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European Medicines Agency

Advice from the Expert Panels on high-risk medical devices

Mandate[1] and advice provided to the Medical Device Coordination Group

[1] According to the section 6.3 of the Rules of procedure of the European Commission expert panels on medical devices and in vitro diagnostic medical devices.

Table of Contents

1. Legal basis for the request

Article 106 (10) (a) and (b) of Regulation (EU) 2017/745 on medical devices.

2. Party requiring the advice

The Medical Device Coordination Group (MDCG).

3. Scientific context and background information

Devices used for human blood grouping in the context of transfusions, to determine the safety and compatibility with potential recipients, or in the context of determining feto-maternal blood group incompatibility, are *in vitro* diagnostic medical devices in accordance with the definition provided in Article 2(2) of Regulation (EU) 2017/746 (IVDR). In the IVDR, these devices are identified as having a higher risk as they are, in accordance with rule 2 of Annex VIII, classified as class C, whilst some of them are classified as class D (devices intended for the determination of the following markers : ABO system [A (ABO1), B (ABO2), AB (ABO3)] — Rhesus system (RH1(D), RHW1, RH2 (C), RH3 (E), RH4 (c), RH5 (e) — Kell system [KEL1 (K)] — Kidd system [JK1 (Jka), JK2 (Jkb)] — Duffy system (FY1 (Fya), FY2 (Fyb)]). This covers both devices for the detection of blood group antigens and of antibodies against these markers. The guidance on classification rules for IVDs (MDCG 2020-16 Rev.2) clarifies this point and provides examples.

In addition to the determination of the ABO system blood group, the detection and identification of irregular antibodies as well as crossmatching tests are crucial pretransfusion steps to ensure recipient safety. There are several types of irregular antibodies. Some are directed against antigens in the highest-risk blood groups listed in the indents of rule 2 of Annex VIII IVDR. Devices used for the detection of irregular antibodies and for crossmatching consist of a panel of red blood cells, an antiglobulin and a physical support. These components may be placed on the market either together or separately. The technique is called the indirect antiglobulin test. The antiglobulin (also referred to as anti-human globulin or AHG) is used to potentiate the possible antigen-antibody reaction and thus, depending on the red blood cells used, to show the presence or absence of these irregular antibodies, whether directed against the antigens of the blood groups explicitly listed in Rule 2 or not.

It should be noted that indirect antiglobulin tests used for the detection of irregular antibodies are also used for detection of antibodies in the diagnosis of haemolytic disease in newborns (in particular antibodies against RH1), which is typically part of in the determination of feto-maternal blood group incompatibility.

The MDCG has a number of questions concerning the use of these tests and the use of antiglobulin, in particular whether indirect antiglobulin tests can be considered as detecting the markers of the Rhesus, Kell, Kidd, or Duffy system.

Relevant rules of Annex VIII of Regulation (EU) 2017/746 and elements of the IVD classification guidance MDCG 2020-16 Rev.2

Rule 2 Annex VIII of Regulation (EU) 2017/746 lays down that:

"Devices intended to be used for blood grouping , or to determine feto-maternal blood group incompatibility or tissue typing to ensure the immunological compatibility of blood, blood components, cells, tissue or organs that are intended for transfusion or transplantation or cell administration, are classified as class C except when intended to determine any of the following markers:

– ABO system [A (ABO1), B (ABO2), AB (ABO3)]

– Rhesus system [RH1 (D), RHW1, RH2 (C), RH3 (E), RH4 (c), RH5 (e)]

– Kell system [KEL1 (K)]

– Kidd system [JK1 (Jka), JK2 (Jkb)]

– Duffy system [FY1 (Fya), FY2 (Fyb)],

in which case they are classified as class D."

