigen is not respected for FFP.
- If ABO-identical FFP is not available, ABO-compatible FFP must be transfused (see Table 8.2).

Table 8.2 FFP compatibility rules

Patient's blood group	Blood group of FFP
O	O, A, B and AB
A	A and AB
B	B and AB
AB	AB

Non-ABO-identical FFP transfusions must remain the exception. If an exception is made this needs to be clearly stated.

8.3 Selection of ABO/RH1 in platelet concentrates

- The following recommendations apply to adults and children:

- The selection of ABO blood group and RH1 antigens for PC is determined by the recipient's ABO/RH1 blood group and availability.
- When RH1-positive PC are given to RH1-negative patients, consideration should be given to administering RHIG prophylaxis to girls and women <50 as there is a risk of sensitisation. This seems to be higher with pooled products than with apheresis products. The indication for RHIG prophylaxis must be weighed against the risk of allosensitisation on a case-by-case basis.
- Blood group only has to be determined once (in emergency situations PC can also be transfused without ABO blood group determination).
- When pathogen-inactivated PC with Amotosalen-based Intercept are transfused, irradiation for prophylaxis of graft-versus-host disease is not necessary (other processes may be added in the future depending on the approval).

8.4 Selection of ABO/RH1 in specific situations

For administration to newborns and intrauterine transfusions, refer to the corresponding sections in chapter 7. See chapter 9 for exchange, emergency and massive transfusions.

9 Procedure and selection of blood products in specific clinical situations

9.1 Autologous transfusion

In order to avoid mix-ups, the same pre-transfusion tests must be performed as for homologous transfusions (see § 5 and guidelines for quality assurance in transfusions issued by the Swiss Working Group on Quality Assurance in the Use of Blood Products [22]).

9.2 Exchange transfusions

Immunohaematological testing and the provision of blood for exchange transfusions should be performed by a specialised laboratory.

The transfusion recommendations listed in § 7.4.2, 7.4.3, 8 and 9.7 apply to exchange transfusions.

If a new blood product is produced (e.g. from pRBC and FFP), the haematocrit content should be determined and reported to the client.

9.3 Emergency transfusion

This chapter applies to situations in which there is not enough time to carry out full pre-transfusion testing. The framework conditions and responsibilities for emergency transfusions must be regulated internally beforehand and documented [22].

If possible, emergency transfusions should always also be administered using products that are blood group identical and in all cases taking known antibodies into account. Whenever possible, a first blood sample should be taken before transfusions/infusions.

9.3.1 Selection of ABO and RH1 blood groups in emergency transfusions

- No known blood group determination (without T&S, XM and DAT testing): blood group O pRBC and AB plasma must be transfused (see § 9.4 “Massive transfusions”).
- One blood group determination (tube or blood group card) available: RH1-identical and blood-group-O pRBC can be transfused.
- Two blood group determinations from at least one sample that is not older than 96 hours (without ABS) are available: products can be switched to the patient’s own blood group immediately if the results are unequivocal (caution: the blood group may be difficult to interpret because of mixed fields and dilutions that occur during emergency transfusions).

9.3.2 Other pre-transfusion tests

- An ABS and, if necessary, DAT should then be performed without delay on the pre-transfusion blood sample taken from the patient.
- The doctor responsible for the transfusion must be informed about previous incompatible transfusions. The doctor in charge also decides whether further incompatible transfusions will be administered. See § 9.5 for warm antibodies.

9.4 Massive transfusions

9.4.1 General

- A massive transfusion is defined as more than four pRBC (in adults) within one hour, or the exchange of more than 50% of the blood within three hours, or full exchange within 24 hours.
- As soon as the massive transfusion protocol is no longer necessary, the standard pre-transfusion test sequence defined in § 5 becomes effective again.

    - If full pre-transfusion testing could not be carried out, see chapter Emergency transfusion (see § 9.3).

- When performing massive transfusions, a XM should if possible be carried out with a pre-transfusion sample if alloantibodies are present.

9.4.2 Selection of ABO/RH1 blood groups in massive transfusions

As soon as the ABO blood group, RH1 and ABS are available, the following applies:

- If the ABO group of the transfused pRBC was compatible with but not identical to the patient's ABO blood group, the patient's own blood group can be used at any time. Otherwise section 8.1.1. also applies here analogously.
- In case of a massive transfusions, RH1-positive pRBC may exceptionally be given to an RH1-negative patient (or a patient whose RH1 is not known) after the doctor performing the transfusion has been consulted or if doing so is permitted by internal directives.
  - This requires that:
    - the required number of units of RH1-negative pRBC are likely to be difficult to obtain;
    - no anti-RH1 antibodies have been identified in the patient or are known;
    - the patient is a man or a woman over 50.
  - Once the acute bleeding has stopped, the transfusion should be switched to RH1-negative pRBC as soon as possible. Alloimmunisation and boostering should be excluded after 96 hours at the latest if RH1-positive pRBC are continued to be given. An ABS should be performed between 6 and 12 weeks after an incompatible transfusion (see § 5.3).
  - Everything possible must be done to avoid giving RH1-positive pRBC to girls and women under 50 who are RH1 negative (see also § 8.1.2).

9.5 Autoimmunohaemolytic anaemia

- There are various autoantibodies (of the warm [IgG], cold [IgM] and mixed [IgG and IgM] type) which necessitate different precautions during transfusion.
- Patients with suspected or confirmed AIHA who are in need of transfusion should be referred to a doctor experienced in transfusion medicine.
- The autoantibodies present in the IAT may mask alloantibodies that are additionally present. Before a transfusion is given, it must be ensured that no clinically relevant alloantibodies are present. A reference laboratory may have to be consulted.
- If a transfusion has been given in the last 4 months:
  - it is impossible to distinguish between alloantibodies and autoantibodies without extensive molecular biological testing;
  - for transfusions in patients with erythrocyte autoantibodies: see § 8.1.3.3. RH1/KEL1 compatible pRBC transfusions are desirable;
  - if clinically relevant cold agglutinins are present, blood products should be administered at 37°C; properly tested equipment intended for this purpose should be used;
  - in an emergency situation, in which there is no time to wait for the laboratory results, the transfusing doctor must be informed about the risk, see also chapter 9.3. If known, the pRBC should be chosen in accordance with the RH/KEL1 phenotype and, if appropriate, the extended phenotype.

9.6 Chronic transfusion requirement

See § 8.1.3.3 for the selection of pRBC.

- For patients with sickle cell anaemia, a XM with each pRBC should be considered even if irregular antibodies are not present.
- RH variants are more common in patients of African origin. For this reason it is recommended to perform thorough molecular biological testing of the RH genotype of patients with sickle cell anaemia. It is also recommended to determine the patient's extended genotype and phenotype.

9.7 Transfusion of irradiated pRBC

- pRBC may be irradiated up to a maximum of day 28 after they have been obtained. An irradiated pRBC must be transfused within 14 days and no later than day 28 after being obtained.
- Patients at risk of hyperkalaemia: irradiated pRBC should be transfused as soon as possible and no longer than 24 hours after irradiation.
- pRBC must be irradiated for intrafamily transfusions (first and second degree).
- Other indications must be defined internally by each hospital.

