Reagent Red Blood Cells
Referencells® (Pooled Cells)
For ABO Serum Grouping

MOLDOVA EUROPEANĂ

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Intended Purpose:
Referencells® (Pooled Cells) is intended for detection of ABO isohemagglutinins by qualitative hemagglutination tube and microplate tests by manual and automated methods for the purpose of blood transfusion to determine safety and compatibility with potential recipients. For laboratory professional use in testing blood specimens collected from patients and donors.

Summary of the Test:
Because of the importance of the ABO groups in transfusion, serum or reverse grouping, employing cells of known ABO groups, is used as an adjunct to red blood cell or forward typing (using Anti-A and Anti-B).[1-3] As a minimum, serum grouping tests must employ at least A_1 and B red blood cells to detect the anti-A or anti-B. Additional serum grouping red blood cell reagents can be used to resolve serum and red blood cell grouping discrepancies. A_2 red blood cells are most commonly used to identify anti-A_1 in the sera of group A people. Group O red blood cells are used to identify agglutination due to non-ABO agglutinins.

Principle of the Test:
The ABO system is the only blood group system where persons, older than 6 months of age, consistently and predictably produce antibodies to antigens that they lack. As a consequence, ABO grouping is performed with serum as well as red blood cells. Serum is systematically tested against Referencells reagent red cells. Agglutination of A_1, A_2 or B cells constitutes a positive test and is the result of a reaction between an antigen and its respectively antibody. No agglutination may indicate either the absence of antibody (providing the test red blood cells possess the corresponding antigen) or that an antibody, if present, is in concentrations too low to be detected by the serologic technique employed. The ABO group of a serum or plasma specimen should match that of the red blood cells. agglutination of group O red blood cells shows the presence of a cold-reactive antibody other than anti-A and anti-B and indicates the reactions with A and B cells may not be due to anti-A or anti-B[4,5].

Reagents:
- Referencells – 1 (Group A_2)
- Referencells – 2 (Group A_1 and B)
- Referencells – 4 (Group A_1, A_2, B and O)

Each cell vial contains a 2-4% suspension of pooled C-D-E- red blood cells suspended in a buffered preservative solution containing adenosine and adenine to retard hemolysis and/or loss of antigenicity during the dating period. EDTA is added to inhibit complement activation and to prevent hemolysis when red blood cells are tested with fresh serum. Chloramphenicol (0.25 mg/mL), neomycin sulfate (0.1 mg/mL), and gentamycin sulfate (0.05 mg/mL) are added as preservatives. The diluent does not interfere with complement mediated hemolysis.

Storage:
- Store at 1 to 10°C when not in use.
- Do not freeze or expose to elevated temperatures.
- Do not use beyond the expiration date which is expressed as CCYY-MM-DD (year-month-day).

Precautions and Warning:
- For *in vitro* diagnostic use.
- For laboratory professional use only.
- Avoid contaminating this product during use. Contamination will adversely affect the product's performance during its shelf life.
- Do not use contaminated reagents.
- Do not use leaking vials.
- Do not use unlabeled vials.
- Suspend red blood cells before use by gently inverting each vial several times.
- Reagent red blood cells should not be used if the red blood cells darken, spontaneously clump, or if there is significant hemolysis.
- Slight hemolysis may occur with age. In this instance, the red blood cells may be washed and suspended in saline immediately prior to use.

Key:
Underline = Addition or significant change; ▲ = Deletion of text

NOTE: Washing will remove the EDTA contained in the diluent. Thus, Referencells that are washed before testing may hemolyze in fresh sera that contain hemolytic anti-A or anti-B. Handle and dispose of the reagent red blood cells as if potentially infectious.

CAUTION: All blood products should be treated as potentially infectious. Source material from which this product was derived was found non-reactive when tested in accordance with EN 13641:2002. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.
Handle and dispose of reagent as if potentially infectious.

LATEX The packaging of this product (Dropper Bulbs) contains dry natural rubber.

