HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER
MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank		
Document:	Internal Policy and Procedure		
Title:	Column Technology and the Gel Micro Typing System		
Applies To:	All Blood Bank Staff		
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1. PURPOSE:

1.1 To demonstrate the column technology using gel micro typing system and its application.
1.2 To ensure the reliability and reproducibility of the gel micro typing system test results.
1.3 To standardize reading, grading and recording of gel reactions.
1.4 To determine the correct ABO group of an individual and ensure the reliability of the result.
1.5 To detect unexpected antibodies to red cell antigens.
1.6 To perform identification and titration of unexpected antibodies to red cell antigens.
1.7 To determine the RBC antigens of an individual in special situations and to ensure the reliability of the result.
1.8 To demonstrate in vivo coating of red cells with globulins, in particular IgG and C3d.
1.9 To detect the presence of blood group antibodies in an intended recipient's plasma/serum directed towards antigens present on donor's red blood cells.

2. DEFINITONS:

2.1 MTS: micro typing system. It's a registered mark for Micro Typing Systems®, Inc. a division of Ortho-Clinical Diagnostics, Inc.

3. POLICY:

3.1 A standardised procedure to perform, interpret, and report gel reactions will contribute to uniformity and reproducibility of test results.
3.2 Manufacturer's directions should always be followed.

4. PROCEDURE:

4.1 **Principle:**
- 4.1.1 A specific red blood cell solution is added to the gel contained in a special micro tube. The gel acts as a trap, the free red blood cells pellet in the bottom of the tube while agglutinates are trapped (fixed) in the top of the gel.
- 4.1.2 The whole procedure can be done manually or through an automated system.
  - 4.1.2.1 Manual method: using either "DiaMed-ID system®" Biorad work station or "ortho™Workstation" as an alternative.
  - 4.1.2.2 Automated method: using "ortho vision® Analyser".
  - 4.1.2.3 Reagents availability is a determining factor in method selection.
- 4.1.3 Strict adherence to procedures and recommended equipment is essential.

4.2 **Different Gel tests;** There are 3 types of Gel:
- 4.2.1 Neutral Gels:
  - 4.2.1.1 No specific antiserum used e.g. the ID card "NaCl, Enzyme test and Cold Agglutinins".
  - 4.2.1.2 Mode of Action: gel traps the agglutinates during centrifugation.
  - 4.2.1.3 Types of tests: Reverse ABO grouping, antibody screening and identification by saline and enzyme technique, compatibility tests.

4.2.2 Specific Gels:
- 4.2.2.1 Gel plus anti-sera e.g. anti-A, anti-B, anti-AB, Anti-D, anti-C, anti- K....etc.
- 4.2.2.2 Types of tests: For antigen determination e.g. ABO, Rh, Kell blood group.

4.2.3 Antiglobulin Gels:
- 4.2.3.1 Gel plus mono or polyspecific antiglobulin e.g. anti- IgG+C3d
- 4.2.3.2 Types of tests: Compatibility testing, Antibody screening, Antibody identification by Coombs technique.

4.3 Sample Material:

4.3.1 Anticoagulated samples drawn into citrate, EDTA, or CPD-A allow ideal access to plasma and cells and provide the best productivity and ease of use in the ID system. Plasma is not detrimental to the quality of antibody detection.

4.3.2 Samples drawn into plain tubes (no anticoagulant) may also be used.

4.3.3 When the use of serum instead of plasma is required, the serum must be well cleared, by centrifugation at 1,500 g for 10 minutes, before use to avoid fibrin residues, which may interfere with the reaction pattern.

4.3.4 Freshly drawn sample is preferred.

4.3.5 No special preparation of the patient is required prior to sample collection.

4.4 Preparation of blood sample:

4.4.1 Cell suspension:
- 4.4.1.1 It is best to prepare cell suspensions from cell concentrates (packed cells) to maintain greater consistency. The use of whole blood to prepare cell suspensions will be more variable as the PCV differs between blood samples.
- 4.4.1.2 Allow the diluent to reach room temperature before use (except for cold agglutinins). Prepare a 0.8 - 1 % suspension of red cells as follows:-
  - 4.4.1.2.1 10 ul red cell concentrate + 1.0 ml ID-Diluent 2 or
  - 4.4.1.2.2 20 ul whole blood of blood bag segment + 1.0 ml ID-Diluent 2
- 4.4.1.3 Mix well.

4.4.2 Plasma or serum:
- 4.4.2.1 After separation, plasma or serum should be stored at 2-8 °C for a maximum of 48 hours, thereafter at -20 °C.
- 4.4.2.2 Allow sample to reach room temperature before use (except for cold agglutinins).

4.5 Pipetting:

4.5.1 Pipetting technique is important to get optimal results from the ID system.

4.5.2 Cells should be added first, using the pipette held at an acute angle. Allow the tip to rest on the edge of the well and dispense the solution into one side of the cup. The fluid should run down into the cup and form a meniscus over the micro-tube to create a 'bubble' between the cells and the test gel.

4.5.3 Secondly, pipette the test sample (plasma or serum) using the pipette held upright, directly above the cells and slightly to one side of the cupule. Pipetting directly onto the gel surface or into the cell suspension may lead to false results.

4.6 Interpretation:

4.6.1 Positive: agglutinated cells form a red line at the top of the gel or agglutinates dispersed within the gel matrix.

4.6.2 Negative: A compact well delineated button of cells at the bottom of the micro tube.

4.7 Reading and Recording for Gel Technique:

4.7.1 Procedure:
- 4.7.1.1 After centrifugation, remove the cards from the centrifuge and observe each card for the following signs of improper centrifugation.
  - 4.7.1.1.1 Unagglutinated red cells observed in the gel are usually caused by an interrupted centrifugation cycle.
  - 4.7.1.1.2 A line of red cells streaming down one side and forming a "J" appearance is caused by improperly seated card in the card holders.
  - 4.7.1.1.3 If the cards show a sign of improper centrifugation, repeat the test. Do not re-centrifuge the cards (sometimes, re-centrifugation may be needed with some reagent patches).

4.7.1.2 Observe both the front and back of each microtube in the gel card.
4.7.1.3 Read Macroscopically.
4.7.1.4 Grade the reactions.

4.7.2 Grade the reactions;

4.7.2.1 Refer to the interpretation guide (e.g. ID-System Quick Guide) for diagram showing ranges of reactions.

4.7.2.2 Record reactions as described below.

Well reaction grade (Record)	Reaction Description
0 or Negative	Unagglutinated red cells forming a well-delineated compact pellet in the bottom of the microtube. See procedural note 4.7.2.3.1, if a few unagglutinated cells are trapped at the top or sides of the gel.
+/- (weak)	Unagglutinated red cells forming a well-delineated compact pellet in the bottom of the microtube. See procedural note 4.7.2.3.1, if a few unagglutinated cells are trapped at the top or sides of the gel.
1+	Agglutinates predominantly observed in the lower half of the microtube. Unagglutinated red cells form a pellet in the bottom of the microtube.
1+	Agglutinates dispersed throughout the length of the gel column. A few agglutinates may be observed in the bottom of the microtube. See procedural note 4.7.2.7.2
3+	Majority of agglutinates trapped in the upper half of the microtube. See procedural note 4.7.2.7.3, 4.
4+	A solid band of red cell agglutinates on top of the gel. A few agglutinates may filter into the gel, but remain near the predominant band. See procedural note 4.7.2.7.5.
Mixed field	A band of red cell agglutinates on top of the gel, accompanied by a pellet of agglutinated cells in the bottom of microtube. See procedural note 4.7.2.7.6.