With the consent of the attending physician, these time limits may be deviated from in exceptional cases. Such exceptional cases are situations in which the benefit of the deviation outweighs the potential risk of transfusion delay. Such exceptional cases must be well documented.

9.8 Procedure and selection of blood products if allergic/anaphylactic transfusion reactions occur and in IgA-deficient patients

There is some controversy in the literature surrounding the relationship between a lack (plasma concentration <70 mg/dl [0.7 g/l]) or a deficiency (plasma concentration <0.05 mg/dl) of IgA in patients (with and without anti-IgA antibodies) and allergic/anaphylactic transfusion reactions [16; 17]. In a Swiss study of 15,000 blood donors, IgA deficiency was identified with a frequency of about 1:850 [18].

- In the event of a transfusion-related serious allergic/anaphylactic reaction, investigation of whether the patient is IgA-deficient is recommended.

Caution: The blood sample for determination of IgA content should be taken prior to transfusion (plasma/pRBC/PC) and the administration of immunoglobulin.

The IgA content of pRBC and PC products (and the content of all other plasma components) can be minimised by "washing". The administration of washed pRBC/PC or plasma from IgA-deficient donors as a precautionary measure can be considered if an IgA-deficient patient experiences a serious allergic transfusion reaction. The latter can also be used in exceptional cases for transfusions that can be planned well in advance.

Please contact your blood transfusion service for sources of these special products.

9.9 Procedure and selection of blood products for patients being treated with monoclonal antibodies

Anti-CD38 is used in the therapy of haemato-oncological and auto-immune diseases. Anti-CD38 can cause a positive antibody screening result for up to 6 months after it has been discontinued because RBC also express CD38 weakly. The strength of the reactions with test cells treated with papain and trypsin is weak to negative.

Valid results of ABS must be available before therapy with monoclonal antibodies such as anti-CD38 begins. It is also recommended to perform extended antigen typing or genotyping.

- If a sample is sent to a reference laboratory, the diagnosis and the drug must be stated on the order form.

- In the presence of a negative ABS, by appropriate method (e.g., tube or DTT, trypsin, or alternative procedure to inhibit interference), pRBC (compatible with ABO/RH1 / RH/KEL1 / KEL3 (Kp^a)) can be released by T&S.
- Alternatively, phenotypically or genotypically compatible pRBC (RH1, KEL1, KEL3 [Kp^a], JK [Jk], FY [Fy], MNS3 [S] und MNS4 [s]), can be released without an ABS by T&S.

9.10 Transplantation

9.10.1 Organ transplants

If a majorly ABO-incompatible organ transplant is performed, the ABO blood group of the plasma must be compatible with the recipient and with the organ.

Alloantibodies produced by passenger lymphocytes (from the transplanted organ) must be taken into account for transfusion purposes for as long as they are detectable.

9.10.2 Allogeneic stem cell transplantation (from a donor)

The following information is required for the transfusion:

- At least the ABO/RH1 and RH/KEL1 phenotype of the donor / donors
- Date of transplantation
- Transplant centre
- Recipient's blood group (ABO/RH1 and RH-KEL1 phenotype) and transfusion history of the last 4 months
- In case of a positive DAT after an ABO incompatible HSCT, an additional A or B test cell must be prepared with the eluate.

If no information is available, blood-group-O-irradiated pRBC and AB plasma must be transfused.

The kinetics (disappearance and appearance) of anti-A/B isoagglutinins varies greatly from one person to another. Incompatible anti-A/B isoagglutinins may reappear if the disease recurs or the transplanted organ is rejected.

It is important to follow the transfusion recommendations of the transplant centre.

9.11 Sickle cell disease

This clinical situation can affect all patients with phenotypes homozygote HbSS, compound heterozygote HbS-β thalassaemia (HbS-β+ or HbS-β°thalassaemia), HbSC, HbS OArab, HbS Lepore, HbSD and HbSE. Transfusions may be necessary depending on the form and clinical characteristics. The supply of blood for these patients presents an immunohaematological challenge for three reasons:

- There is considerable genetic diversity between the patients (African origin) and the donor population.
- Alloimmunization and severe immune haemolytic reactions can occur more frequently.
- *RHD* and *RHCE* variants are more frequent than in the Caucasian population [31; 32].

The recommendations are therefore as follows:

- Previous results of pre-transfusion tests and a transfusion history must be obtained to organize the patient care as well as possible.
- If no previous phenotype/genotype data are available, the following tests should be performed:
- Extended phenotyping: RH1, RH2, RH3, RH4, RH5, KEL1, KEL2, JK1, JK2, FY1, FY2, MNS1, MNS2, MNS3 and MNS4 (RhD, C, E, c, e, K, k, Jk^a, Jk^b, Fy^a, Fy^b, M, N, S and s) if there has been no transfusion within the past four months.

- Extended genotyping: KEL*01.01, KEL*02, JK*01, JK*02, FY*01, FY*02, FY*02.N.01, GYPA*01, GYPA*02, GYPB*03 and GYPB*04
- Genotyping can optionally be extended with the alleles *DO*01*, *DO*02*, *KEL*02.03*, *KEL*02* (c.841C, c.1790T), *KEL*02.06* (Do^a, Do^b, Kp^a, Kp^b, Js^a and Js^b).
- In addition, the most frequent and most relevant variants of the *RHD* and *RHCE* genes should be investigated. Extended genotyping should also be carried out if the extended phenotype is already known.
- Where a patient has been transfused > 12 units pRBC without forming alloantibodies or autoantibodies, forgoing in-depth testing of the RHD and RHCE genes can be considered [33].
- The antibody testing should be carried out using the enzyme technique (e.g. papain) in addition to IAT. In particular, in the event of the occurrence of a vaso-occlusive crisis after transfusions, an inadequate increase in haemoglobin or a suspected transfusion reaction, alloimmunization should be excluded as the cause. This can be done with additional tests such as elution despite negative DAT or compatibility testing with eluate.
- Certain alloantibodies, which in most cases play a minor role in transfusion medicine (see Table 8.1.3.2), should be generously taken into account in sickle cell patients (e.g. LE1 in papain), even when they can no longer be detected.
- The following antigens should be taken into account as a preventive measure in every RBC transfusion: RH1, RH2, RH3, RH4, RH5, KEL1, KEL2, JK1, JK2, FY1, FY2, MNS3 and MNS4 (RhD, C, E, c, e, K, k, Jk^a, Jk^b, Fy^a, Fy^b, S, s). If this is not possible, the prescribing doctor must be informed of the risk of possible immunization.
- Detection of an initial irregular antibody or autoantibody should be considered a warning sign: The patient may be a responder and could be at risk of forming further alloantibodies, which could result in a transfusion shortage.
- Release of pRBC using the T&S procedure is explicitly advised against. Compatibility for all pRBC to be transfused is recommended, even if no irregular antibodies are present. This ensures that the risk of a transfusion reaction by an anti-private antibody is minimized.
- Since certain antibodies rapidly fall back below the limit of detection, antibody testing should be carried out again 10 to 21 days after each transfusion.
- Every vaso-occlusive crisis that occurs within 21 days of a transfusion should be considered as potential alloimmunization, which must be actively investigated [34].