Specimen Collection and Preparation:
Draw a blood specimen using an acceptable phlebotomy technique. In manual tests, or in tests using semiautomatic instruments, fresh serum or plasma (EDTA, heparin, ACD, CPD, CPDA-1, CP2D) may be used. Testing should be performed as soon as possible following collection to minimize the chance that falsely positive or falsely negative reactions will occur due to improper storage or contamination of the specimen. Should delays in testing occur, store specimens at 1–10 °C. Specimens collected in EDTA can be stored for 10 days, ACD 21 days, heparin 3 days, no anticoagulant (serum) 21 days. Donor blood collected in CPD, CP2D or CPD-A1 can be tested until expiration (e.g., 21 days for CPD/CP2D, and 35 days for CPDA-1). Alternatively, serum or plasma can be separated from red blood cells and stored frozen for 10 years.[6] Weakly reactive antibodies may deteriorate and become undetectable in samples stored at room temperature for several days before testing or in samples stored for prolonged periods at 1–10 °C. Do not use samples drawn into tubes with neutral gel separators; false-positive results may occur with such samples.

Referencells® generated valid results with no interference using samples having triglycerides up to 1000 mg/dL, bilirubin up to 30 mg/dL, hemolysis up to 2+, albumin up to 5.2 g/dL, cholesterol up to 400 mg/dl, and plasma hemoglobulin up to 20 g/dL. All positive phenotypes showed agglutination strength of 3+ to 4+ and antigen negative phenotypes were negative.

Procedure:
Materials Provided:
Referencells – 1 REF 0002342 1 vial of A_2 cells (1x10 mL)
Referencells – 2 REF 0002345 1 vial each of A_1 and B cells (2x10 mL)
Referencells - 4 REF 0002338 1 vial each of A_1, A_2, B and O cells (4x10 mL)

Additional Materials Required But Not Provided:

Tube method:
1. Test tubes (10x75 mm or 12x75 mm) and a test tube rack
2. Transfer pipettes
3. Serological centrifuge*
4. Interval timer
5. Isotonic saline or phosphate-buffered (approximately 15 mM) isotonic saline, pH 6.5-7.5

Microplate or microwell methods:
1. Transfer pipettes or pipetting system*
2. Galileo Microplates REF 0066050
3. CMT Plates REF 0089000
4. Centrifuge*
5. Isotonic saline or phosphate-buffered (approximately 15 nM) isotonic saline, pH 6.5-7.5
6. Mechanical microplate shaker* (optional)
7. Microplate reader* (optional)
8. Galileo Echo* (software version 2.1) (as applicable) (REF 0087000)
9. Echo Lumena* (as aplicable) (REF 0086998)
10. Galileo NEO* (software 3.1) (as applicable) (REF 0064600)
11. NEO Iris* (as applicable) (REF 0064598)

*It is the user's responsibility to validate an accessory device for its intended use. Validation results should be maintained as part of the laboratory's records for review by regulatory agencies.

Test Methods:

Tube Method

1. Label 1 test tube for each of the Referencells to be tested.
2. Add 2 drops of serum or plasma to each tube.
3. Gently invert each reagent several times to completely suspend the red blood cells.
   Add 1 drop of each reagent to the appropriately labeled tubes. Mix the contents of each tube thoroughly. **NOTE:** Room temperature incubation for 5–60 minutes may be necessary to enhance reactions due to weakly reactive ABO antibodies.
4. Centrifuge each tube 15–30 seconds at 900–1000x *g* or a time, appropriate for the centrifuge used, that produces the strongest reaction of antibody with antigen-

positive red blood cells yet allow easy suspension of antigen-negative red blood cells.

5. Gently suspend each red blood cell button and examine for agglutination. Record results.

Microplate Method

1. Label the plate or strip to be tested.
2. Gently invert each reagent several times to completely suspend the red blood cells.
3. Add 25–50 uL (±5 uL) of each Referencells to separate wells.
   NOTE: Referencells are manufactured as 2–4% suspensions. Some microplate users prefer suspensions of approximately 1%. If a lighter suspension is desired, dilute an aliquot of each Referencells reagent with isotonic saline. Dilution of the reagents will reduce the content of EDTA, therefore red cells may hemolyze in the presence of hemolytic anti-A or anti-B. Referencells diluted in saline should be used within 24 hours.
4. Add 2 drops (100±5 uL) of the patient's or donor's serum or plasma to each of the wells. Mix the contents of each well gently but thoroughly by manually tapping the plate or with a microplate shaker. **NOTE:** Room temperature incubation for 5–60 minutes may be necessary to enhance reactions due to weakly reactive ABO antibodies.
5. Centrifuge the tests at 150–250x *g* for 60 seconds, or for an appropriate time and speed to produce positive results with antibody-positive serum or plasma and negative results with antibody-negative serum or plasma.
6. Agitate the wells to suspend the cell buttons by manually tapping the plate or with a mechanical microplate shaker. Gently suspend each red blood cell button and examine for agglutination. Record results. An optical aid can be used to examine the reactions in each well, if desired.

