Comité Libanais de Transfusion Sanguine

RÉPUBLIQUE DU LIBAN
MINISTÈRE DE LA SANTÉ PUBLIQUE

Good Laboratory Practices in Red cell ImmunoHematology

April 2022

VERSION 1

LCBT-QUAL-002

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Objectives and Scope

The objective of this document is to define the technical procedures for implementing the red cell immunohematological testing of donations.

This document concerns the blood donation testing.

General Procedure

Blood donations testing is performed on venous blood samples collected from the same phlebotomy site as the donation. Only tubes labelled with the donation ID (primary tubes) are accepted by the laboratory to enter the testing process.

Blood donations testing involves the automation and computerization of tests according to the terms defined in the automation and computerization chapter (Principles of Good Transfusion Practices October 2012).

The operating personnel takes, under the responsibility of the Blood Transfusion Center (BTC) director, the final decision regarding the analytical validation regardless of the automation and computerization level.

All technical procedures for any given reagent or equipment used in immunohematology must be initially validated.

Each new reagent must undergo a method validation. Each new reagent lot must be also validated before use. The critical parameters that require validation are specific to each of the above processes.

Biological validation must be performed using an automated validation system that allows the analysis of the concordance between:

- Tests results repeated on the exact same blood sample;
- Current and previous test results;
- Current and previous antibody screen results.

A specific procedure, whether computerized or not, must be defined in case of non-conformity or discrepancy.

Specific procedures

1. The ABO-RH1 (RhD) grouping

a. Definition

This test consists in determining simultaneously the ABO and RH 1 (RhD) phenotypes.

b. Methods of implementation

Principle

Performing the ABO-RH1 blood group

ABO blood grouping is based on two complementary and inseparable tests:

- A globular test which consists of searching for A (AB01) and B (AB02) antigens using the following monoclonal reagents: anti-A (anti-AB01), anti-B (anti-AB02) and anti-AB (anti-AB03) :
- A plasma test which consists of searching for anti-A and Anti-B antibodies with A1 and B test cells. At least one of these cells must be of RH: -1 phenotype.

RH1 blood grouping must involve the use of a monoclonal anti-RH1 reagent and a control that lack any antibody activity, but whose ability to agglutinate sensitized red cells is strictly identical to the anti-RH1 reagent.

The anti-RH1 reagent must detect most RH1 variants, particularly the partial RH1 antigens in category VI and weak RH1 type 2. For the first donation, an additional control test (indirect antiglobulin test) must be performed for RH: -1 samples.

During the globular test, the anti-B reagent used must not cross-react with the acquired B antigen.

One of these two reagents, anti-A or anti-AB, must be able to recognize Ax red blood cells.

Determining the ABO-RH1 blood group

Its definition depends on the following conditions:

- If all procedures including the modalities of verification and recording of samples and requests are strictly realized through automated and computerized systems, the ABO-RH1 phenotype can be determined by performing one test, using one batch of reagents and test cells by one technician;
- For all other cases, it is based on two tests performed by tow technicians using two different batches of reagents (not having common clones) and test cells. The manual entry of results must be double checked by two different personnel.

"Valid ABO-RH1 grouping: Blood grouping is routinely performed (except during life-threatening emergencies) on two different samples (one determination per sample) and if the patient blood group is previously known in the healthcare facility, then one sample becomes sufficient."

Internal Quality Controls (IQC)

For ABO determination, the analytical system must be monitored using a set of control samples whose phenotypes are previously known (completely realized by a different technique), comprising at least:

- One group A sample;
- One group B sample;
- One group O sample.

For RH1 determination, controls samples must include at least:

- One RH: 1 sample;
- One RH: -1 sample.

Interpretation and validation of results, management of discrepancies

Interpretation and validation of results

The final decision for analytical validation rests with the operating personnel who, under the responsibility of the BTC director, must ensure that the technical conditions for performing all analysis comply with the applicable procedures and that they guarantee the optimum quality of the results.

Analytical validation is based on:

- Conforming IQC results;
- Absence of cross-reaction with each reagent.
- Absence of double population. It is imperative to report to the laboratory any recent transfusion history (less than four month) when the tests are ordered.
- Absence of discrepancies between two determinations
- Absence of discrepancies with previous results
- Agreement between the globular and the plasma tests

Management of discrepancies

The director of the BTC must intervene if a discrepancy is detected during the analytical validation phase of ABO-RH1 grouping.