10 Adverse transfusion reactions

This document mentions only those adverse transfusion reactions that occur in the context of immunohaematological testing of patients' samples. Information about further classification and investigation of transfusion reactions can be found on the Swissmedic website (Haemovigilance: https://www.swissmedic.ch/swissmedic/en/home/humanarzneimittel/market-surveillance/haemovigilance/haemovigilance-meldesystem.html)

10.1 General

Adverse transfusion reactions and transfusion-related incidents must be investigated in accordance with the applicable legal requirements for haemovigilance [1].

- The doctor performing the transfusion must be aware of the various causes of transfusion reactions and initiate steps to investigate them.
- Adverse transfusion reactions must be reported to the laboratory that performed the immunohaematological testing so that the circumstances can be investigated without delay.
- Blood products that have led to adverse transfusion reactions, and all other blood products that could be affected, must be withdrawn from circulation immediately and may not be released again until the investigations have been completed (see § 10.3).

10.2 Investigation of suspected haemolytic transfusion reactions

10.2.1 Material

- The following materials are required to investigate a possible haemolytic transfusion reaction:
  - Pre-transfusion blood samples from the recipient
  - Segments and/or blood bags of all currently transfused blood products
  - A sample taken from the recipient immediately after the transfusion reaction occurred

10.2.2 Immunohaematological investigations

- Possible administrative errors and sample mix-ups must be investigated.
- The following investigations of samples of the patient's blood obtained pre- and post-transfusion must be performed:
  - Visual inspection of the patient's plasma/serum for haemolysis before and after transfusion
  - Full ABO/RH1 blood group determination
  - ABS
  - DAT determination. If the DAT is positive, elution of the post-transfusion blood sample is performed. If the DAT is negative, elution is still indicated if there are signs of haemolysis. In case of ABO incompatibility, e.g. PC or after administration of IVIG, the eluate should additionally be prepared with an A or B test cell.
  - XM with all pRBC transfused in the last 6 hours
- Investigation of all transfused blood products (pRBC or segment):
  - Visual inspection (colour and homogeneity)
  - AB/RH1 antigen check of the segments of the pRBC and, where indicated, RH/KEL1 phenotype and other blood group antigens
  - For FFP, reverse typing is performed using the bags

- Further blood products should, if clinically possible, not be transfused until the investigations have been completed

10.2.3 Further investigations

If an adverse transfusion reaction occurs, it is the responsibility of the doctor performing the transfusion to arrange further investigations.

10.3 Reporting system

Adverse transfusion reactions are reported to Swissmedic either by the haemovigilance officer or the doctor who performed the transfusion. If an adverse transfusion reaction is suspected of being related to the quality of the blood product, the manufacturer (Regional Blood Transfusion Service) must be informed immediately so that all other potentially affected products (e.g. from the same donor) can be blocked or recalled.

11 Standards for molecular blood group typing

Chapter 11 is divided into the following sections:

A – Applications
B – Personnel qualifications
C – Cuality assurance
D – External proficiency testing
E – Analysis processes
P – Processing
R – Reporting
Z – Appendix

Sections **A** – Applications, **P** – Processing, **R** – Reporting and **Z** – Appendix were written independently by a subgroup of the Immunohaematology expert group.

Sections **B, C, D** and **E** were adopted word for word from the corresponding chapters of the European Federation for Immunogenetics (EFI) standards for histocompatibility & immunogenetics testing (HLA), version 8 (effective from 01.01.2020).

The original EFI standard was abridged by omitting certain subheadings. The remaining chapters and subheadings use the same wording as the EFI standards (direct use of future EFI standards).

The EFI has given its consent to the use of its guidelines for chapter 11 “Standards for molecular blood group typing”.

Overview of the nomenclature for molecular blood group typing in other chapters of this document.

1) **3.3.2 External quality controls**
2) **5 Immunohaematological investigation**
3) **5.1.2 Result and interpretation of ABO blood group determination**
4) **5.1.3 Result and interpretation of RH1 antigen determination**
5) **7.1.3 Pregnant women with RH1 variants**
6) **7.1.4 Foetal *RHD* determination from maternal blood**
7) **7.3 Testing of children over four months of age**
8) **8.1.3 Selection of other blood group antigens**
9) **8.1.3.3 Further indications for the selection of phenotyped/genotyped pRBC**

			A. Applications of molecular blood group detection			
			* donor genotyping is not topic of this recommendation	comments and examples	reci-pients	do-nors*
A	1		Clarification of serological prevalues			
A	1	1	ABO antigen and isoagglutinin dicrepancies		+	+
A	1	2	*RHD* categories and partials		+	+
A	1	3	Antigens reacting discrepant with different moAB (all blood groups)		+	+
A	2		Presence of antibodies (all blood groups)			
A	2	1	Presence of allo-antibody		+	+
A	2	2	Presence of auto-antibody		+	+
A	3		Determination of weakly agglutinating antigens			
A	3	1	Determination of *RHD*01W.01/.02/.03 (RHD*weak D type 1/2/3)*	recommended for girls and women under the age of 50	+	+
A	3	2	Determination of RH:W1 other than *RHD*01W.01/.02/.03 (RHD*weak D type 1/2/3)*		+	+
A	3	3	Determination of antigens with weak agglutination of all blood groups		+	+
A	4		Determination of antigens only detectable by adsorption/elution			
A	4	1	Detection of RH1 antigens only detectable by adsorption/elution ("*RHD*01EL,* Del")	also in screening for *RHD* in RH:–1	–	+
A	4	2	Detection of antigens only detectable by adsorption/elution of all blood groups		+	+
A	5		Clarification of geno-/phenotype discrepancies		+	+
A	5	1	Case phenotype correct positive, genotype false negative	e.g. alleles with "primer-binding-site" mutations	+	+
A	5	2	Case phenotype correct negative, genotype false positive	"null alleles", recognised by carrying an N in ISBT term	+	+
A	5	3	Case phenotype false positive, genotype correct negative	*RHD*01N.06* (DCeS) with pseudo RH2 (C), though genetically RH2 (C) negative. MNS15 (St^a) / GYP*401 alleles of MNS	+	+
A	5	4	Case phenotype false negative, genotype correct positive	e.g. RHD*01EL.01	+	+

A 6		Screening for *RHD* among RH:–1			
A 6	1	Detection of RH1-negative *RHD-CE-D* hybrid alleles		–	+
A 6	2	Detection of unexpressed (RH:–1) *RHD* genes		–	+
A 7		Detection of blood groups in case no commercial reagents for serological detection are available			
A 7	1	Detection of Dombrock blood group system	DO1 (Do^a) / DO2 (Do^b), LU18 (Lu18) / LU19 (Lu19) …	+	+
A 7	2	Rare blood group antigens / high frequency antigen (HFA) negatives	DI1 (Di^a) / DI2 (Di^b), SC1 (Sc1) / SC2 (Sc2) …	+	+
A 7	3	Rare blood group antigens of defined ethnicities	e.g. RH10 (V), RH20 (VS), RH31 (Rh31), IN1 (In^a) / IN2 (In^b)	+	+
A 8		Prenatal			
A 8	1	Prenatal detection of blood groups from fetal material		+	–
A 9		Blood group assessment in special clinical situations			
A 9	1	Mol. BG determination in polytransfused patients		+	–
A 9	2	Mol. BG determination in DAT-positive individuals		+	–
A 9	3	Monoclonal hematopoiesis (loss of BG alleles)		+	–
A 9	4	Post stem cell transplantation		+	–
A 9	5	Chronic transfusion needs (thalassemia, sickle cell disease, MDS, etc.)		+	–
A 10		Use of alternative sample material			
A 10	1	If indicated, alternative sample material may be used		+	–