4.7.2.3 Do not use half grade, superscript or "plus signs" (i.e. +, ++, +++, or ++++).

4.7.2.4 See procedural note 4.7.2.7 if the reaction in the microtube is not described in the table above.

4.7.2.5 No agglutination or hemolysis of the red cells is a negative test result.

4.7.2.6 Agglutination or hemolysis of the red cells is a positive test result.

4.7.2.7 Procedural notes:

4.7.2.7.1 Debris, fibrin or other artifacts associated with serum or frozen specimens may cause a few unagglutinated cells to be trapped on the top or sides of the gel. These tests should be interpreted as negative. Plasma or serum specimens previously frozen should be centrifuged prior to use.

4.7.2.7.2 When interpreting 2+ reactions, consider the upper and lower position of agglutinated red cells in the gel. Size of the red cell pellets in the bottom of the microtube may vary.

4.7.2.7.3 A 3+ reaction appears as a thick group of agglutinates or band, with some red cells dispersed below the predominant band in the upper half of the gel column.

4.7.2.7.4 A 3+ reaction may also be characterized by an even distribution of agglutinates in the upper portion of the gel. Occasionally, a few unsensitized cells may migrate to the bottom of the microtube.

4.7.2.7.5 A strong 4+ agglutinations form a band of agglutinates and become trapped on or near the top of the gel. Occasionally a few unsensitized red cells may migrate to the bottom of the tube, but the middle of the gel should remain free from agglutination.

4.7.2.7.6 Consider the following when interpreting a reaction was mixed field:

4.7.2.7.6.1 The clinical history of the recipient and type of testing performed should be considered (e.g., mixed field reactions are usually not considered when performing an antibody screen because the cells are not pooled.

4.7.2.7.6.2 Strong cold agglutinins may give a mixed field appearance. These reactions are not truly mixed field and should be interpreted as positive.

4.7.2.7.7 Consider the following trouble shouting tips when reactions in gel microtubes are difficult to grade;

4.7.2.7.7.1 Rouleaux is a property of test serum resulting in a characteristic pattern of red cell aggregation. It can occur if sufficient quantities of abnormal proteins are present in the test sample and may infrequently cause difficulties in gel test interpretation. Rouleaux shall be confirmed using tube hemaglutination methods and saline replacement performed when necessary.

4.7.2.7.7.2 Too few or too many cells in the microtube may cause false positive or false negative reactions. They may be due to one or both of the following errors:

4.7.2.7.7.2.1 Improperly prepared cell suspension.

4.7.2.7.7.2.2 Adding the incorrect quantities of cells to the upper chamber.

4.7.2.7.7.3 In this case, repeat the test(s) ensuring correct quantities using new cell suspensions.

4.7.2.7.7.4 Insufficient centrifugation and/or centrifugation when the cards were not properly seated in the centrifuge holders may cause one or more of the following reactions:

4.7.2.7.7.4.1 A line of cells streaming down one side of the microtube.

4.7.2.7.7.4.2 The red cell pellet shifted from the bottom of the microtube.

4.7.2.7.7.4.3 Unagglutinated cells observed throughout the gel (appearing pink or hazy) in all microtubes on the card.

4.7.2.7.7.5 In this case, repeat the test(s) ensuring correct placement of the cards and centrifugation time.

4.8 **Notes:**

4.8.1 Test cell reagents for antibody screening containing pooled cells can show a double cell population appearance depending on the antibody present. This is considered to be a positive result.

4.8.2 The cards must be centrifuged for exactly 10 minutes at about 70g. False-negative results are obtained if centrifugation is too long or fast. False-positive results are obtained if centrifugation is too slow or short.

4.8.3 Cold Antibodies:

4.8.3.1 Cold antibodies may be enhanced by incubating the ID card at or below room temperature and the detection of many antibodies, such as Lewis, HI, may also be optimized by using enzyme techniques. (MNS and Duffy antigens are damaged by enzymes).

4.8.3.2 For ID tests at 4°C for cold agglutinins, special care is needed: before use place the ID cards at 4°C for 2 hours and use similarly cooled reagents and samples .

4.9 **Limitations:**

4.9.1 Bacterial and other contaminants of materials used can cause false positive and negative reactions. Do not use reagents which have become turbid or show precipitates.

4.9.2 Fibrin residues in the red cell suspension may trap non-agglutinated cells causing a fine pink line "red-line" on the top of the gel while most are seen at the bottom of the microtube after centrifugation.

4.9.3 Use of other test cells reagents, suspension solutions for red cells or other test sera other than recommended by the manufacturer may modify the reactions patterns.

4.9.4 Too heavy red cell suspensions can cause false positive or false negative results.

4.9.5 The ABD confirmation tests do not replace a complete ABO/Rh type determination. It should only be used as a confirmatory test of previously determined ABO/Rh type. ABO/Rh and reverse typing is validated by a negative control "**ctl**" test.

4.9.6 Do not use ID-Cards which show signs of drying, have bubbles, damaged seals, drops of gel or supernatant in the upper part of the microtubes or on the underside of the aluminium foil.

4.9.7 **Caution:** The source materials, from which these products were manufactured, were found non-reactive for HBs Ag, HCV and HIV (1+2) when tested with licensed reagents. However, no known test method can assure that infectious agents are absent. Products from human blood should be considered potentially infectious.

4.9.8 Polyagglutinable cells may react with all human antisera. Further investigation of such reactions is required.

4.9.9 Certain drugs are known to cause positive Coombs tests .

4.9.10 Some pathological conditions are also reported to cause positive Coombs tests

4.10 **Storage of the ID Cards:**

4.10.1 The cards should be stored in the correct way as recommended by the manufacturer.

4.10.2 The reagent gel is stable until the date of expiry.

4.10.3 Centrifugation of the cards before using may be needed to pack unsettled contents.

4.11 **Test cell reagents for the id-system:**

4.11.1 Introduction And Principles:

4.11.1.1 The reliability of antibody detection is largely dependent on the availability of test cells with appropriate antigens and on the sensitivity of the test methods used.

4.11.1.2 The requirements for antigen configuration are stringent: it must allow the safe detection of all clinically significant antibodies.

4.11.1.3 For the Rh system, MNSs, Duffy and Kidd, the antigens must be in homozygous form. The Lewis antigens must be present, as should the rare antigen Kpa.

4.11.1.4 It is generally considered most effective to perform screening tests by both anti-human globulin (AHG) and enzyme test procedures. Due to higher sensitivity of the indirect antiglobulin test (IAT) with procedures such as the ID-System, the enzyme test has become somewhat less important.

4.11.1.5 However, enzyme techniques are useful when increased sensitivity in antibody screening is desired or where more than one antibody may be present. They enhance the reactions of certain antibodies, notably in the Rh, Kell and Kidd systems, whereas antibodies to enzyme-sensitive antigens may not be detected, notably in the Duffy and MNS Systems.