The guidance MDCG 2020-16 Rev.2 further mentions the following:

"Rule 2 applies equally to donor and recipient testing. This rule classifies blood grouping devices into two classes depending on the likelihood that a blood group marker could cause an immunogenic response or a severe haemolytic transfusion reaction. The red blood cell markers listed in this rule are critical for ensuring immunological compatibility and safe transfusion of blood and blood components. Devices related to these markers, either intended as screening, diagnostic, confirmatory or supplemental devices are class D devices.
These class D devices includes those intended for:

- *The determination of the expression of ABO and Rhesus (Rh) D, Weak D, C, E, c, e in donor and recipient e.g. by serological testing or molecular genotyping.*
- *The determination of partial D, as these D antigen positive patients are at risk of anti-D alloimmunization.*
- *The detection of anti-A and anti-B antibodies for reverse ABO typing, as ABO blood grouping requires both forward (antigen) and reverse (antibody) typing.*
- *Screening, detection or identification of red cell antibodies for the Rh system (anti RH antibodies), Kell system (anti-KEL1 antibodies), Kidd system (anti-JK1 and anti-JK2 antibodies) and Duffy system (anti-FY1 and anti-FY2 antibodies*
- *Typing of specific red blood cell antigens (KEL1, JK1, JK2 , FY1, FY2).*

Devices intended for identifying markers, other than the red blood cell markers listed in this rule, which are either intended as screening, diagnostic, confirmatory or supplemental devices for blood grouping, tissue typing, or to ensure the immunological compatibility of blood, blood components, cells, tissue or organs that are intended for transfusion or transplantation or cell administration are class C devices.

All devices intended for HLA tissue typing are classified under this rule as class C devices when they are intended to be used for blood grouping, or tissue typing to ensure the immunological compatibility of blood, blood components, cells, tissue or organs that are intended for transfusion or transplantation or cell administration."

4. Relevant medical field and areas of competence required

In vitro diagnostic medical devices, blood and tissue donations, antibodies, antigens, indirect antiglobulin tests.

5. Specific thematic panel or panel sub-group best suited to address the request for advice (if applicable)

In vitro diagnostic medical devices (IVD) panel.

6. Scope of the advice

The MDCG has requested the Expert Panels to address the following questions:

1) In the context of transfusion and foeto-maternal blood grouping incompatibility, what is the clinical use of indirect antiglobulin tests?

2) Can indirect antiglobulin tests be understood as devices which determine any of the following markers: the Rhesus system (RH1(D), RHW1, RH2 (C), RH3 (E), RH4 (c), RH5 (e), Kell system [KEL1 (K)], Kidd system [JK1 (Jka), JK2 (Jkb)], Duffy system (FY1 (Fya), FY2 (Fyb)])?

3) What antibodies are understood to fall under the term "irregular antibodies"?

4) Is it practically possible to have an indirect antiglobulin test that detects only certain antibodies i.e. those markers listed in Q2 or markers other than those listed in Q2? Would such tests have a clinical use?

5) Concerning anti-human globulin placed on the market as a standalone product: What could be the different clinical uses (non-exhaustive list)?

7. Timelines for providing the advice

Start of the advice: 11/10/2023

Advice delivered to the MDCG: 11/12/2023

8. Consultation or collaboration with other scientific bodies for the preparation of the advice (if necessary)

N/A

9. Complexity of the task (according to the criteria established in Table 2 of the Commission Implementing Decision (EU) 2019/1396 Annex)

☑ Category I — simple matter

☐ Category II — complex matter

☐ Category III — very complex matter

10. Advice provided by the IVD Expert Panel

10.1. In the context of transfusion and foeto-maternal blood grouping incompatibility, what is the clinical use of indirect antiglobulin tests?

The direct antiglobulin test (DAT) and the indirect antiglobulin test (IAT) are two forms of the antiglobulin test. The IAT, also known as the indirect Coombs test, is a vital immunohaematological assay used in the context of transfusion and fetal-maternal blood grouping incompatibility. It plays a critical role in identifying and managing conditions such as blood transfusion reactions and haemolytic disease of the newborn (HDN). This test detects the presence of antibodies in a patient's serum that can react with specific red blood cell (RBC) antigens. The IAT can be performed with both plasma and serum; today, plasma is usually used.

The test is crucial for the detection of so-called incomplete antibodies, i.e., immunoglobulins of the IgG type, which cannot bridge the gap between two erythrocytes and do not cause agglutination without antiglobulin serum. Depending on the intended clinical (diagnostic) use, it may be an antibody screening test, a serological tolerance test, or further tests to determine antibody specificity. The test's purpose depends on the question being asked, not on the test method. Therefore, it is normally not possible to request an "indirect antiglobulin test" in the laboratory. The parameter tested is "antibodies against erythrocyte antigens in the patient's plasma".