			B. Personnel qualifications Effective from January 1st, 2020	
			Comment: [ ] … rectangular brackets indicate changes with respect to the EFI standards, e.g. [BG vs. ~~HLA~~] in these “Standards for Molecular Blood Group Typing”	
			Comment: most current versions of the ISBT Blood Group Allele Tables (plus actual version number) are given at: http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/	
B	5		Competency evaluation and continuous education	
B	5	2	The Laboratory Director and the technical staff must participate in continuing education relating to each category [~~for which~~ of molecular blood group typing (e.g. single sample typing, blood group sequencing …) ~~HLA EFI accreditation is sought~~]	[BG vs. HLA]

						C. Quality assurance		
						Comment: [ ] … rectangular brackets indicate changes with respect to the EFI standards, e.g. [BG vs. ~~HLA~~] in these "Standards for Molecular Blood Group Typing"	[BG vs. HLA]	comment added
						Comment: most current versions of the ISBT Blood Group Allele Tables (plus actual version number) are given at: http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/	[BG vs. HLA]	comment added
C	2					Technical		
C	2	1	3			Laboratories performing amplification of nucleic acids must use:		
C	2	1	3	1		A dedicated work area with restricted traffic flow		
C	2	1	3	2		Physical barriers to prevent DNA contamination, including the use of dedicated:		
C	2	1	3	2	1	Equipment		
C	2	1	3	2	2	Laboratory coats		
C	2	1	3	2	3	Disposable supplies		
C	2	1	4			Pre-amplification procedures must be performed in an area which excludes amplified DNA that has the potential to serve as a template for amplification in any of the genetic systems tested in the laboratory		
C	2	1	5			All activities occurring from and including thermal cycling must take place in the post-amplification area		

	D. External proficiency testing	
	Comment: [ ] … rectangular brackets indicate changes with respect to the EFI standards, e.g. [BG vs. ~~HLA~~] in these "Standards for Molecular Blood Group Typing"	
D 1	Procedure of External Proficiency Testing	
D 1 1	Registration for EPT schemes	
D 1 1 1	The laboratory must participate in EPT programme(s) to cover	
D 1 1 1 1	All the accredited laboratory applications [of Molecular Blood Group Typing as exemplified by e.g. Instand e.V., or UK NEQAS [~~HLA typing, antibody screening and identification, crossmatching, etc.~~]	[BG vs. HLA]
D 1 2	The laboratory must prospectively define core and supplemental techniques according to the Accreditation Application	
D 1 3	The laboratory must	
D 1 3 1	Prospectively document the relevant EPT schemes or workshops on an annual basis	
D 1 3 2	Have a predetermined policy for testing EPT samples and must document this prior to the annual commencement of the EPT cycle	
D 1 4	EPT sample must be	
D 1 4 1	Tested by the same techniques as routinely employed for clinical samples, either individually or in combination	
D 1 4 2	Interpreted in a manner comparable to routine clinical samples	
D 1 5	Minimum number of samples for EPT per year	
D 1 5 1	The minimum number of samples applies to all techniques used to produce a final result:	
D 1 5 1 1	Blood Group Genotyping: [2 times per year, 4 samples each, specificities as currently requested by Instand e.V., or UK NEQAS]	[BG vs. HLA]
D 2 1	For phenotyping/genotyping schemes participants must report:	
D 2 1 1	The antigen specificities and alleles identified	
D 2 1 2	The method(s) used	
D 3	Laboratory performance	
D 3 5	Participating laboratories must ensure that all the following EPT-related documents are maintained and are made available to [~~EFI~~] inspectors [of the Swiss Accreditation Service (SAS)] for assessment:	[BG vs. HLA]

D 3 5 1	All data and analyses produced for all techniques
D 3 5 2	Results submitted to the EPT
D 3 5 3	EPT summary/scheme reports
D 3 5 4	Certificates generated by the EPT Provider
D 3 5 5	Outcomes of investigations of any unsatisfactory results
D 3 5 6	Corrective or preventive actions

						E. Analysis processes
E	2	7				Thermal Cyclers
E	2	7	1			Accuracy of thermal cycling instruments:
E	2	7	1	1		Must be verified by annual thermal verification of the block using a calibrated device designed specifically for this purpose
E	1	5				Reagents for nucleic acid analysis
E	1	5	3			The appropriate performance of individual products must be documented before results using these reagents are reported for:
E	1	5	3	1		Each shipment, and
E	1	5	3	2		Each lot
E	1	5	4			For commercial kits, the following information must be documented:
E	1	5	4	1		Source
E	1	5	4	2		Lot number
E	1	5	4	3		Expiry date
E	1	5	4	4		Storage conditions
E	1	5	4	5		Test each lot and shipment of commercial kits against at least one DNA sample of known type
E	1	5	5			Reagents from different lots of commercial kits must not be mixed, unless either:
E	1	5	5	1		Specified by the manufacturer, or
E	1	5	5	2		Validated and documented with appropriate quality control in the laboratory
E	1	5	6			Inhouse Primers
E	1	5	6	1		The specificity of primer combinations and the annealing positions must be defined
E	1	5	6	2		Laboratories must:
E	1	5	6	2	1	Have a policy for quality control of each lot or shipment of primers
E	1	5	6	2	2	Confirm the specificity and quantity of the amplified product using reference material
E	1	5	6	2	3	
E	4	5	1			Nucleic acid extraction
E	4	5	1	1		The method used for nucleic acid extraction:
E	4	5	1	1	1	Must be published and documented
E	4	5	1	1	2	Must be validated in the laboratory
E	4	5	1	2		Purity and concentration of nucleic acids:
E	4	5	1	2	1	Must be sufficient to ensure reliable test results
E	4	5	1	2	2	Should be determined for each sample, or