4.11.2 Reagents: All test cell reagents are of human origin

4.11.2.1 For antibody screening, single donors, blood group O:

4.11.2.1.1 ID-DiaCell I-II-III.

4.11.2.2 For antibody identification, single donors, blood group O:

4.11.2.2.1 ID-DiaPanel 11 test cells for IAT and NaCl test

4.11.3 Further Materials Required:

4.11.3.1 ID- Dispenser

4.11.3.2 ID- Pipetor

4.11.3.3 ID- Tips (pipetor tips)

4.11.3.4 ID- Working Table

4.11.3.5 Suspension Tubes

4.11.3.6 ID- Incubator 37 °C

4.11.3.7 ID- Centrifuge

4.11.4 Use Of The Id-Test Cell Reagents:

4.11.4.1 All test cell reagents are for use with the ID-Cards of the ID-System only.

4.11.4.2 Strictly follow the test procedures as described in the specific package inserts of the ID-Cards to be used.
4.11.4.3 Always gently resuspend the red cells, by inverting the vial several times before use and also before placing the vials into a pipetting automate.
4.11.4.4 Make sure that the test cells are at room temperature (18-25 °C) when in use.
4.11.4.5 During the working procedures check that the test cell reagents remain in suspension. If there is settling of the cells, resuspend again.
4.11.4.6 When recording the reactions, ensure that the lot number of the antigen table corresponds with the lot number of the reagent vials.
4.11.4.7 After use, close the vials and place them in the refrigerator.

4.12 **LISS/Coombs ID-Card:** (Indirect and direct antiglobulin test):

4.12.1 Introduction:

4.12.1.1 Polyspecific anti-human globulin (AHG) reagents are used for routine alloantibody detection and identification, compatibility tests and the direct antiglobulin test (DAT).
4.12.1.2 The most important function of the polyspecific AHG reagent is to detect the presence of IgG. The importance of anti-complement in the AHG reagent is debatable since antibodies detectable only by their ability to bind complement are rather rare. However, anti-C3d activity is important for the DAT in the investigation of autoimmune haemolytic anaemia (AIHA).
4.12.1.3 A positive DAT generally indicates that the red cells are coated in vivo with immunoglobulin and/or complement.
4.12.1.4 The microtubes of the ID-Card 'LISS/Coombs" contain polyspecific AHG, to be used for antibody screening, antibody identification, crossmatch and the DAT.
4.12.1.5 For the indirect antiglobulin test (IAT), labour intensive washing procedures are eliminated, due to the fact that the red cell suspension is added to the microtube before the plasma/serum, creating a barrier over the gel suspension, thus avoiding neutralization of the AHG by serum IgG proteins.
4.12.1.6 The anti-human globulin IgG used in the ID-Card 'LISS/Coombs" is not heavy chain specific and thus may also be capable of reacting with the Kappa (k) and Lambda (l) light chains of IgA and IgM molecules.
4.12.1.7 The ID-Card 'LISS/Coombs" is suitable for the DAT, for the compatibility test, for antibody screening and identification with "ID-DiaCell and 'ID-DiaPanel".

4.12.2 Reagents:

4.12.2.1 ID-Card 'LISS/Coombs" with 6 microtubes containing polyspecific AHG (rabbit anti-IgG and monoclonal anti-C3d) within the gel matrix.

4.12.3 Additional Reagents Required:

4.12.3.1 ID-Diluent 2; modified LISS for red cell suspension.
4.12.3.2 ID-DiaCell, ID-DiaPanel: Test cell reagents.

4.12.4 Further Materials Required:

4.12.4.1 ID- Dispenser.
4.12.4.2 ID- Pipetor.
4.12.4.3 ID- Tips (pipetor tips).
4.12.4.4 ID- Working Table.
4.12.4.5 Suspension Tubes.
4.12.4.6 ID- Incubator 37 °C.
4.12.4.7 ID- Centrifuge.

4.12.5 Preparation Of Blood Sample:

4.12.5.1 Red cell suspension for DAT or autocontrol: Prepare a 0.8 - 1 % red cell suspension in ID-Diluent 2 as follows:
4.12.5.1.1 Allow the diluent to reach room temperature before use.
4.12.5.1.2 Dispense 1.0 mL of ID-Diluent 2 into a clean tube.
4.12.5.1.3 Add 10 uL of packed red cells, mix gently.
4.12.5.1.4 The cell suspension may be used immediately.
4.12.5.2 Red cell suspension for crossmatch procedures:
4.12.5.2.1 Prepare a 0.8% red cell suspension in ID-Diluent 2 as above (4.12.5.1).

4.12.5.2.2 Where whole blood directly from a segment of the blood bag is used, add 20 uL of blood to the 1.0 mL of ID-Diluent 2.

4.12.5.3 Plasma or serum for Indirect antiglobulin test (IAT) procedures:

4.12.5.3.1 Where samples are not for immediate testing they should be stored at 2-8 °C after separation for a maximum of 48 hours, thereafter at -20 °C.

4.12.6 Test Procedures:

4.12.6.1 Direct antiglobulin test (DAT):

4.12.6.1.1 Identify the appropriate microtubes of the ID-Card 'LISS/Coombs" with the patient's or donor's name or number.

4.12.6.1.2 Remove the aluminium foil from as many microtubes as required by holding the ID card in the upright position.

4.12.6.1.3 Pipette 50 uL of the red cell suspension to the appropriate microtube.

4.12.6.1.4 Centrifuge the ID-Card for 10 minutes in the ID-Centrifuge.

4.12.6.1.5 Read and record the results.

4.12.6.2 Antibody screening (IAT): Use the ready-to-use test cell reagent "ID-DiaCell". Allow the test cell reagents and samples to reach room temperature before use.

4.12.6.2.1 Identify the appropriate microtubes of the ID-Card 'LISS/Coombs" with the patient's or donor's name or number.

4.12.6.2.2 Remove the aluminium foil from as many microtubes as required by holding the ID card in the upright position.

4.12.6.2.3 Pipette 50 uL of each test cell reagent to the appropriate microtube (marked with the corresponding test cell).

4.12.6.2.4 When an autocontrol is to be included, pipette 50 uL of the sample's own red cell suspension to the appropriate microtube.

4.12.6.2.5 Add 25 uL of the patient's or donor's plasma or serum to each microtube.

4.12.6.2.6 Incubate the ID-Card for 15 minutes at 37 °C in the ID-Incubator.

4.12.6.2.7 Centrifuge the ID-Card for 10 minutes in the ID-Centrifuge.

4.12.6.2.8 Read and record the results.

4.12.6.3 Antibody Identification (IAT): Use the ready-to-use test cell reagent "ID-DiaPanel". Allow the test cell reagents and samples to reach room temperature before use.

4.12.6.3.1 Identify two ID-Card 'LISS/Coombs" with the patient's or donor's name or number.

4.12.6.3.2 Remove the aluminium foil from as many microtubes as required by holding the ID card in the upright position.

4.12.6.3.3 Pipette 50 uL of each ID-DiaPanel test cell to the appropriate microtube (marked 1 to 11).

4.12.6.3.4 Pipette 50 uL of the sample's own red cell suspension to the 12thmicrotube (Autocontrol "AC").

4.12.6.3.5 Add 25 uL of the patient's or donor's plasma or serum to all 12 microtubes.

4.12.6.3.6 Incubate the ID-Card for 15 minutes at 37 °C in the ID-Incubator.

4.12.6.3.7 Centrifuge the ID-Card for 10 minutes in the ID-Centrifuge.

4.12.6.3.8 Read and record the results.

4.12.6.4 Compatibility test:

4.12.6.4.1 Identify the appropriate microtubes of the ID-Card 'LISS/Coombs" with the patient's and donor's name or number.