Clinical use of IAT in Transfusion Medicine:

- Compatibility Testing: IAT is used in pre-transfusion testing to ensure compatibility between the recipient's serum and the donor's RBCs. When a patient requires a blood transfusion, IAT helps identify any unexpected antibodies in the recipient's serum that may react with donor RBC antigens. This is crucial for preventing haemolytic transfusion reactions, which can be life-threatening. The IAT helps ensure safe and compatible transfusions, as described in various clinical guidelines and textbooks on blood banking and transfusion medicine.
- Crossmatching: The IAT is an essential component of the crossmatch procedure. In this process, the recipient's serum is mixed with donor RBCs to verify that no unexpected antibodies are present that could lead to RBC destruction upon transfusion. The IAT helps confirm the absence of such antibodies, ensuring a safe crossmatch.

Clinical use of IAT in Fetal-Maternal Blood Grouping Incompatibility:

- RhD Incompatibility: One of the most well-known applications of IAT in fetal-maternal blood grouping incompatibility is the detection of RhD incompatibility between a RhD-negative mother and a RhD-positive fetus. This condition can lead to haemolytic disease of the newborn (HDN), which can be prevented through appropriate monitoring and interventions. IAT is used to screen the mother's serum for the presence of anti-RhD antibodies that may cross the placenta and harm the fetus. Early detection of these antibodies allows for proper management to prevent HDN, as outlined in obstetrics and gynaecology textbooks.
- Other Blood Group Incompatibilities: In addition to RhD incompatibility, IAT is used to detect other blood group incompatibilities such as ABO and other RBC antigen disparities between the mother and fetus. Identifying maternal antibodies with IAT can help healthcare providers assess the risk of haemolysis in the newborn and take appropriate measures to manage and prevent complications.

The cited resources [1-3] provide comprehensive information on the clinical use of the indirect antiglobulin test in the context of transfusion medicine and fetal-maternal blood grouping incompatibility.

10.2. Can indirect antiglobulin tests be understood as devices which determine any of the following markers: the Rhesus system (RH1(D), RHW1, RH2 (C), RH3 (E), RH4 (c), RH5 (e), Kell system [KEL1 (K)], Kidd system [JK1 (Jka), JK2 (Jkb)], Duffy system (FY1 (Fya), FY2 (Fyb)])?

The indirect antiglobulin test (IAT) is a laboratory technique that detects antibodies in a person's serum or plasma against specific antigens on RBCs. It is a widely used method in immunohematology for assessing blood group compatibility, including markers from various blood group systems. Antibodies against the markers/antigens mentioned (Rhesus system, Kell system, Kidd system, and Duffy system) are indeed among those that can be detected using the IAT.

- Rhesus (Rh) System: The Rh system includes RhD (D), RhC (C), RhE (E), Rhc (c), and Rhe (e) antigens. The indirect antiglobulin test is used to detect the presence of antibodies against these antigens in serum. This is particularly important in cases of Rh incompatibility between the mother and fetus during pregnancy [4].
- Kell System: The Kell system includes the K (KEL1) antigen. Antibodies against the K antigen can be detected using the indirect antiglobulin test. These antibodies can cause haemolytic transfusion reactions and haemolytic disease of the newborn [3].
- Kidd System: The Kidd system includes the Jka (JK1) and Jkb (JK2) antigens. The presence of anti-Jka or anti-Jkb antibodies can be determined using the indirect antiglobulin test. These antibodies can cause haemolytic transfusion reactions [5].
- Duffy System: The Duffy system includes Fya (FY1) and Fyb (FY2) antigens. The indirect antiglobulin test can be used to detect anti-Fya and anti-Fyb antibodies. This system is significant in blood transfusion and transplantation medicine [6].

The cited resources [3-6] provide scientific evidence and literature support for the use of the indirect antiglobulin test in detecting antibodies against various blood group markers, including those from the Rhesus, Kell, Kidd, and Duffy systems. The IAT is a crucial tool in blood banking and transfusion medicine for ensuring blood compatibility and preventing adverse transfusion reactions.