E	4	5	1	2	3	If not determined for each sample, the laboratory must have tested and validated this policy		
E	4	5	1	3		If the DNA is not used immediately after purification, suitable methods of storage must be available that will protect the integrity of the material		
E	4	5	2			Electrophoresis		
E	4	5	2	1		[~~Optimal~~] Electrophoretic conditions must be documented	[BG vs. HLA]	“optimal” deleted from Standards for Molecular Blood Group Typing
E	4	5	2	2		The laboratory must establish criteria for accepting each slab or capillary gel migration, and each lane of a gel or capillary injection		
E	4	5	2	3		When the size of an amplicon is a critical factor in the analysis of data, size markers that produce discrete electrophoretic bands spanning and flanking the entire range of expected fragment sizes must be included in each gel		
E	4	5	3			Analysis		
E	4	5	3	2		The method of allele assignment must be designated		
E	4	5	3	3		The [ISBT Blood Group Allele Tables ~~IMGT/HLA database~~] must be:	[BG vs. HLA]	changed IMGT/HLA to ISBT
E	4	5	3	3	1	Documented		
E	4	5	3	3	2	Updated at least once a year with the most current version of the [ISBT Blood Group Allele Tables ~~IMGT/HLA database~~]	[BG vs. HLA]	changed IMGT/HLA to ISBT
E	4	5	3	4		If a manual allele call or interpretation of positive/negative reactions is performed for SSOP or SSP, two independent interpretations of primary data must be performed, except under justified special emergency situations		
E	4	5	4			Contamination control (“wipe-test”)		
E	4	5	4	3		If amplified product is detected, there must be:		
E	4	5	4	3	1	Written description of how to eliminate the contamination		
E	4	5	4	3	2	Measures taken to prevent future contamination		
E	4	5	4	3	3	Evidence of elimination of the contamination		
E	4	7				Sequence-specific primers (SSP)		
						Comment: in-house developed tests are addressed, versus for commercial products, responsibility for correct allele detection lies within the manufacturers.	[BG vs. HLA]	comment added
E	4	7	1			Each amplification reaction must include controls to detect technical failures (e.g. an internal control such as additional		

					primers or templates that produce a product that can be distinguished from the typing product)		
E	4	7	3		The laboratory must use the following data in the interpretation phase of the typing:		
E	4	7	3	1	Information derived from the validation process		
E	4	7	3	2	Information derived from previous typings with the same lot of primers		
E	4	9			Sanger sequencing		
E	4	9	1		Sequencing templates:		
E	4	9	1	1	Must have sufficient purity, specificity, quantity and quality to provide interpretable sequencing data		
E	4	9	1	2	Should be purified after amplification to eliminate the presence of dNTPs, Taq polymerase and amplification primers		
E	4	10	6	2	For each run the size of fragments must be documented and the selection must be specified		
E	4	9	2		Sequencing reaction:		
E	4	9	2	1	The specificity of the template in combination with the sequencing primer ([ISBT Blood Group Allele locus (gene) and alleles ~~HLA locus and alleles~~)] must be defined	[BG vs. HLA]	changed IMGT/HLA to ISBT
E	4	9	2	2	Quantity and quality of templates, sequencing primers and sequencing reagents must be sufficient to provide interpretable primary sequencing data		
E	4	9	2	3	The conditions for the sequencing reaction must be documented and appropriate for obtaining reliable primary sequencing data		
E	4	9	3		Nucleotide assignment		
E	4	9	3	2	The signal-to-noise ratio must be sufficient to ensure reliable nucleotide assignments		
E	4	9	5		Allele assignment	[BG vs. HLA]	overlap with reporting
E	4	9	5	2	Criteria for allele assignment must be established	[BG vs. HLA]	overlap with reporting (go to NCBI BLAST plus check ISBT allele tables)
E	4	13			Other methods		
E	4	13	1		If alternative methods (e.g. SSCP, heteroduplex, DGGE) are used for [Molecular Blood Group ~~HLA~~] typing, there must be established procedures in place which:	[BG vs. HLA]	changed IMGT/HLA to ISBT
E	4	13	1	1	Must be validated		

E	4	13	1	2	Must include sufficient controls to ensure accurate assignment of types for every sample
E	4	13	1	3	Must comply with all relevant standards from section E **(Nucleic Acid Analysis)**

P	P. Processing of molecular data
	Comment: most current versions of the ISBT Blood Group Allele Tables (plus actual version number) are given at: http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/
P 1	Molecular Blood Group Typing may start from any appropriate source of molecular raw data, e.g. SNP typing, sequencing and others done on resources such as RNA and DNA
P 2	Raw molecular data must be translated to “haplotype alleles”, commonly described by the term “alleles” within this document
P 2 1	Current versions of the allele names as proposed by the ISBT terminology committee must be used, whenever available
P 2 2	In case of the discovery of new alleles and description of blood group alleles with non-existent ISBT names, <Trivial Names> for alleles must be used
P 2 2 1	Naming of new alleles with Trivial Names should be done in a way to avoid confounding with existent (and potential future) ISBT allele names
P 2 2 2	There should be written records for each newly discovered allele (with a Trivial Name)
P 2 2 3	Newly discovered alleles should be reported in peer-reviewed journals, the obtained sequences submitted to nucleotide databases and the discovery be reported to the respective point persons of the ISBT terminology committee
P 3	The two parental alleles must be described as a <Genotype>
P 3 1	Homozygosity may best be described by naming the respective allele only
P 3 2	Homozygosity for *RHD* (and similar genes) may best be inferred by RH box analysis or quantitative methods
P 3 3	Proven homozygosity for *RHD* (and similar genes) may be declared naming the respective *RHD* alleles twice
P 3 4	Untested zygosity determination for *RHD* (and similar genes) may be indicated similarly to serology by a dot <RHD/ " ">
P 3 5	If indicated, a third allele name per gene locus may be given in case of duplicated genes on one haplotype (e.g. *GYP*401*)
P 5	There should be written records for each genotype assignment to the Predicted Blood Group Phenotype (“interpretation matrices”), also considering newly discovered alleles (with Trivial Names)
	R. External reporting of results
R 1	Methods used, e.g. SNP typing, sequencing, and others, and type of material investigated (RNA, DNA), must be declared
R 1 2	When reporting SNP results, genetic positions of polymorphisms tested must be indicated as given by the ISBT terminology
R 2	Current versions of the allele names as proposed by the ISBT terminology committee must be used, whenever available
R 3	In case of the discovery of new alleles and description of blood group alleles with non-existent ISBT names, <Trivial Names> for alleles must be used

R	4	The two parental alleles must be described as a <Genotype>
R	5	Every genotype must be translated into a <Predicted Blood Group Phenotype>
R	6	All above-mentioned documentations may be commented, especially for rare alleles and uncommon genotype occurrences
R	7	There should be a transfusion recommendation, especially for rare alleles, uncommon genotype occurrences and newly discovered alleles (with Trivial Names)
		Z. Commonly known BG polymorphism
Z	1	APPENDIX 1: commonly recognised alleles with known BG phenotypes