4.12.6.4.2 Remove the aluminium foil from as many microtubes as required by holding the ID card in the upright position.

4.12.6.4.3 Pipette 50 uL of the donor red cell suspensions to the appropriate microtubes.

4.12.6.4.4 For the autocontrol, Pipette 50 uL of the patient's own red cell suspension to the appropriate microtube.

4.12.6.4.5 Add 25 uL of the patient's plasma or serum to each microtubes.

4.12.6.4.6 Incubate the ID-Card for 15 minutes at 37 °C in the ID-Incubator.

4.12.6.4.7 Centrifuge the ID-Card for 10 minutes in the ID-Centrifuge.

4.12.6.4.8 Read and record the results.

4.12.7 Interpretation Of The Results:

4.12.7.1 Direct antiglobulin test (DAT) (see DCT policy LB-IPP-200):

4.12.7.1.1 A negative reaction indicates absence of detectable IgG antibodies or C3d complement component on the red cells.

4.12.7.1.2 A positive reaction (± to 4+) indicates that the patient's red cells are sensitized (red cells coated With IgG antibodies and/or C3d).

4.12.7.2 Antibody screening: (see "Antibody Screening, Identification, And Titration" policy LB-IPP-199)

4.12.7.2.1 A negative reaction indicates the absence of detectable irregular antibodies in the patient's or donor's serum or plasma.

4.12.7.2.2 A positive reaction (± to 4+) indicates the presence of irregular antibodies. Enter the reactions obtained on the antigen table. Verify that the lot number of the 3 test cell reagents 'ID-DiaCell' corresponds to the lot number indicated on the antigen table.

4.12.7.2.3 Following the reaction pattern and the antigen configuration, the type of antibody present may be indicated. Perform the usual further tests to identify the antibody (upon availability).

4.12.7.2.4 A positive reaction with one or more test cells and a negative autocontrol suggest the presence of a specific antibody.

4.12.7.2.5 When there is a positive reaction with all test cells and a positive autocontrol but with one or more test cells showing a stronger positive reaction than the autocontrol, the patient sample should be submitted for further testing, to investigate the possibility of an underlying allo-antibody.

4.12.7.3 Antibody Identification: ((see "Antibody Screening, Identification, And Titration" policy)

4.12.7.3.1 A positive reaction indicates the presence of irregular antibodies. Enter the reactions obtained on the antigen table. Verify that the lot number of the test cell reagents 'ID-DiaPanel' corresponds to the lot number indicated on the antigen table.

4.12.7.3.2 Following the reaction pattern and the antigen configuration, the type of antibody present can, in most cases, be identified (autocontrol must be negative).

4.12.7.3.3 A positive reaction with all 'ID-DiaPanel" test cells and a negative autocontrol may be due to non-specific reactions or may indicate the presence of an alloantibody directed against a high frequency antigen.

4.12.7.3.4 A positive reaction with all 'ID-DiaPanel' test cells and the autocontrol but with one or more test cells showing stronger reactions than the autocontrol, may indicate an underlying allo-antibody and further investigation should be undertaken.

4.12.7.4 Compatibility test (see "Cross Matching Techniques: policy LB-IPP-205)

4.12.7.4.1 A negative reaction indicates compatibility of the donor blood with the recipient.

4.12.7.4.2 A positive reaction (± to 4+) indicates incompatibility of the donor blood with the recipient, due to presence of antibodies directed against antigens on the donor red cells. Further investigation to identify the antibody specificity should be performed.

4.13 **DiaClon ABO/D& ortho gel cards + reverse grouping:**
(Determination of the ABO/ Rh blood groups combined with reverse grouping)

4.13.1 Introduction:

4.13.1.1 ABO blood group typing, using anti-A and anti-B test sera, is known as direct or forward grouping test.

4.13.1.2 Reverse grouping uses red cell reagents of known ABO antigen specificity to indicate the presence or absence of anti-A and anti-B isoagglutinins, the results of which determine the reverse group.

4.13.1.3 Discrepancies between forward and reverse grouping require further investigation.

4.13.1.4 Classification of blood groups must be based on both forward and reverse grouping.
4.13.1.5 The ID-Card 'DiaClon ABO/D + Reverse Grouping" allows combined testing of forward and reverse grouping as well as RhD determination.

4.13.2 Reagents:
4.13.2.1 ID-Card "DiaClon ABO/D **or ortho gel cards** + Reverse Grouping" contains monoclonal anti-A, anti-B and anti-D within the gel matrix.
4.13.2.2 The microtube ctl is the negative control.
4.13.2.3 Two microtubes with "neutral gel" serve for reverse grouping with A1 and B cells.
4.13.2.4 The anti-B of monoclonal origin does not react with the acquired B antigen.
4.13.2.5 The anti-D used has been selected so as not to react with DVI variants.

4.13.3 Additional Reagents Required:
4.13.3.1 ID-Diluent 2: modified LISS for red cell suspensions.
4.13.3.2 Test cell Reagents: ID-DiaCell or ortho A1 and B in a 0.8% suspension, in 10 ml vials, ready-to-use.

4.13.4 Further Materials Required:
4.13.4.1 ID- Dispenser
4.13.4.2 ID- Pipetor
4.13.4.3 ID- Tips (pipetor tips)
4.13.4.4 ID- Working Table
4.13.4.5 Suspension Tubes
4.13.4.6 ID- Incubator 37 °C
4.13.4.7 ID- Centrifuge

4.13.5 Preparation Of Blood Sample:
4.13.5.1 Red cell suspension (for ABO/D determination): Prepare a 5% red cell suspension in ID-Diluent 2 as follows:
4.13.5.1.1 Allow the diluent to reach room temperature before use.
4.13.5.1.2 Dispense 0.5 ml of ID-Diluent 2 into a clean tube.
4.13.5.1.3 Add 50 ul of whole blood or 25 ul of packed cells, mix gently.
4.13.5.1.4 The cell suspension may be used immediately.
4.13.5.2 Plasma or Serum for reverse grouping:
4.13.5.2.1 Where samples are not for immediate testing they should be stored at 2-8 °C after separation for a maximum of 48 hours, thereafter at -20 °C.

4.13.6 Test Procedure:
4.13.6.1 Allow the test cell reagent to reach room temperature before use.
4.13.6.2 Identify the ID-Card with the unique patient or donor number / details as appropriate.
4.13.6.3 Remove the aluminum foil from as many microtubes as required by holding the ID-Card in the upright position.
4.13.6.4 Pipette 50 ul of of ortho or "ID-DiaCell A1" to microtube 5 (A1).
4.13.6.5 Pipette 50 ul of of ortho or 'ID-DiaCell B" to microtube 6 (B).
4.13.6.6 Pipette 50 ul of the patient serum or plasma to both microtubes 5 and 6. Incubation for 10 minutes at room temperature is recommended
4.13.6.7 Pipette 10 or 12.5 ul of the patient's red cell suspension to the microtubes 1-4 (A, B, D, ctl).
4.13.6.8 Centrifuge the ID-Card for 10 minutes in the ID-Centrifuge of biorad or 5 minutes of orthoworkstation . .
4.13.6.9 Read and record the reactions.