The management is then based on:

- A safe attitude in terms of investigation:
    - Do not release the result;
    - Provide a provisional transfusion advice in case of emergency.
- A new blood group determination:
- If the discrepancy is resolved, the result becomes valid;
- If not, further investigations are required
- A coherent attitude during investigation that takes into account:
    - The clinical context;
    - The obtained pattern of reactivity;
    - The results of the controls:
        - The “auto” control, which consists of testing the subject’s plasma against

his/her own red blood cells under the same technical conditions;

- The « allo » and possibly the « A2 » controls, which consist of testing under the same technical conditions, the subject's plasma against a range of O and A2 test cells whose antigenic constitution will allow the detection of anti-erythrocyte antibodies, other than anti-A and anti-B, likely to interfere with the plasma test;
- The « reagent » control which consist of testing, under the same technical conditions, the subject's red blood cells against a control reagent that has no antibody activity.

2. The RH-KEL 1 (Rh-K) phenotyping

a. Definition

This test includes the study of the RH2 (C). RH3 (E), RH4 (c), RH5 (e) and KEL1 (K) antigens.

b. Methods of implementation

Principle

Performing the RH-KEL 1 phenotype

It requires the use of anti-RH2, anti-RH3, anti-RH4, anti-RH5 and anti-KEL1 reagents along their adequate controls. The use of monoclonal reagents is mandatory.

Determining the RH-KEL 1 phenotype

The RH-KEL 1 phenotype is determined using one batch of reagents and performed by one technician on one sample. The manual entry of results must be double checked by two different personnel.

Internal Quality Controls

The analytical system must be monitored using a set of control samples whose phenotypes are previously known (completely realized by a different technique), comprising for each subgroup the following red cells:

- anti-RH2: one sample RH :2,4 and one sample RH: -2,4;
- anti-RH3: one sample RH :3.5 and one sample RH: -3,5:
- anti-RH4: one sample RH :2,4 and one sample RH :2.-4;
- anti-RH5: one sample RH :3,5 and one sample RH :3, -5;
- anti-KEL 1: one sample KEL :1 and one sample KEL: -1.

Interpretation and validation of results, management of discrepancies

Interpretation and validation of results

Analytical validation is based on:

- Conforming IQC results;
- Absence of cross-reaction with each reagent.
- Absence of double population. It is imperative to report to the laboratory any recent transfusion history (less than four month) when the tests are ordered.
- Absence of discrepancies between two determinations
- Absence of discrepancies with previous results
- Negative reaction obtained with the reagent control.

Management of discrepancies

The director of the BTC must intervene if a discrepancy is detected during the analytical validation phase of RH-KEL 1 phenotyping.

The management is then based on:

- A safe attitude in terms of investigation:
    - Do not release the result;
    - Provide a provisional transfusion advice in case of emergency.
- A new phenotype determination:
- If the discrepancy is resolved, the result becomes valid;
- If not, further investigations are required
- A coherent attitude during investigation that takes into account:
    - The clinical context;
    - The obtained pattern of reactivity (including adequate controls).

3. Extended Phenotype

a. Definition

This test consists of testing for one or more red cell antigens other than those identified by the ABO-RH1 grouping and RH-KEL 1 phenotyping.

b. Methods of implementation

Principle

Performing the extended phenotype

For a given blood system, testing for each antigen is based on the use of a specific reagent along its adequate control.

Determining the extended phenotype

Its definition depends on the following conditions:

- The extended phenotype is determined using one batch of reagents by one technician on one sample.
- The manual entry of results must be double checked by two different personnel.

Internal Quality Controls

The analytical system must be monitored for each group using two control samples whose phenotypes are previously known (completely realized by a different technique). One of these samples must be negative and the other « heterozygous ».

Interpretation and validation of results, management of discrepancies

Interpretation and validation of results

Analytical validation is based on:

- Conforming IQC results;
- Absence of cross-reaction with each reagent.
- Absence of double population. It is imperative to report to the laboratory any recent transfusion history (less than four month) when the tests are ordered.
- Absence of discrepancies between two determinations
- Absence of discrepancies with previous results
- Negative reaction obtained with the reagent control.

Management of discrepancies

The director of the BTC must intervene if a discrepancy is detected during the analytical validation phase of the extended phenotype.

The management is then based on:

- A safe attitude in terms of investigation:

- Do not release the result;
- Provide a provisional transfusion advice in case of emergency.