Appendix Z: Commonly known BG polymorphism with known effects on BG phenotypes

Blood Group System	ISBT #	Blood Group	Gene (HGNC)	on	Chromo-some	allele name 1	allele name 2	nt position	nt 1	nt 2	amino a.	anti-gens	allele ct.	SNP ct.	rs #
ABO	001	ABO A vs O1	*ABO*	9q	9q34.2	*ABO*wt*	*ABO*O.01*	261	G	del G	fsThr88Pro	1	2	1	rs8176719
ABO	001	ABO A vs O2	*ABO*	9q	9q34.2	*ABO*wt*	*ABO*O.02*	802	G	A	Gly268Arg	-	1	1	rs41302905
ABO	001	ABO A vs B	*ABO*	9q	9q34.2	*ABO*wt*	*ABO*O.02*	803	G	C	Gly268Ala	1	1	1	rs8176747
MN	002	M / N	*GYPA*	4q	4q31.21	*GYPA*01*	*GYPA*02*	59	C	T	Ser20Leu	2	2	1	rs7682260
Ss	002	S / s	*GYPB*	4q	4q31.21	*GYPB*03*	*GYPB*04 (wt)*	143	C	T	Thr48Met	2	2	1	rs7683365
RhD	004	RhD+ / RhD-	*RHD / RHCE*	1p	1p36.11	*RHD*01 (wt)*	*RHD*01N.01*	455	A	[C]	Asn152[Thr]	2	2	1	rs17418085
RhD	004	RhD+ / RhD-	*RHD / RHCE*	1p	1p36.11	*RHD*01 (wt)*	*RHD*01N.01*	787	G	[A]	Gly263[Arg]	-	-	1	rs3118454
RhD	004	RhD+ / RhD-	*RHD / RHCE*	1p	1p36.11	*RHD*01 (wt)*	*RHD*01N.01*	1362	A	[T]	-	-	-	1	no rs
RhD	004	RhD+ / RhD-	*RHD / RHCE*	1p	1p36.11	*RHD*01 (wt)*	*RHD*04N.01*	504-541	-	ins 37 bp	-	-	1	1	no rs
RhD	004	RhD+ / RhD-	*RHD / RHCE*	1p	1p36.11	*RHD*01 (wt)*	*RHD*01N.06*	1006	G	C	Gly336Gly	-	1	1	no rs
RhD	004	RhD+ / RhD partial	*RHD*	1p	1p36.11	*RHD*01 (wt)*	*RHD*05.01, or RHD*DV.1*	667	T	G	Phe223Val	-	-	-	rs1053356
.	.	.	.	.	.	.	.	697	G	C	Glu233Gln	-	-	-	rs1053359
RhD	004	RhD+ / RhD partial	*RHD*	1p	1p36.11	*RHD*01 (wt)*	*RHD*05.07, or RHD*DV.7*	667 ... to	T	G	Phe223Val	-	-	-	rs1053356
RhD	004	RhD+ / RhD partial	*RHD*	1p	1p36.11	*RHD*01 (wt)*	*RHD*06.01, or RHD*DVII.1*	505 ... to	A	C	Met169Leu	-	-	-	rs17421137
RhD	004	RhD+ / RhD partial	*RHD*	1p	1p36.11	*RHD*01 (wt)*	*RHD*07.01, or RHD*DVII.1*	329	T	C	Leu110Pro	-	-	-	rs121912762
RhD	004	RhD+ / RhD weak	*RHD*	1p	1p36.11	*RHD*01 (wt)*	*RHD*01W.1, or RHD*weak D type 1*	809	T	G	Val270Gly	-	-	-	rs121912763
RhD	004	RhD+ / RhD weak	*RHD*	1p	1p36.11	*RHD*01 (wt)*	*RHD*01W.2, or RHD*weak D type 2*	1154	G	C	Gly385Ala	-	-	-	rs71652374
RhD	004	RhD+ / RhD weak	*RHD*	1p	1p36.11	*RHD*01 (wt)*	*RHD*01W.3, or RHD*weak D type 3*	8	C	G	Ser3Cys	-	-	-	rs144969459
RhD	004	RhD+ / RhD Del	*RHD*	1p	1p36.11	*RHD*01 (wt)*	*RHD*01EL.01, or RHD*DEL1*	1227	G	A	Lys409Lys	-	-	-	rs549616139
RhD	004	RhD+ / RhD Del	*RHD*	1p	1p36.11	*RHD*01 (wt)*	*RHD*01EL.08, or RHD*DEL8*	486+1 = IVS3+1g>a	g	a	splice mutant	-	-	-	rs371990272
RhD	004	RhD+ / RhD weak, partial, Del	*RHD*	1p	1p36.11	*RHD*01 (wt)*	*RHD*11, or RHD*weak partial 11*	885	G	T	Met295Ile	-	-	-	rs371803235
RhCE	004	Rhc / RhC	*RHCE*	1p	1p36.11	*RHCE*01 (03) (wt)*	*RHCE*02 (04)*	i2+3095	-	ins 109 bp	-	2	2	1	no rs
RhCE	004	Rhc / RhC	*RHCE*	1p	1p36.11	*RHCE*01 (03) (wt)*	*RHCE*02 (04)*	307	C	T	Ser103Pro	-	-	1	rs676785
RhCE	004	RhC, Rhc / RhC^{w}	*RHCE*	1p	1p36.11	*RHCE*all*	*RHCE*02.08*	122	A	G	Gln41Arg	1	1	1	rs138268848
RhCE	004	Rhe / RhE	*RHCE*	1p	1p36.11	*RHCE*01 (02)*	*RHCE*03 (04)*	676	G	C	Pro226Ala	2	2	1	rs609320
Lutheran	005	Lu^{a} / Lu^{b}	*BCAM*	19q	19q13.32	*LU*01*	*LU*02 (wt)*	230	A	G	His77Arg	2	2	1	rs28399653
Kell	006	K / k	*KEL*	7q	7q34	*KEL*01*	*KEL*02 (wt)*	578	T	C	Met193Thr	2	2	1	rs8176058
Kell	006	Kp^{a} / Kp^{b}	*KEL*	7q	7q34	*KEL*02.03*	*KEL*02 (wt)*	841	T	C	Trp281Arg	2	1	1	rs8176059
Kell	006	Js^{a} / Js^{b}	*KEL*	7q	7q34	*KEL*02.06*	*KEL*02 (wt)*	1790	C	T	Pro597Leu	2	1	1	rs8176038
Duffy	008	Fy^{a} /Fy^{b}	*DARC*	1q	1q23.2	*FY*01, or FY*A*	*FY*02, or FY*B*	125	G	A	Gly42Asp	2	2	1	rs12075
Duffy	008	Fy^{b} / Fy^{x}	*DARC*	1q	1q23.2	*FY*02*	*FY*02M*	265	C	T	Arg89Cys	-	1	1	rs34599082
Duffy	008	$Fy^{a,b}$ / Fy null	*DARC*	1q	1q23.2	*FY*02*	*FY*02N.01*	P-67t>c	T	C	-	1	1	1	rs2814778
Kidd	009	Jk^{a} /Jk^{b}	*SLC14A1*	18q	18q11-q12	*JK*01, or JK*A*	*JK*02, or JK*B*	838	G	A	Asp280Asn	2	2	1	rs1058396
Diego	010	Di^{a} / Di^{b}	*SLC4A1*	17q	17q21.31	*DI*01*	*DI*02 (wt)*	2561	T	C	Leu854Pro	2	2	1	rs2285644
Wright	010	Wr^{a} / Wr^{b}	*SLC4A1*	17q	17q21.31	*DI*02.03*	*DI*02 (wt)*	1972	A	G	Glu658Lys	2	2	1	rs75731670
Cartwright	011	Yt^{a} / Yt^{b}	*ACHE*	7q	7q22.1	*YT*01 (wt)*	*YT*02*	1057	C	A	His353Asn	2	2	1	rs1799805
Scianna	013	SC:1, SC:2	*ERMAP*	1p	1p34.2	*SC*01 (wt)*	*SC*02*	169	G	A	Gly57Arg	2	2	1	rs56025238
Dombrock	014	Do^{a} / Do^{b}	*ART4*	12p	12p12.3	*DO*01*	*DO*02 (wt)*	793	A	G	Asn265Asp	2	2	1	rs11276
Colton	015	Co^{a} / Co^{b}	*AQP1*	7p	7p14.3	*CO*01.01 (wt)*	*CO*02*	134	C	T	Ala45Val	2	2	1	rs28362692
Landsteiner-Wiener	016	LW^{a} / LW^{b}	*ICAM-4*	19p	19p13.2	*LW*05 (wt)*	*LW*07*	299	A	G	Gln100Arg	2	2	1	rs77493670
Indian	023	In^{a} / In^{b}	*CD44*	11p	11p13	*IN*01*	*IN*02 (wt)*	137	G	C	Arg46Pro	2	2	1	rs121909545
Vel	n.a.	Vel+ / Vel-	*SMIM1*	1p	1p36.32	*[SMIM1*Vel+]*	*[SMIM1*Vel-]*	64-80	-	del 17 bp	-	1	2	1	rs566629828
[Hum. Platelet AG 1]	n.a.	[HPA-1a / b]	*ITGB3*	17q	17q21.32	*[ITGB3*001] (HPA-1a) (wt)*	*[ITGB3*002] (HPA-1b)*	176	T	C	Leu59Pro	2	2	1	rs5918
[Hum. Platelet AG 5]	n.a.	[HPA-5a / b]	*ITGA2*	5q	5q11.2	*[ITGA2*001] (HPA-5a) (wt)*	*[ITGA2*002] (HPA-5b)*	1600	G	A	Glu534Lys	2	2	1	rs10471371