4.13.7 Interpretation Of The Results:
4.13.7.1 Reactions for blood groups ABO:
4.13.7.1.1

Anti - A	Anti - B	Blood Group
3+ to 4+	Negative	A
Negative	3+ to 4+	B
3+ to 4+	3+ to 4+	AB
Negative	Negative	O

4.13.7.1.2 Weaker reactions than 3+ may indicate A or B subgroups. For correct interpretation, a complete grouping test should be performed (anti-A, anti-B, anti-AB).

4.13.7.1.3 In the presence of weak or very weakly expressed antigens, the reaction can be negative.

4.13.7.1.4 The microtube ctl must show a negative reaction.

4.13.7.1.5 If the ctl is positive, the ABO determination is not valid and repeat the test.

4.13.7.2 Reactions for reverse grouping:

4.13.7.2.1

A1 cells	B cells	Blood Group
1+ to 4+	Negative	B
Negative	1+ to 4+	A
1+ to 4+	1+ to 4+	O
Negative	Negative	AB

4.13.7.2.2 If questionable reactions are obtained, repeat reverse grouping with 4 red cell reagents (A1, A2, B and O) (upon availability).

4.13.7.3 Reactions for RhD:

4.13.7.3.1

3+ to 4+	± to 2+	Negative
Rh D positive	**Rh D weak** positive	**Rh D negative**

4.13.7.3.2 ±, trace or weak reactions is considered as negative for recipients.

4.13.7.3.3 Weak D may give a negative reaction.

4.13.7.3.4 Do not perform further testing for weak or partial-D in patients.

4.13.7.3.5 The microtube ctl must show a negative reaction.

4.13.7.3.6 If the ctl is positive, the RhD determination is not valid

4.13.8 Remarks:

4.13.8.1 The negative control must always show a negative reaction.

4.13.8.1.1 If the negative control is positive, wash the red cells first in warm isotonic saline solution or ID-Diluent 2, before preparing the red cell suspension. Then proceed as under "Preparation of blood sample" and 'Test procedure". If the negative control subsequently shows a negative result, the reactions can be interpreted.

4.13.8.1.2 If the negative control remains positive, the results of the ABO/Rh determination should be considered invalid and further investigations following recommended techniques should be undertaken to ascertain the reason, before valid antigen typing can be assured.

4.13.8.2 Full forward and reverse grouping requires the use of anti-A, -B, -AB, and A1, A2, B and O cells. The ID-Card "DiaClon ABO/D + Reverse Grouping" does not contain anti-AB and allows the use of A1 and B cells only.

4.13.8.3 For reverse grouping, an incubation of at least 10 minutes at 18-25 °C prior to centrifugation will enhance the reactions and minimize repeat testing due to weak isoagglutinins.

4.14 **DiaClon ABO/Rh and ortho for newborns DVI+:**

(Determination of the ABO/ Rh blood groups with direct antiglobulin test (DAT) for newborns)

4.14.1 Introduction:

4.14.1.1 Anti-A, anti-B and anti-AB test sera are necessary to detect the presence or absence of A/B antigens on human red cells.

4.14.1.2 Since A and B antigens are not fully developed at birth, weaker reactions may occur with red cells of newborns than of adults and subgroups often cannot be identified.

4.14.1.3 The serum of adults contains antibodies directed against the A and B antigens absent from their own red cells. Both antibodies appear after the first 4 to 6 months of life. As a result, reverse grouping is not usually undertaken on newborn blood samples.

4.14.1.4 Confirmation of the newborn's blood group is indicated when the A and B antigen expression is fully developed (2-4 years).

4.14.1.5 The D antigen as well as weak D is fully developed at birth. The determination of the RhD status of the newborn's blood group is important if the mother is RhD negative.

4.14.1.6 A direct antiglobulin test (DAT) on newborn blood samples has become a standard procedure, since it is of importance to know if the newborn's red cells have been coated with maternal antibodies in-utero.

4.14.2 Reagents:

4.14.2.1 ID-Card "DiaClon or ortho ABO/Rh for Newborns DVI+" contains monoclonal anti-A, anti-B, anti-AB and anti-D, within the gel matrix. The microtube (ctl) is the negative control.

4.14.2.2 The anti-human globulin serum is a blend of rabbit anti-IgG and monoclonal anti-C3d.

4.14.2.3 The anti-D test sera were selected so as to react with DVI variants.

4.14.2.4 The anti-B of monoclonal origin does not react with the acquired B antigen.

4.14.3 Additional Reagents Required:

4.14.3.1 ID-Diluent 2: modified LISS for red cell suspensions.

4.14.4 Further Materials Required:

4.14.4.1 ID- Dispenser.

4.14.4.2 ID- Pipetor.

4.14.4.3 ID- Tips (pipetor tips).

4.14.4.4 ID- Working Table.

4.14.4.5 Suspension Tubes.

4.14.4.6 ID- Incubator 37 °C.

4.14.4.7 ID- Centrifuge.

4.14.5 Sample Material:

4.14.5.1 Cord or heel prick samples may be used. It is normally not necessary to wash the cells before use. Where cord samples are used, care must be taken to avoid contamination with Wharton's jelly. Preferably, blood samples should be drawn into Citrate, EDTA, or $CPDA_1$.

4.14.5.2 Prepare a 0.8 - 1 % red cell suspension in ID-Diluent 2 as follows:

4.14.5.2.1 Allow the diluent to reach room temperature before use.

4.14.5.2.2 Dispense 1.0 mL of ID-Diluent 2 into a clean tube.

4.14.5.2.3 Add 10 uL of packed red cells, mix gently.

4.14.5.2.4 The cell suspension may be used immediately.

4.14.6 Test Procedure:

4.16.6.1 Identify the ID-Card with the unique patient or donor number/details as appropriate.

4.16.6.2 Remove the aluminium foil from as many microtubes as required by holding the ID-Card in the upright position.

4.16.6.3 Pipette 50 ul of the red cell suspension to all 6 microtubes of the ID-Card.

4.16.6.4 Centrifuge the ID-Card for 10 minutes in the ID-Centrifuge of biorad and 5 minutes for orthoworkstation..

4.16.6.5 Read and record the results.

4.14.7 Interpretation Of The Results:

4.14.7.1 Reactions for blood groups ABO:

4.14.7.1.1

Anti - A	Anti - B	Anti - AB	Blood Group
1+ to 4+	Negative	1+ to 4+	A
Negative	1+ to 4+	1+ to 4+	B
1+ to 4+	1+ to 4+	1+ to 4+	AB
Negative	Negative	Negative	O

4.14.7.1.2 In the presence of weak or very weakly expressed antigens the reaction can be negative.

4.14.7.1.3 Important: The microtube ctl must show a negative reaction. If the ctl is positive, the ABO determination is not valid. Repeat the test as described under Remarks (4.14.8.1).