10.3. What antibodies are understood to fall under the term "irregular antibodies"?

"Irregular antibodies," also known as "unexpected antibodies" or "unexpected red cell antibodies," refer to antibodies against red blood cell (RBC) antigens that are not commonly encountered in the general population (in contrast to isoagglutinins). Irregular antibodies are typically formed in response to exposure to non-self RBC antigens, either through transfusion, pregnancy or transplantation. These antibodies can cause complications during blood transfusions, pregnancy and transplantation, so they need to be carefully identified and managed. These antibodies may be directed against less common RBC antigens or may be due to unique antigenic variants or mutations. Irregular antibodies can pose challenges in blood transfusion and compatibility testing because they are not routinely screened for and may lead to complications if not detected and managed appropriately.

Irregular antibodies can target various RBC antigens, and the specific antibodies and their prevalence can vary regionally based on the ancestry makeup of the population. Some of the well-known irregular antibodies include:

- Antibodies to High-Incidence Antigens: High-incidence antigens are RBC antigens that occur in more than 99% of the population. Antibodies against these antigens are considered irregular. For example, the Kell (K), Duffy (Fya, Fyb, Fy3), Kidd (Jka, Jkb), and MNS (M, N, S, s, U) blood group systems include high-incidence antigens.

- Antibodies to Low-Incidence Antigens: Some RBC antigens are rare (less than 1% of the population), and antibodies against these antigens are also considered irregular. Examples include antibodies to the Lutheran (Lua, Lub) and Diego (Di a, Di b) blood group systems.
- Antibodies to Variant Antigens: Irregular antibodies can also develop against variant RBC antigens, which are due to genetic mutations or changes in the structure of common antigens. For example, the anti-Dib antibody is directed against a variant form of the RhD antigen (Dib) and is considered an irregular antibody.
- Autoimmune Antibodies: Some irregular antibodies are autoimmune in nature, meaning they are directed against a person's own RBC antigens. Autoimmune antibodies can be irregular and may lead to autoimmune haemolytic anaemia.

In terms of blood group systems affected:

- Rhesus (Rh) system: Antibodies against Rh antigens, such as anti-C, anti-D, anti-E, anti-c, anti-e, etc.
- Kell system: Antibodies against Kell antigens, such as anti-K, anti-k, etc.
- Kidd system: Antibodies against Kidd antigens, such as anti-Jka and anti-Jkb.
- Duffy system: Antibodies against Duffy antigens, such as anti-Fya and anti-Fyb.
- Lewis system: Antibodies against Lewis antigens, such as anti-Lea and anti-Leb.
- MNSs system: Antibodies against MNSs antigens, such as anti-M, anti-N, anti-S, and anti-s.
- P1PK system: Antibodies against P1PK antigens, such as anti-P1 and anti-P.
- Lutheran system: Antibodies against Lutheran antigens, such as anti-Lua and anti-Lub.
- Diego system: Antibodies against Diego antigens, such as anti-Dia and anti-Dib.
- Duffy and Kell combination: Antibodies targeting both Duffy and Kell antigens, which can be especially challenging.

These irregular antibodies are typically identified through laboratory testing, including techniques like the IAT, and their presence can significantly affect the compatibility of blood for transfusion and the management of maternal-fetal blood group incompatibility during pregnancy. Blood banks and clinical laboratories carefully screen for irregular antibodies in patients and donors to ensure safe and compatible transfusions and to manage pregnancies where there is a risk of haemolytic disease of the newborn (HDN) due to maternal antibodies.

Literature references [1-3, 7] for the concept of irregular antibodies can be found in textbooks and scientific publications related to transfusion medicine and blood banking.

10.4. Is it practically possible to have an indirect antiglobulin test that detects only certain antibodies i.e. those markers listed in Q2 or markers other than those listed in Q2? Would such tests have a clinical use?

It is agreed that it is possible in practice to design and perform indirect antiglobulin tests (IATs) that specifically detect antibodies against certain blood group markers or antigens. In the field of transfusion medicine and blood banking, IATs are routinely used to target a wide range of blood group antigens, including those mentioned in previous question (e.g., Rh, Kell, Kidd, Duffy, etc.).