Automated Methods

For microplate testing with automated instrumentation, including isohemagglutinin titers, refer to instructions provided in the instrument operator manual.

NOTE: Magnetic stirballs (REF 0006226) are used to keep the red blood cells in suspension when used with automation. If the stirballs are not added to the vials, then the reagent red blood cells will not be mixed and the results may be invalid or incorrect. Do not touch the stirballs. The stirballs must be added directly to the reagent vials using the dispenser provided (REF 0006074). Contamination of cellular reagents can occur if the stirballs are touched.

Stability of Reaction:

Following centrifugation, all tests should be read immediately, and results interpreted without delay. Delays may result in dissociation of antigen-antibody complexes leading to falsely negative, or at most, weakly positive reactions. Microplate tests should be interpreted immediately following resuspension to avoid erroneous test results due to the settling or dissociation of red cell agglutinates.

Quality Control:

To confirm the reactivity of the A_1, A_2 and B red blood cells, it is recommended they be tested each day of use with the appropriate weakly reactive ABO antibody (e.g. corQC Test System REF 0002410). Lack of reactivity indicates a reagent is not suitable for use. For microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

Interpretation of Results:

Positive test: agglutination of red blood cells

Negative test: no agglutination

EXPECTED SERUM GROUPING RESULTS

	Reagent Red Blood Cells			
Blood Group	**A_1**	**A_2**	**B**	**O**
O	+	+	+	0
A_1	0	0	+	0
A2	0	0	+	0
A_2 with anti-A_1	+	0	+	0
B	+	+	0	0
A_1B	0	0	0	0
A_2B with Anti-A_1	+	0	0	0

Limitations:

1. Falsely positive or falsely negative test results can occur form bacterial or chemical contamination of test materials, inadequate incubation time or temperature, improper centrifugation, or omission of sample or reagent.
2. Reagent A_1, A_2 and B red blood cells possess antigens other than A or B. It is possible that on occasion a particular serum will contain a saline phase agglutinin defining one of these antigens. Non-ABO-related agglutination may interfere with serum grouping tests. Direct agglutination of a negative control (group O red blood cells) by a test sample suggests that the agglutination of A or B cells with the sample should be further investigated.
3. Referencells Group O (Pooled Cells) do not meet requirements for Reagent Red Bood Cells intended for antibody detection of unexpected antibodies.
4. Negative reactions may be obtained with one or more reagent red blood cells if the sample contains antibodies in concentrations that are too low to be detected by the test method employed. Decreased antibody activity to A and B antigens has been reported with samples from debilitated or elderly patients or patients who are less than 6 months old.
5. The ABO antibodies of most group A, B or O adults agglutinate A1, A2 and B cells strongly (3-4+). Reactions of 2+ or less may indicate the reaction is due to an antibody other than anti-A or anti-B. Thus, weakly positive reactions should be evaluated carefully to ensure no ABO discrepancy exists and the correct ABO group is assigned.
6. Umbilical samples may contain maternal anti-A and/or anti-B and will not give reliable serum grouping results.
7. Infrequently, falsely positive results may occur in the presence of antibodies directed to components of the red blood cell diluent. These unwanted reactions can usually be avoided by utilizing Reagent Red Blood Cells that have been washed with saline prior to testing.
8. With reference to the microplate method, new and unused plastic microplates are capable of passively adsorbing cells and serum proteins to their surfaces. This nonspecific adsorption can lead to erroneous test results. To overcome this characteristic, microplates should be treated prior to use to block nonspecific adsorption.
9. The reactivity of Reagent Red Blood Cells may diminish over the dating period. The rate at which antigen reactivity (ie, agglutinability) is lost is partially dependent upon the individual donor characteristics that are neither controlled nor predicted by the manufacturer.
10. For isohemagglutinin (anti-A and anti-B) titers, the clinical interpretation and significance of the cut-off must be established by the user.