4.14.7.2 Reactions for RhD:

4.14.7.2.1

3+ to 4+	± to 2+	Negative
Rh D positive	**Rh D weak** positive	**Rh D negative**

4.13.7.3.1 A negative reaction indicates the absence of detectable antibodies on the newborn's red cells.

4.13.7.3.2 A positive reaction (± to 4+) indicates that the newborn's red cells are sensitized (red cells coated with antibodies).

4.14.8 Remarks:

4.14.8.1 The negative control (ctl) must always show a negative reaction. If the negative ctl is positive, proceed as follows:

4.14.8.1.1 Wash the red cells first with isotonic saline solution (or ID-Diluent 2) before preparing the 0.8% cell suspension.

4.14.8.1.2 Then proceed as under 'Preparation of blood sample" and 'Test procedure".

4.14.8.2 If the negative control remains positive, safe interpretation of the ABO and RhD groups cannot be made and further investigation is required.

4.15 ID-antigen profile I, II, III (For Antigen determination):

4.15.1 Introduction:

4.15.1.1 Transfusions are made with ABO, RhD and compatible blood, in certain cases Rh phenotypes and Kell status are also taken into account. Other blood group antigens are generally not considered.

4.15.1.2 However, when a clinically significant antibody is present, appropriate antigen negative blood should be used. For such cases, the rapid availability of fully typed donor blood is of great advantage. The ID-System facilitates complete antigen profiling.

4.15.1.3 The ID-Cards:

4.15.1.3.1 "ID-Antigen Profile I" is suitable for the determination of the antigens P1 (P1), Le(a) (LE1), Le(b) (LE2), Lu(a) (LU1) and Lu(b) (LU2);

4.15.1.3.2 "ID-Antigen Profile II" for the antigens k (KEL2), Kp (a) (KEL3), Kp (b) (KEL4), Jk(a) (JK1) and Jk(b) (JK2) and

4.15.1.3.3 "ID-Antigen Profile III" for the antigens M (MNS1), N (MNS2), S (MNS3), s (MNS4), Fy(a) (FY1) and Fy(b) (FY2).

4.15.1.4 In the ID-Cards "ID-Antigen Profile I + II", the gel suspensions contain the corresponding antibodies, requiring only the addition of the red cell suspension (in ID-Diluent 1).

4.15.1.5 In the ID-Card "ID-Antigen Profile III", the first 2 microtubes contain neutral gel and the last 4 gel with polyspecific anti-human globulin(AHG): The corresponding antibodies (ID-test sera, specially adapted for the ID-System) are added after the red cell suspension (in ID-Diluent 2).

4.15.2 Reagents:

4.15.2.1 ID-Card "ID-Antigen Profile III", 2 microtubes with neutral gel and 4 microtubes with polyspecific anti-human globulin (rabbit anti-IgG and monoclonal anti-C3d) within the gel matrix (to be used with the specific ID-test sera).

4.15.2.2 ID-Card "ID-Antigen Profile I", containing monoclonal anti-P1 antibodies, monoclonal anti-Lea, monoclonal anti-Leb, anti-Lua and anti-Lub, polyclonal antibodies from human serum, within the gel matrix. The microtube ctl is the negative control.

4.15.2.3 ID-Card "ID-Antigen Profile II", containing anti-k, anti-Kpa and anti-Kpb, polyclonal antibodies from human serum, monoclonal anti-Jka and monoclonal anti-Jkb, within the gel matrix. The microtube ctl is the negative control.

4.15.2.4 ID-test sera (for "ID-Antigen Profile III"), ready-to-use, in 1.4 ml or 5.0 ml vials: anti-M monoclonal and anti-N monoclonal; anti-S, anti-s, anti-Fya and anti-Fyb, polyclonal antibodies from human serum.

4.15.3 Additional Reagents Required:

4.15.3.1 ID-Diluent 1: modified bromelin solution for red cell suspension.

4.15.3.2 ID-Diluent 2: modified LISS for red cell suspension.

4.15.4 Further Materials Required:

4.15.4.1 ID- Dispenser

4.15.4.2 ID- Pipetor

4.15.4.3 ID- Tips (pipetor tips)

4.15.4.4 ID- Working Table
4.15.4.5 Suspension Tubes
4.15.4.6 ID- Incubator 37 °C
4.15.4.7 ID- Centrifuge

4.15.5 Preparation Of Blood Sample:

4.15.5.1 ID-Antigen Profile I and II: Prepare a 5% red cell suspension in ID-Diluent 1 as follows:

4.15.5.1.1 Allow the diluent to reach room temperature before use.
4.15.5.1.2 Dispense 0.5 ml of ID-Diluent 1 into a clean tube.
4.15.5.1.3 Add 50 ul of whole blood or 25 ul of packed red cells, mix gently.
4.15.5.1.4 Incubate the red cell suspension for 10 minutes at room temperature (18-25 °C).
4.15.5.1.5 Use within 15 minutes after incubation.

4.15.5.2 ID-Antigen Profile III: Prepare a 0.8-1% red cell suspension in ID-Diluent 2 as follows:

4.15.5.2.1 Allow the diluent to reach room temperature before use.
4.15.5.2.2 Dispense 1.0 ml of ID-Diluent 2 into a clean tube.
4.15.5.2.3 Add 10 ul of packed cells or 20 ul of whole blood, mix gently.
4.15.5.2.4 The cell suspension may be used immediately.

4.15.6 Test Procedure: Allow the ID-Cards and reagents to reach room temperature (18-25 °C) before use.

4.15.6.1 ID-Antigen Profile I and II:

4.15.6.1.1 Identify the ID-Card with the unique patient or donor number/details as appropriate.
4.15.6.1.2 Remove the aluminium foil from as many microtubes as required by holding the ID-Card in the upright position.
4.15.6.1.3 Pipette 10 or 12.5 uL of the red cell suspension in ID-Diluent 1 to all microtubes.
4.15.6.1.4 Centrifuge the ID-Card for 10 minutes in the ID-Centrifuge.
4.15.6.1.5 Read and record the results.

4.15.6.2 ID-Antigen Profile III:

4.15.6.2.1 Identify the ID-Card with the unique patient or donor number/details as appropriate.
4.15.6.2.2 Remove the aluminium foil from as many microtubes as required by holding the ID-Card in the upright position.
4.15.6.2.3 Pipette 50 ul of the red cell suspension in ID-Diluent 2 to all microtubes.
4.15.6.2.4 Add 50 ul of the ID-test sera to the appropriate microtubes.
4.15.6.2.5 Incubate the ID-Card for 10 minutes at room temperature (18-25 °C).
4.15.6.2.6 Centrifuge the ID-Card for 10 minutes in the ID-Centrifuge.
4.15.6.2.7 Read and record the results.

4.15.7 Interpretation Of The Results:

4.15.7.1 Positive reactions of 1+ to 4+ indicate presence of the corresponding antigen.
4.15.7.2 A double population must also be considered as positive. However, a double population may also indicate the presence of cells positive and negative for the corresponding antigen (e.g. Post transfusion, if the antigen configuration of the transfused blood was different from the patient's).
4.15.7.3 Negative reactions indicate absence of the corresponding antigen.
4.15.7.4 Strong 4+ reactions are very rare.
4.15.7.5 Anti-Jka may react more strongly with Jk (a+b-) than with Jk (a+b+) red blood cells (dosage effect).