- A new phenotypic determination:
- If the discrepancy is resolved, the result becomes valid;
- If not, further investigations are required.

- A coherent attitude during investigation that takes into account:
  - The clinical context;
  - The pattern of reactivity obtained.

4. Red cell antibody detection

a. Definition

Using screening cells of human origin, antibodies against red cell antigens other than A and B are detected and identified in serum or plasma samples.

c. Methods of implementation

Principle

Red cell antibody detection consists of two steps and their sequence is under the responsibility of the BTC director:

- A screening step during which the laboratory will report if no antibodies are detected as: Negative Antibody Screen.

If the test is positive, the relevant red cell antibody (ies) must be identified.

This step involves using at least three group O test cells that allows the detection of antibodies directed against the following antigens: RH1 (D), RH2 (C), RH3 (E), RH4 (c), RH5 (e), KEL 1 (K), KEL 2 (Cellano), KEL 4 (Kpb), FY1 (Fya), FY2 (Fyb), JK1 (Jka), JK2 (Jkb), MNS1 (M), MNS2 (N), MNS3 (S), MNS4 (s), LE1 (Lea), LE2 (Leb), P1 (P1) and LU2 (Lub).

The Red Cell Antibody screen must include the following RH phenotypes: RH: 1, 2, -3, -4, 5; RH: 1, -2, 3 ,4, -5 and RH: -1, -2, -3, 4, 5.

Furthermore, screening test cells with “homozygous” expression for antigens must be used and preferably for FY2 and MNS4 antigens.

Reagent red cells should not be pooled under no circumstances.

- An Identification step which consists in determining the specificity of the antibody (ies) by comparing the pattern of negative and positive reactions obtained with the pattern of antigens present on the panel red cells.

For the antibody identification that are performed in another laboratory, an unopened and non-centrifuged sample,

if possible, must be used.

This step requires the use of at least 10 group O test cells other than the three screening cells.

These cells must include the following antigens: RH1, RH2, RH3, RH4, RH5, RH6, RH8 (Cw), KEL1, KEL2, KEL3 (Kpa), KEL 4, FY1, FY2, JK1, JK2. MNS1, MNS2, MNS3, MNS4, LE1, LE2, P1, LU1 (Lua) and LU2.

At least two test cells must include the following phenotypes: KEL1, FY: 1, -2, FY -1, 2, JK: 1, -2, JK: -1.2. MNS: 3.-4, MNS: -3, 4 and P: -1.

This test should allow the identification of one common antibody or orient to the concomitant presence of several antibodies.

Procedures

The method for antibody screen and identification is an indirect antiglobulin test (polyspecific antihuman globulin or anti-IgG).

It may be useful or even essential to also use enzymatic techniques especially in the case of difficult identification (combination of alloantibodies) and during investigation of immune-mediated transfusion reactions.

Internal Quality Controls

The analytical system must be monitored using control samples (negative or positive) of known specificities and titers with at least one antibody having a titer < or = to 4 along its corresponding antigen expressed heterozygously on one test cell. For antibody screening, it is recommended to use two control samples: one with a frequent antibody (for example, anti-RH1/D), the other with an antibody directed against a rare antigen (anti-FY1/Fya) in order to ensure the quality and the good preservation of test cells over time.

Interpretation and validation of results

Identifying one or several red cell antibodies requires:

- The validation of the antibody specificity by obtaining a positive reaction with all test cells expressing the corresponding antigen (at least three) and a negative reaction with all test cells lacking the antigen (at least three) The rule of three does not apply in case there is a combination of anti-RH antibodies.

If this rule does not apply, the interpretation must take into account the « homozygous or heterozygous » expression of the red cells used. At this point, an additional step with a larger number of test cells must be performed;

- The elimination of any additional antibodies:
    - By implementing further techniques;
    - By the presence of antigens, on negative panel test cells, available on the screening cells that

do not correspond to the previously identified specificity (ies);

- Checking that the antigen corresponding to each identified antibody is absent, if their reagents are commercially available.

In case of invalid results, the red cell antibody identification should be supplemented by ABO-RH1 grouping and RH-KEL1 phenotyping.

5. Major Cross-Match

a. Definition

It involves testing the recipient's serum or plasma with the donor's red cells obtained from a segment of the tubing attached to the blood unit.

b. Methods of implementation

Principle

Major cross-match is performed in three steps.