Specific Performance Characteristics:

Prior to release, each lot of Referencells is tested with Anti-A, Anti-B and Anti-A1 lectin according to the insert method. Each donor sample has been shown to be D-C-E-c+e+ by two independent laboratories using no less than two sources of antibody. All suspensions are tested and shown to have a negative direct antiglobulin test using polyspecific anti-human globulin. The performance of this product is dependent upon adhering to the IFU recommended methodologies.

Precision:

Manual Repeatability and Reproducibility

The repeatability and reproducibility of Referencells in manual tube method was assessed by testing sample panel members in quadruplicate, by three (3) technologists, two (2) runs per day, for five (5) days over fourteen (14) days using two (2) lots of Referencells® – 4. Three (3) panel members, group A, B, and O were tested with A1, A2, and B cells, and one (1) panel member containing anti-c was tested with O cells.

There was 100% (point estimate, PE) results agreement when evaluated by replicate, technologist, test run, lot and day.

Agreement Analysis	Inter-precision
Sample Repeatability	100% (480/480)
Reproducibility by Technologist	100% (2880/2880)
Reproducibility by Run	100% (2880/2880)
Reproducibility by Day	100% (2880/2880)
Lot-to-Lot Reproducibility (intra-precision)	100% (2880/2880)

NEO Iris/Galileo NEO Repeatability and Reproducibility

Three (3) lots of Referencells were tested. A panel of four (4) members were tested in quadruplicated on three (3) NEO Iris systems on five (5) nonconsecutive days over fourteen (14) days. Three (3) panel members, group A, B, and O were tested with A1, A2, and B cells, and one (1) panel member containing anti-c was tested with O cells.

Agreement Analysis	Inter-precision	Intra-precision
Sample Repeatability	100%	100%
Instrument Reproducibility	100%	N/A
Reproducibility by Run	100%	100%
Reproducibility by Day	100%	100%
Lot-to-Lot Reproducibility	N/A	100%

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ECHO Lumena/Galileo Echo Precision
Two (2) lots of Referencells were tested. A panel of five (5) members were tested in duplicate on three (3) Galileo Echo (v2.1) systems in three (3) runs per day, on five (5) nonconsecutive days over fourteen (14) days.

Agreement Analysis	Inter-precision	Intra-precision
Sample Repeatability	100%	100%
Instrument Reproducibility	100%	N/A
Reproducibility by Run	100%	100%
Reproducibility by Day	100%	100%
Lot-to-Lot Reproducibility	N/A	100%

Sensitivity and Specificity
Diagnostic sensitivity and specificity was performed using both donor and patient samples with manual and automated test methodologies.

Comparison to CE-marked Device
A study was conducted that compared performance of CE-marked A1, A2, and B Reagent Red Blood Cells to Referencells®. 944 specimens were tested with Referencells® A1 and B on Galileo® and 150 specimens were tested with Referencells® A2 by tube method and compared to results obtained with CE-marked comparator A1, A2 and B reagent red blood cells tested by an automated method.

Reagent	Agreement (Point Estimate)		
	N	Initial	Resolved
A1 Cells	944	99.8%*	100%
A2 Cells	944	100%	100%
B Cells	150	100%	100%

*There were two (2) discrepant specimens. One (1) specimen, group B with weak A1 isohemagglutinin, demonstrated 2+ reactivity with Referencells A1 and no reactivity with the comparator A1 cells. Repeat testing of the comparator A1 cells was 1+. One (1) specimen, group A, gave false-positive 1+ reactivity with the comparator A1 cells; results edited to negative after visual review by operator.

Manual Method:
Manual tube testing was performed using three (3) lots of Referencells – 4 (A1, A2, B and O Cells). A total of three hundred, twenty-two (322) samples consisting of three hundred (300) samples from donors, patients and plasma from blood segments, and twenty-two (22) samples of cold reactive autoagglutinins, were tested by two (2) technologists. Each sample results in four (4) test results for each blood group. Cold reactive autoagglutinins samples were tested only with O cells to confirm sensitivity.

Reagent	Positive Percent Agreement		Negative Percent Agreement	
	N	Point Estimate	N	Point Estimate
A_1 Cells	177	100%	123	100%
A_2 Cells	177	100%	123	100%
B Cells	233	100%	67	100%
O Cells	22	100%	300	100%

Automated Methods:
Combined data from NEO Iris® and Echo Lumena® automated testing are presented in the tables below. The data compare testing performed using Referencells on NEO Iris/Echo Lumena with agreement (point estimate) based upon ABO red blood cell (forward) grouping results.