4.15.8 Remarks:

4.15.8.1 ID-Antigen Profile I and II:

4.15.8.1.1 The negative control must always show a negative reaction.
4.15.8.1.2 If the negative control is positive, wash the red cells first in warm isotonic saline solution or ID-Diluent 2, before preparing the red cell suspension then repeat the negative control test.
4.15.8.1.3 If the negative control is negative, proceed to the required antigen test.

4.15.8.1.4 If the negative control remains positive, further investigations should be undertaken to ascertain the reason, before valid antigen typing can be assured.

4.15.8.1.5 ID-Antigen Profile I: Serologically, the P1 phenotype exhibits variation in strength on the red cells, which appears to be under genetic control.

4.15.8.2 ID-Antigen Profile III:

4.15.8.2.1 Prior to testing for the presence of an antigen, it should be assured that the red cells are free from in vivo or in vitro coating by autoantibodies and/or complement components, which may react with the AHG, producing falsely positive results. Proceed to the direct antiglobulin test (DAT) as follows:

4.15.8.2.1.1 Identify the ID-Card (LISS/Coombs) with the unique patient or donor number/details as appropriate.

4.15.8.2.1.2 Take off the aluminium foil by holding the ID-Card in the upright position.

4.15.8.2.1.3 Pipette 50 uL of the red cell suspension (in ID-Diluent 2) to a microtube.

4.15.8.2.1.4 Centrifuge the ID-Card for 10 minutes in the ID-Centrifuge.

4.15.8.2.1.5 Read and record the results.

4.15.8.2.2 If the DAT is negative, proceed to the antigen test.

4.15.8.2.3 If the DAT is positive:

4.15.8.2.3.1 Wash the cells with warm isotonic saline solution, ID-Diluent 2 or other recommended techniques, before preparing the cell suspension in ID-Diluent 2. Then repeat the DAT.

4.15.8.2.3.2 If the DAT is negative, proceed to the antigen test.

4.15.8.2.3.3 If it remains positive, the autoantibody should be eluted following recommended techniques before proceeding to the antigen test.

4.15.9 Limitations:

4.15.9.1 Lewis antigens may be lost as a result of disease. Lewis antigens are often greatly reduced during pregnancy.

4.15.9.2 The very rare Fy (x) gene is responsible for the production of extremely weak Fyb antigens that may give a negative reaction with Fyb antisera.

4.16 **Rh-Subgroups + Cw + K ID-Card** (Determination of Rh phenotypes with CW and K):

4.16.1 Introduction:

4.16.1.1 Besides the RhD Antigen, other important antigens of the Rh system are: C (RH2), Cw (RH8), E (RH3), c (RH4), e (RH5).

4.16.1.2 Appropriate antigen-positive red cells may stimulate antibody production in C, Cw, c, E and e -negative individuals, and once formed, the antibodies may cause destruction of corresponding red cells. The K antigen is strongly immunogenic. Anti-K has been reported as the cause of hemolytic transfusion reactions, both immediate and delayed, and hemolytic disease of the newborn.

4.16.1.3 The determination of Rh and K phenotypes are, therefore, important during pregnancy, for previously transfused patients, for patients with known irregular antibodies and for phenotyping donor blood prior to transfusion.

4.16.1.4 The ID-Card "Rh-Subgroups + Cw + K" offers a complete profiling of the Rh phenotype and includes Cw and K typing in one easy procedure.

4.16.2 Reagents:

4.16.2.1 ID-Card "Rh-Subgroups + Cw + K" contains a mixture of polyclonal and monoclonal antibodies anti-E and anti-CW, polyclonal antibodies anti-C, anti-c, anti-e and anti-K, from human serum within the gel matrix.

4.16.3 Additional Reagents Required:

4.16.3.1 ID- Diluent 1: Bromelin solution for red cell suspension.

4.16.4 Further Materials Required:
- 4.16.4.1 ID- Dispenser.
- 4.16.4.2 ID- Pipetor.
- 4.16.4.3 ID- Tips (pipetor tips).
- 4.16.4.4 ID- Working Table.
- 4.16.4.5 Suspension Tubes.
- 4.16.4.6 ID- Centrifuge.

4.16.5 Preparation Of Blood Sample: Prepare a 5% red blood cell suspension in ID-Diluent 1 as follows:
- 4.16.5.1 Allow the diluent to reach room temperature before use.
- 4.16.5.2 Dispense 0.5 ml of Diluent 1 into a clean, identified tube.
- 4.16.5.3 Add 50 ul of whole blood or 25 ul of packed cells, mix gently.
- 4.16.5.4 Incubate the red cell suspension for 10 minutes at room temperature.
- 4.16.5.5 Use within 15 minutes after incubation.

4.16.6 Test Procedure:
- 4.16.6.1 Identify the ID-Card with the unique patient or donor number/details as appropriate.
- 4.16.6.2 Remove the aluminium foil from as many microtubes as required by holding the ID-Card in the upright position.
- 4.16.6.3 Add 10 or 12.5 ul of the red cell suspension to all microtubes of the ID-Card.
- 4.16.6.4 Centrifuge the ID-Card for 10 minutes in the ID-Centrifuge.
- 4.16.6.5 Read and record the reactions.

4.16.7 Interpretation Of The Results:
- 4.16.7.1 A positive reaction (1+ to 4+) indicates presence of the corresponding antigen.
- 4.16.7.2 Reactions of ≤ 2+ may indicate the presence of weak or variant forms of antigen.
- 4.16.7.3 A negative reaction indicates absence of the corresponding antigen.

4.16.8 Remarks:
- 4.16.8.1 If the sample concerned was not tested with the ID-Card "ABO/Rh", a negative control should be included.
  - 4.16.8.1.1 If the control is negative, proceed to the required antigen test.
  - 4.16.8.1.2 If the control is positive, proceed as follows:
    - 4.16.8.1.2.1 Wash the cells in warm (37 °C) isotonic saline solution or ID-Diluent 2, before preparing the cell suspension.
    - 4.16.8.1.2.2 Repeat the negative control.
    - 4.16.8.1.2.3 Where the control remains positive, Rh phenotyping is recommended to be undertaken using ID-Cards containing monoclonal antibodies. A direct antiglobulin test (DAT) should be performed to initiate further investigation of the sample.
  - 4.16.8.1.3 Mutations in the blood group gene may give rise to weak or variant forms of an antigen, which may result in unexpectedly weak or negative results.
  - 4.16.8.1.4 The anti-C reacts also with the C^w antigen.

4.17 **DiaClon Complete Crossmatch ID-Card** (Compatibility Testing):

4.17.1 Introduction:
- 4.17.1.1 The methods used in compatibility tests should include those that will demonstrate ABO and RhD incompatibility and detect clinically significant unexpected antibodies.
- 4.17.1.2 The ID-Card "DiaClon Complete Crossmatch " is configured to meet the above requirements in one easy step.
  - 4.17.1.2.1 ABD-Confirmation of both donor and recipient.
  - 4.17.1.2.2 Major compatibility in anti-human globulin (AHG) and enzyme test.
  - 4.17.1.2.3 Autocontrol (AHG test).