Bibliography

1. Verordnung über die Arzneimittel (VAM). Art 37- 39. SR 812.212.21, Inkrafttreten 21. April 2018: Stand am 1. April 2020 (http://www.admin.ch/ch/d/sr/c812_212_21.html).
2. Kriterien zum Betreiben von medizinisch-analytischen Laboratorien (KBMAL), Ver2016 (http://qualab.ch/QUALAB_d.htm).
3. Verordnung über die Bewilligungen im Arzneimittelbereich (Arzneimittel-Bewilligungsverordnung, AMBV), Art 16 - Hämovigilanz. SR 812.212.1, Inkrafttreten 14.11.2018: Stand am 1. Januar 2020 (http://www.admin.ch/ch/d/sr/c812_212_1.html).
4. Bundesgesetz über Arzneimittel und Medizinprodukte (Heilmittelgesetz, HMG) SR 812.21, Inkrafttreten 01.01.2002 (Stand 01.01.2020), (http://www.admin.ch/ch/d/sr/812_21/index.html).
5. Guide to the preparation, use and quality assurance of blood components. Published by the European Directorate for the Quality of Medicines & Healthcare of the Council of Europe. Actual version.
6. White J. Pre-transfusion testing. Vox sanguinis. 2009, 4:37–44.
7.
7. Guidelines for blood grouping and antibody testing in pregnancy. British Committee for Standards in Haematology Blood Transfusion Task Force. Gooch A, Parker J, Wray J et al. Transfus Med. 2007, 4:252–262.
8. Empfehlungen Anti-D-Rh-Prophylaxe. Schweiz Med Forum, 2006, 6:749–751.
9. Prévention de l'allo-immunisation Rhésus-D fœto-maternelle. Recommandations pour la pratique clinique. Collège national des gynécologues et obstétriciens français. 2005 (http://www.cngof.asso.fr/).
10. Noizat-Pirenne F., Verdier M., Lejealle A. et al. Weak D phenotypes and transfusion safety: where do we stand in daily practice? Transfusion. 2007,47:1616–1620.
11. Flegel, Willy A. Genetik des Rhesus-Blutgruppensystems. Deutsches Ärzteblatt 2007; 104: A-651–657.
12. Guidelines on transfusion for fetuses, neonates and older children. British Journal of Haematology. 2016,175, 784–828.
13. New HV, Stanworth SJ, Engelfriet CP et al. Neonatal transfusions – International Forum. Vox Sang, 2009, 96: 62–85.
14. EudraLex – Volume 4. Good manufacturing practice (GMP) Guidelines http://ec.europa.eu/health/documents/eudralex/vol-4/index_en.htm
15 Sandler S. G., Eder A. F., Goldman M., and Winters J.L.: The entity of immunoglobulin A-related anaphylactic transfusion reactions is not evidence based. Transfusion. 2015;55:199–204.
16. Anani W., Triulizi D., Yazer M.H., and Qu L. Relative IgA-deficient recipients have an increased risk of severe allergic transfusion reactions. Vox Sanguinis. 2014;107:389–392.
17. Hustinx H., Scholl N., Gowland P., Krieg R., Stolz M., Fontana S., Niederhauser C. Screening of Swiss blood donors for IgA deficiency and its significance for the

investigation of anaphylactic transfusion reactions. Swiss Medical Forum 2009;9 (Suppl. 46).

18. T. Türkmen, D. Qiu, N. Cooper, U. Sachs, W. Wössmann, D. Schranz, K.-P. Zimmer, H. Ehrhardt, H. Hackstein, and G. Bein, Red blood cell alloimmunization in neonates and children up to three years of age.Transfusion. 2017 Nov;57(11):2720–2726. doi: 10.1111/trf.14273. Epub 2017 Sep 6.

19 M. Pai, R. Cook, R. Barty, J. Eikelboom, K. Lee, N. Heddle; Exposure to ABO-nonidentical blood associated with increased in-hospital mortality in patients with group A blood. Transfusion, 2016 Mar;56(3):550–557.

20. Leitfaden für die Qualitätssicherung in der Transfusionspraxis, Schweizerische Arbeitsgruppe Qualitätssicherung in der Anwendung von Blutprodukten.

21. Expertenbrief Nr. 68. Empfehlungen zur Anti-D-Immunglobulin-Gabe in der Schwangerschaft (= Anti-D-Prophylaxe). M. Hodel, S. Lejon Crottet, L. Raio, R. Zimmermann, O. Lapaire, G. Canellini, C. Henny, C. Niederhauser, S. Waldvogel, S. Fontana.

22. Leitlinien Inspektionen von Blutlagern. Swissmedic: 17.01.2020.

23.. Marion E. Reid, Christine Lomas-Francis and Martin L. Olsson. The Blood Group Antigen Facts Book (2012), Academic Press.

24. International Society of Blood Transfusion (ISBT) (https://www.isbtweb.org/).

25. Vorschriften B-CH SRK, Kapitel 10 «Herstellung», § 10.9.2 «Bestrahlen labiler Blutprodukte».

26. Standards for Blood Banks and Transfusion Services, 33rd ed, , AABB, Bethesda, April 2017

27. Guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories. Milkins C, Berryman J, Cantwel C et al. British Committee for Standards in Haematology. Transfus Med. 2013, 23:3-35. (http://www.bcshguidelines.com). Update 25th April 2017

28. The Direct Antiglobulin Test: Indications, Interpretation, and Pitfalls. Parker V et Tormey CA. Arch Pathol Lab Med 2017; 141 (2): 305–310.