1. Selection of units according to good distribution practices

This takes into account:

- The recipient immuno-hematological status which is based, in the absence of a previous history, on determining the:
  - The ABO-RH1 group;
  - The phenotypes for RH-KEL1 and/or other immunogenic antigens whom serological compatibility must be respected;
- The validity period of the antibody screen that must be in accordance with the regulations;
- The red cell antibody identification in case of a positive screen;
- The existence of transfusion protocols specific to the relevant clinical situation.

2. Preparation of RBCs form the tubing segment

The purpose of this step is to prepare the donor's RBC prior to testing under the same technical conditions of the antibody screen.

Particular attention should be paid to the identification of samples and tubing using the barcode number of the blood product unit.

3. Procedure

The technical conditions are identical to those used for the antibody screen.

Internal Quality Controls

The analytical system must be monitored using control samples identical to those applied for the antibody screen.

Interpretation and validation of results

A standard operating procedure shall define the modalities for issuing cross-matched blood products according to the cross-match results:

- Conforming IQC results;
- In the absence of reactivity: the unit is declared compatible. Its release is authorized with a specific identification according to the applicable regulations;
- In case of reactivity: the unit is declared incompatible. Depending on the context, its release may be authorized according to the regulations figuring in the good distribution practices and the blood transfusion committee. Further exploration may also be undertaken in order to explain these results and select new units accordingly.

6. Direct Anti-Globulin test (DAT)

a. Definition

The DAT demonstrate the sensitization of RBC in vivo by IgG antibodies and/or complement. This test should be performed preferably on an anti-coagulated blood sample. It is best to perform this test on a sample collected in a citrate tube.

b. Methods of implementation

Principle

In vivo sensitization of RBC can be detected using human antiglobulin(s) whose Fab fraction recognize the isotypic markers of immunoglobulins or complement fractions specifically attached on RBCs.

This test requires the use of an anti-IgG, an anti-C3d as well as appropriate control reagents.

Internal Quality Controls

The analytical system must be monitored using RBCs previously sensitized in vitro by anti-IG and possibly complement.

Interpretation and validation of results

- Conforming IQC results;

- Absence of cross-reaction with each reagent.
- Reactivity pattern consistent with the pre-established interpretation algorithms.

7. Internal Quality Control

The BTC director must put in place an IQC system which is based on running control samples under the same conditions as those applied to blood samples. IQC samples are run at least daily and their results must be previously known and stable.

8. Automation - Computerization

The minimum characteristics for a system should be defined so that the process of immuno-hematological analysis can be qualified as automated:

Regardless of the automation degree, the quality of the analytical process is directly linked to the preanalytical phase, which involves critical manual operations whose error may affects the reliability of the result:

- Reception of samples and attached documents (prescription, medical follow-up sheet),
- Registering civil status,
- Linking patient – Identification method – sample.

Written standard operating procedures must detail these operations to avoid any input or identification errors. Specific operations should be implemented to verify the data entry and the link between the patient and the corresponding sample.

Consequently, the civil status entered electronically based on the prescription must be verified by a second data entry based on the information recorded on the identified sample.

A system is qualified as "automated" if it can support certain phases of the analytical process that appear critical for the reliability of the results and can automatically and unequivocally link the patient with the corresponding results after identifying accurately the sample.

This concept can therefore be applied to both automated and semi-automated systems as defined in the field of laboratory medicine.

9. Blood group card

The blood group card is a document summarizing the patients' medical data in order to ensure their immunological safety during transfusion.

The following information must appear on the blood group card:

a. Identification of the issuing laboratory

- Name of the laboratory

- Address
- Phone number
- Signature of the director

b. Patient Identification

- Complete birth name and if applicable, marital name
- First name(s), and written in full in case of a composite first name
- Gender
- Date of birth.

In case the marital name changed, the card remains valid if the other identifiers are correct.

c. Blood groups and red cell phenotypes

The result of each determination is followed by the date it was performed.
The use of the international alphanumeric terminology is recommended.

d. Red cell antibody screening

The presence of one or more RBC antibodies is mentioned on the card followed by the test date. There is no need to mention the reagent test cells used nor their manufacturers.

A negative antibody screen is not mentioned on the blood group card.

The use of the international alphanumeric terminology is recommended.

10. Special case of the newborn

Blood grouping in a newborn or infant requires a venous blood sample. It cannot be performed from a cord blood sample.

The blood group document is only valid until the age of six months. It should mention: "newborn blood group - valid until - date of birth + 6 months-".