4.17.2 Reagents:
- 4.17.2.1 ID-Card "DiaClon Complete Crossmatch" with;
  - 4.17.2.1.1 3 microtubes containing monoclonal anti-A, monoclonal anti-B and monoclonal anti-D within the gel matrix, for the ABO/D confirmation of both recipient and donor.
  - 4.17.2.1.2 1 microtube containing neutral gel for the enzyme compatibility test and

4.17.2.1.3 2 microtubes with polyspecific AHG serum (rabbit anti-IgG and monoclonal anti-C3d) for the compatibility test in AHG and the autocontrol (ac).

4.17.2.1.4 The anti-D test sera were selected so as not to react with DVI variants.

4.17.3 Additional Reagents Required:

4.17.3.1 ID-Diluent 2: modified LISS for red cell suspension.

4.17.3.2 ID-Papain: Papain solution for enzyme test.

4.17.3.3 ID-Diluent 1: modified bromelin solution for enzyme test.

4.17.4 Further Materials Required:

4.17.4.1 ID- Dispenser

4.17.4.2 ID- Pipetor

4.17.4.3 ID- Tips (pipetor tips)

4.17.4.4 ID- Working Table

4.17.4.5 Suspension Tubes

4.17.4.6 ID- Incubator 37 °C

4.17.4.7 ID- Centrifuge

4.17.5 Sample Material:

4.17.5.1 Donor: The donor sample must be from a segment, originally attached to the blood bag intended for transfusion, and well mixed.

4.17.6 Preparation Of Blood Sample:

4.17.6.1 For Patient's and donor's red cells

4.17.6.2 Prepare a 0.8-1% red cell suspension in ID-Diluent 2

4.17.7 Test Procedure:

4.17.7.1 with ABO/D test of both donor and patient:

4.17.7.1.1 Allow diluent and samples to reach room temperature before use.

4.17.7.1.2 Identify the ID-Card with the unique patient or donor number/details as appropriate.

4.17.7.1.3 Remove the aluminium foil from as many microtubes as required by holding the ID-Card in the upright position.

4.17.7.1.4 Pipette 50 uL of the patient's red cell suspension to microtubes 1, 2, 3 and 6 (A, B, D, ac).

4.17.7.1.5 Pipette 50 ul of the donor's red cell suspension to microtubes 1, 2, 3, 4 and 5 (A, B, D, Enz., AHG).

4.17.7.1.6 Add 25 ul of the patient's plasma or serum to microtubes 4 and 5 (compatibility test) and microtube 6 (autocontrol).

4.17.7.1.7 Add 25 ul of ID-Diluent 1 or ID-Papain to microtube 4 (enzyme test).

4.17.7.1.8 Incubate the ID-Card for 15 minutes at 37 °C in the ID-Incubator.

4.17.7.1.9 Centrifuge the ID-Card for 10 minutes in the ID-Centrifuge.

4.17.7.1.10 Read and record the results.

4.17.7.2 With ABO/D test of patient only (confirmation of blood group):

4.17.7.2.1 Identify the ID-Card with the unique patient or donor number/details as appropriate.

4.17.7.2.2 Remove the aluminium foil from as many microtubes as required by holding the ID-Card in the upright position.

4.17.7.2.3 Pipette 50 ul of the patient's red cell suspension to microtubes 1, 2, 3 and 6 (A, B, D, AC).

4.17.7.2.4 Pipette 50 ul of the donor's red cell suspension to microtubes 4 and 5 (compatibility test).

4.17.7.2.5 Add 25 ul of the patient's serum or plasma to microtubes 4 and 5 (compatibility test) and microtube 6 (autocontrol).

4.17.7.2.6 Add 25 ul of ID-Diluent 1 or ID-Papain to microtube 4 (enzyme test).

4.17.7.2.7 Incubate the ID-Card for 15 minutes at 37 °C in the ID-Incubator.

4.17.7.2.8 Centrifuge the ID-Card for 10 minutes in the ID-Centrifuge.

4.17.7.2.9 Read and record the results.

4.17.8 Interpretation Of The Results: (Reactions for complete compatibility test)

4.17.8.1 ABD confirmation of both donor and patient:

4.17.8.1.1 ABO/D compatibility is proven when the reaction gives only one cell population: 4+ or negative.

4.17.8.1.2 ABO incompatibility has to be considered when the reaction with anti-A and/or anti-B shows two distinct cell populations: positive and negative (sedimentation of non-agglutinated cells) causing a double cell population phenomenon.

4.17.8.1.3 Rh D incompatibility has to be considered when the reaction with anti-D shows two distinct cell populations (positive and negative).

4.17.8.1.4 When there is a possible incompatibility, ABO and Rh blood groups of both recipient and donor need to be retested.

4.17.8.2 ABD confirmation of patient only:

4.17.8.2.1

Anti - A	Anti - B	Anti - D	Blood Group
3+ to 4+	negative		A
negative	3+ to 4+		B
3+ to 4+	3+ to 4+		AB
negative	negative		O
		3+ to 4+	D positive
		± to +2+	D weak
		Negative	D negative

4.17.8.2.2 ±, trace or weak reactions with anti (D) is considered as D negative and must receive D negative cellular blood components.

4.17.8.2.3 Weaker reactions than 3+ with anti-A or anti-B can indicate the presence of A or B subgroups. In this case interpretation can only be made after a complete ABO blood grouping procedure. In the presence of weak or very weakly expressed antigens, the reaction can be negative.

4.17.8.3 Compatibility test:

4.17.8.3.1 A positive reaction (± to 4+) in one or both microtubes 4 and 5 (enzyme and AHG test) and a negative reaction in microtube 6 (autocontrol) indicates an incompatibility due to the possible presence of antibodies.

4.17.8.3.2 A negative reaction in the microtubes 4 and 5 (enzyme and AHG test) and a negative reaction in microtube 6 indicates the absence of detectable anti-bodies, indicates compatibility.

4.17.8.3.3 A positive reaction in microtube 6 only (autocontrol) may indicate a positive direct antiglobulin test (DAT) of the patient's cells. The patient's sample should be submitted to further testing.

4.17.8.3.4 The patient's sample should be submitted to further testing if a positive reaction in one or both microtubes 4 and 5 and a positive autocontrol is observed.

4.17.9 Remarks:

4.17.9.1 When there is an incompatibility, antibody screening and identification tests should be performed.

4.17.9.2 Strong cold antibodies can be the cause of weak positive reactions, especially in the enzyme test microtube.

4.17.9.3 Some antigens are destroyed or weakened by enzyme treatment. As a result, antibodies against these antigens cannot be detected by enzyme procedures.

5. MATERIALS AND EQUIPMENT:

5.1 As mentioned in each section.

6. RESPONSIBILITIES:

6.1 Blood bank staff members like technician/ specialist have to follow the detailed procedures.

5.1 As mentioned in each section.

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6.1 Blood bank staff members like technician/ specialist have to follow the detailed procedures.

7. APPENDICES:

7.1 N/A

8. REFERENCES:

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8.7 DiaMed-AG. Help File Version 2.05(480) Dated 22/10/96.
8.8 Micro Typing systems, version 2.0, publication number J32851_EN, 2008.
8.9 Good Manufacturing Practice for Blood Establishments, Version 2.0, May 2019, Saudi FDA

9. APPROVALS:

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