These panels are composed of reagent RBCs that express specific antigens, and the IAT is performed by mixing the anti-human globulin with these reagent RBCs after primary incubation of the reagent

RBCs with the patient serum. If the patient's serum contains antibodies that react with the antigens present on the reagent RBCs, agglutination occurs, indicating the presence of specific antibodies.

The clinical use can be as follows:

- Transfusion Medicine: Specific antibody screening panels are essential for ensuring the compatibility of blood transfusions. For example, when a patient requires a blood transfusion, it's crucial to screen for any antibodies in their serum that may react with the antigens on the donor's RBCs to prevent adverse transfusion reactions. This is done by using specific antibody screening panels targeting various blood group antigens.
- Prenatal Testing: In the case of maternal-fetal blood group incompatibility, specific antibody screening panels can be used to detect antibodies in the mother's serum that may pose a risk to the fetus. This information helps healthcare professionals manage and monitor pregnancies that are at risk for haemolytic disease of the newborn (HDN).

There are various commercially available antibody screening and identification panels that target specific blood group antigens. These panels are developed and validated based on the current scientific knowledge and the clinical need for accurate and specific antibody detection.

The cited resources [1,7,8] can provide detailed information on the design and clinical use of specific antibody screening and identification panels in the context of transfusion medicine and blood group antigen testing.

10.5. Concerning anti-human globulin placed on the market as a standalone product: What could be the different clinical uses (non-exhaustive list)?

Anti-human globulin (AHG), mostly known as Coombs's reagent, is a critical component in various clinical and laboratory procedures, particularly in the field of immunohematology. AHG is used to detect and enhance the agglutination reactions between antibodies and especially red blood cells (RBCs) or/and enhances complement dependent reactions in laboratory testing. Here are some of the key clinical uses of AHG:

1. Red blood cell (RBC) related:

- Detection of Incomplete Antibody-Mediated haemolysis: AHG is used to detect and confirm the presence of incomplete antibody-mediated haemolysis, where antibodies have coated RBCs but haven't caused visible agglutination in the direct antiglobulin test (DAT). The use of AHG helps to enhance the sensitivity of RBC- bound antibody detection, particularly in cases of autoimmune haemolytic anaemia or drug-induced haemolytic anaemia.[9]
- Compatibility Testing for Blood Transfusions: AHG is an essential component in crossmatching and compatibility testing to ensure safe blood transfusions. It is used in the antibody screening process to detect unexpected antibodies in a patient's serum, which may react with donor RBCs (indirect antiglobulin test).[1]
- Diagnosis and Monitoring of Haemolytic Disease of the Newborn (HDN): In cases of maternal-fetal blood group incompatibility, AHG is used to assess the presence and level of maternal antibodies in the fetal circulation during pregnancy (IAT), helping in the management of HDN.[10]
- Antigen Typing: AHG can be used to detect weakly expressed RBC antigens, such as weak D (Du) in the RhD system or weak Kell antigens, by enhancing the agglutination reactions.[11]

- Preventing Haemolytic Transfusion Reactions: AHG testing is essential to identify and prevent haemolytic transfusion reactions due to incompatible donor RBCs and recipient antibodies.[3]
- Investigation of Autoimmune Haemolytic Anaemia: AHG is used to confirm the presence of autoantibodies that may be responsible for autoimmune haemolytic anaemia, particularly in warm antibody hemolysis.[12]
- Investigation of Drug-Induced Haemolytic Anaemia: In cases of suspected drug-induced haemolytic anaemia, AHG can be used to detect drug-dependent antibodies that may be responsible for RBC destruction.[13]
- Investigation of Paroxysmal Nocturnal Haemoglobinuria (PNH): AHG testing can be used in the diagnosis and monitoring of PNH to detect complement-mediated haemolysis and the absence of certain RBC surface proteins.[14]

2. Non-RBC related:

- Transplantation and Tissue Typing: AHG can be utilized in solid organ transplantation and tissue typing to detect the presence of anti-human leukocyte antigen (HLA) antibodies, aiding in the assessment of donor-recipient compatibility.[15]

These clinical uses highlight the significance of AHG in immunohematology and transfusion medicine for detecting antibodies, ensuring blood transfusion compatibility, and diagnosing and managing various haemolytic conditions.

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