29. Resolving the daratumumab interference with blood compatibility testing, CI, RT, MD et al. Transfusion 2015;55 :1545–1554.

30. Thornton NM, Grimsley SP. Clinical significance of antibodies to antigens in the ABO, MNS, P1PK, Rh, Lutheran, Kell, Lewis, Duffy, Kidd, Diego, Yt, and Xg blood group systems. Immunohematology. 2019 Sep;35(3):95-101. PMID: 31621367.

31. Chou ST, Alsawas M, Fasano RM, et al. American Society of Hematology 2020 guidelines for sickle cell disease: transfusion support. Blood Adv 2020;4:327-55.

32. Linder GE, Chou ST. Red cell transfusion and alloimmunization in sickle cell disease. Haematologica 2021;106:1805-15.

33. Narbey D, Habibi A, Chadebech P et al. Incidence and predictive score for delayed hemolytic transfusion reaction in adult patients with sickle cell disease. American Journal of Hematology. 2017;92:1340–1348

34. Habibi A, Mekontso-Dessap A, Guillaud C, et al. Delayed hemolytic transfusion reaction in adult sickle-cell disease: presentations, outcomes, and treatments of 99 referral center episodes. Am J Hematol 2016;91:989-94.

Other resources

1. Arrêté du 10 septembre 2003 portant homologation du règlement de l'Agence française de sécurité sanitaire des produits de santé définissant les principes de bonnes pratiques dont doivent se doter les établissements de transfusion sanguine. ORF n°226 du 30 septembre 2003.
2. Arrêté du 15 mai 2018 fixant les conditions de réalisation des examens de biologie médicale d'immuno-hématologie érythrocytaire. JORF n°0116 du 23 mai 2018 https://www.legifrance.gouv.fr/eli/arrete/2018/5/15/SSAP1803010A/jo/texte
3. Richtlinie zur Gewinnung von Blut und Blutbestandteilen und zur Anwendung von Blutprodukten (Richtlinie Hämotherapie). Aufgestellt gemäß §§ 12a und 18 Transfusionsgesetz von der Bundesärztekammer im Einvernehmen mit dem Paul-Ehrlich-Institut. Gesamtnovelle 2017https://www.bundesaerztekammer.de/fileadmin/user_upload/downloads/pdf-Ordner/RL/RiliH_Lese.pdf

Swiss Transfusion SRC (B-CH SRK), its regional blood transfusion services and the Board of the SVTM will be glad to provide further information:

Swiss Transfusion SRC
Waldeggstrasse 51
3097 Liebefeld
www.blutspende.ch
bsd@blutspende.ch

SVTM Secretariat
c/o Blutspende SRK Schweiz AG
Stefanie Mast
Waldeggstrasse 51
3097 Liebefeld
www.svtm-asmt.ch
svtm-asmt@blutspende.ch

Responsible expert group

- Soraya Amar, expert group member (representing B-CH)
- Daniel Bolliger, representing anaesthesiology
- Giorgia Canellini, expert group member (Interregional Blood Transfusion)
- Michael Daskalakis, expert group member (Inselspital)
- Charlotte Engström, expert group member (Regional Blood Transfusion Service Zurich)
- Beat Frey, expert group member (Regional Blood Transfusion Service Zurich)
- Inga Hegemann, representing Zurich University Hospital
- Sofia Lejon Crottet, head of expert group (Interregional Blood Transfusion)
- Behrouz Mansouri, representing SVTM
- Antoinette Monn, representing Zurich City Hospital Waid and Triemli
- Christoph Niederhauser, expert group member (Interregional Transfusion Service)
- Tanja Rüfli, expert group member (Regional Blood Transfusion Service Basel-Stadt/ Basel-Landschaft)
- Belinda Ryser, expert group member (Regional Blood Transfusion Service Tessin)
- Sophie Waldvogel, expert group member (Regional Blood Transfusion Service Geneva)

Addendum 1

ISBT No.	System	Antigen number												
		1	2	3	4	5	6	7	8	9	10	11	12	Total
001	ABO[$]	A	B	A,B	A1	…								4
002	MNS	M	N	S	s	U	He	Mi^a	M^c	Vw	Mur	M^g	Vr	50
003	P1PK	P1	---	p^k	NOR									3
004	RH	D	C	E	c	e	f	Ce	C^W	C^X	V	E^W	G	55
005	LU (Lutheran)	Lu^a	Lu^b	Lu3	Lu4	Lu5	Lu6	Lu7	Lu8	Lu9	…	Lu11	Lu12	27
006	KEL (Kell)	K	k	Kp^a	Kp^b	Ku	Js^a	Js^b	…	…	Ul^a	K11	K12	36
007	LE (Lewis)	Le^a	Le^b	Le^{ab}	Le^{bH}	ALe^b	BLe^b							6
008	FY (Duffy)	Fy^a	Fy^b	Fy3	…	Fy5	Fy6							5
009	JK (Kidd)	Jk^a	Jk^b	Jk3										3
010	DI (Diego)	Di^a	Di^b	Wr^a	Wr^b	Wd^a	Rb^a	WARR	ELO	Wu	Bp^a	Mo^a	Hg^a	22

[$] The ISBT terminology for the ABO blood group system is not used in these recommendations. Each blood group system is defined by the respective ISBT number and by a combination of 2–4 capital letters (ISBT symbol). The Kidd system, for example, has the ISBT symbol JK and the ISBT number 009. The antigen Jk^b is referred to as JK2 by the ISBT nomenclature.

Example 1

	Traditional	ISBT
Antigen	Fy^a	FY1
Phenotype	Fy(a+b–)	FY:1,–2[$$]
Allele	*Fy^a*	*FY*01*
Genotype	*Fy^a Fy^a*	*FY*01/FY*01*
Antibody	Anti-Fy^a	Anti-FY1

Example 2

	Traditional	ISBT
Antigen	K	KEL1
Phenotype	K+k–	KEL:1,–2[$$]
Allele	*K*	*KEL*01.01*
Genotype	*KK*	*KEL*01.01/KEL*01.01*
Antibody	Anti-K	Anti-KEL1

Example 3

	Traditional	ISBT
Antigens	D, C, E, c, e	RH1, RH2, RH3, RH4, RH5
Phenotype	D+C+E+c+e+ (R1R2)	RH:1,2,3,4,5[$$]
Allele	D, CE	RHD*01/RHCE*02/ RHCE*03[$$$]
Genotype	*CDe/cDE*[$$$]	*RHD*01/RHD*01, RHCE*02/RHCE*03*[$$$]
Antibody	Anti-D, -C, -E, -c, -e	Anti-RH1, -RH2, -RH3, -RH4, -RH5

[$$] According to the ISBT nomenclature, serologically weak antigens (weak or partial) are identified phenotypically by respectively W or P before the antigen number, e.g. FY:W2 = phenotype Fy(b+w), RH:P1 = phenotype RhD partial.

[$$$] Most likely genotype

Adapted from reference [25, 26]