Referencells A_1 N=1164		ABO Forward Grouping			Positive % Agreement	100%
		Positive	Negative	Equivocal		
NEO Iris/ Echo Lumena	Positive	651	0	0	Negative % Agreement	99.9%
	Negative	0	512	0		
	Equivocal	0	1	0		

Referencells A_2 N=200		ABO Forward Grouping			Positive % Agreement	100%
		Positive	Negative	Equivocal		
NEO Iris/ Echo Lumena	Positive	117	0	0	Negative % Agreement	99.9%
	Negative	2*	77	2		
	Equivocal	3	1	0		

*After discordant resolution, samples were group B.

Referencells B N=1164		ABO Forward Grouping			Positive % Agreement	99.4%
		Positive	Negative	Equivocal		
NEO Iris/ Echo Lumena	Positive	961	1†	0	Negative % Agreement	99.9%
	Negative	3	187	0		
	Equivocal	10	2*	4		

*After discordant resolution, samples were group A. †Repeat resting did not resolve.

Referencells O N=200		ABO Forward Grouping			Positive % Agreement	N/A
		Positive	Negative	Equivocal		
NEO Iris/ Echo Lumena	Positive	0	0	0	Negative % Agreement	100%
	Negative	0	199	0		
	Equivocal	0	1	0		

Automated ABO Titration Assays
Method comparison studies were performed at two (2) external sites and at Immucor, Inc. as an internal site. Test results were compared for agreement between NEO Iris assays and Galileo NEO assays.

Note: Agreement between methods does not indicate which method is correct.

Initial Results		Equal or within ±1 doubling-dilution			Equal or within ±2 doubling-dilutions		
Assay	N	n	% Agreement	% LCI*	n	% Agreement	% LCI*
TMA1	102	84	82.35	74.96	99	97.06	92.57
TMA2	102	91	89.22	82.87	100	98.04	93.96
TMB	95	84	88.42	81.56	93	97.90	93.52
TLGA1†	98	89	90.82	84.52	98	100.00	96.31
THGA2†	22	21	95.46	80.19	22	100.00	84.56
Both†	102	90	88.24	81.64	102	100.00	96.45
TLGA2	102	95	93.14	87.50	102	100.00	96.45
TLGB††	97	86	88.86	81.93	96	98.97	95.20
THGB††	13	13	100.00	75.29	13	100.00	75.29
Both††	98	87	88.78	82.11	97	98.98	95.25

*Agreement at the 95% one-sided lower confidence interval
Discordant samples were manually diluted and tested by a reference method.

Resolved Results		Equal or within ±1 doubling-dilution			Equal or within ±2 doubling-dilutions		
Assay	N	n	% Agreement	% LCI*	n	% Agreement	% LCI*
TMA1	102	87	85.29	78.26	102	100.00	96.45
TMA2	102	93	91.18	85.11	102	100.00	96.45
TMB	95	85	88.47	82.80	94	98.95	95.10
TLGA1†	98	89	90.82	84.52	98	100.00	96.31
THGA2†	22	21	95.46	80.19	22	100.00	84.56
Both†	102	90	88.24	81.64	102	100.00	96.45
TLGA2	102	95	93.14	87.50	102	100.00	96.45
TLGB††	97	86	88.86	81.93	96	98.97	95.20
THGB††	13	13	100.00	75.29	13	100.00	75.29
Both††	98	87	88.78	82.11	97	98.98	95.25

*Agreement at the 95% one-sided lower confidence interval

EU/EEA/EFTA: Report any serious incident involving this reagent to Immucor Medizinische Diagnostik GmbH, or the distributor, and the National Competent Authority of the Member State in which the user and/or patient is established. Summary of Safety and Performance can be found at www.immucor.com, select Customer Login, Customer Center.

To get a Certificate of Analysis (CoA) or paper copy of the IFU or SDS go to www.immucor.com and enter Customer Login or contact your local Customer Service:

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Symbols Glossary:
The Symbols Glossary (ID No. 400) is provided electronically at http://adextranet.immucor.com/EN/Pages/PackageInserts.aspx

Additional symbols that appear in product labeling:

REAGENT RED BLOOD CELLS
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CE 0197

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