REAGENT RED BLOOD CELLS

REFERENCELLS® Pooled Cells

For ABO Serum Grouping

- IVD Rx ONLY
- 
- 1°C 10°C
- Discard if markedly hemolyzed
- 2-4% Suspension
- No US standard of potency
- Preservatives: chloramphenicol (0.25 mg/mL) neomycin sulfate (0.1 mg/mL) gentamycin sulfate (0.05 mg/mL)

CAUTION: ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. THE PACKAGING OF THIS PRODUCT (DROPPER BULBS) CONTAINS DRY NATURAL RUBBER.

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REAGENT RED BLOOD CELLS

Referencells® – 4

(Group A_1, A_2, B and O)

Referencells® – 2

(Group A_1 and B)

Referencells® – 1

(Group A_2)

for ABO Serum Grouping

IMMUCOR

Intended Use:

Referencells (Pooled Cells) are intended for use in tube and microplate ABO serum grouping tests.

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.

Reagents:

Referencells - 4 is a four-vial set of one vial each of A_1, A_2, B and O cells.

Referencells – 2 is a 2-vial set of A_1 and B cells.

Referencells – 1 is a single vial reagent of A_2 cells.

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.

No US standard of potency.

Precautions:

For in vitro diagnostic use.

Store at 1-10°C when not in use. Do not freeze or expose to elevated temperatures. 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 beyond the expiration date. Do not use leaking vials. Do not use unlabeled vials. The format for the expiration date is expressed as CCYY-MM-DD (year-month-day).

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.

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 NEGATIVE WHEN TESTED IN ACCORDANCE WITH CURRENT FDA REQUIRED TESTS. NO KNOWN TEST METHODS CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS. 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, specimens should be stored at 1-10°C if possible. Alternatively, serum or plasma can be separated from red blood cells and stored frozen. 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.

Procedure:

Materials Provided:

Referencells in dropper vials ready for use

Additional Materials Required:

All methods:

1. Donor or patient sample
2. Marking pens

Tube methods:

1. 10 x 75 mm or 12 x 75 mm test tubes 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. Microplates, microwells or Immucor Hemagglutination/Dilution Strips
3. Centrifuge*
4. Isotonic saline or phosphate-buffered (approximately 15 mM) isotonic saline, pH 6.5-7.5
5. Mechanical microplate shaker* (optional)
6. Microplate reader* (optional)
7. Galileo* (as applicable)
8. Galileo Echo* (as applicable)
9. Echo Lumena* (as applicable)
10. NEO Iris* (as applicable)

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

*It is the users 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.

Tube Test 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.
4. Add 1 drop of each reagent to the appropriately labeled tubes. Mix the contents of each tube thoroughly.
5. Centrifuge each tube.* Gently suspend each red blood cell button and examine for agglutination. Record results.**

*Suggested centrifugation time: 15-30 seconds at 900-1000 x g or a time, appropriate for the centrifuge used, that produces the strongest reaction of antibody with antigen-positive red blood cells, yet allows easy suspension of antigen-negative red blood cells.
**Room temperature incubation for 5-60 minutes may be necessary to enhance reactions due to weakly reactive ABO antibodies.

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.
5. Centrifuge the tests at 150-250 x 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.)

* Room temperature incubation for 5-60 minutes may be necessary to enhance reactions due to weakly reactive ABO antibodies.
For microplate testing with automated instrumentation, including isohemagglutinin titers, refer to instructions provided in the instrument operator manual.

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. 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

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Limitations:

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.

Reagent A_1, A_2 and B red blood cells posess 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.

Referencells Group O red blood cells (Pooled Cells) DO NOT MEET THE REQUIREMENTS OF THE FDA for reagent red blood cells intended for antibody screening for unexpected antibodies.

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.

The ABO antibodies of most group A, B or O adults agglutinates A_1, A_2 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.

Umbilical samples may contain maternal anti-A and/or anti-B and will not give reliable serum grouping results.

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.

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. Immucor Hemagglutination/Dilution Strips are pretreated by the manufacturer and require no further treatment.

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.

US license does not apply to the group O control cell.

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 Immucor Referencells is tested with Anti-A, Anti-B and Anti-A_1 lectin according to the insert method. The performance of this product is dependent upon adhering to the insert's recommended methodology. Each donor sample has been shown to be D-C-E-c+e+ by two independent laboratories using no less than two donor sources of antibody. All suspensions are tested and shown to have a negative direct antiglobulin test using polyspecific anti-human globulin. For additional information or for technical support, contact Immucor at 855-IMMUCOR (466-8267).

The expiration date is set at 67 days from the date of manufacture which is the earliest date that blood is withdrawn from any donor used in a component of the product.

Performance Characteristics on Galileo Echo and Echo Lumena:

Method comparison studies were performed at four external clinical sites. Specimens were tested on Galileo Echo and/or Echo Lumena and Galileo Neo. Specimens that gave initial equivocal (?) test well results with Referencells were retested on the analyzer that gave the initial equivocal results. Test results were evaluated for agreement between analyzers. Combined results from all sites are summarized in the following tables:

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

A1 Cells N=5044		Galileo Neo				
		Positive	Negative	Equivocal		
Galileo Echo	Positive	3039	52	0	Positive % Agreement	99.3%
	Negative	21	1932	0	PPA (95% Lower Bound One-Sided CI)	99.0%
	Equivocal	0	0	0	Negative % Agreement	97.4%
					NPA (95% Lower Bound One-Sided CI)	96.7%

Results are for North America Market assays. Galileo Echo testing performed with software v2.1. NPA lower 95% CI did not meet 99% due to 52 false-positive results. ABO serum (reverse) grouping results that are not concordant with ABO cell (forward) grouping results result in NTD (No Type Determine) interpretation.

A1 Cells N=5322		Galileo Neo				
		Positive	Negative	Equivocal		
Echo Lumena	Positive	3190	19	0	Positive % Agreement	99.2%
	Negative	25	2088	0	PPA (95% Lower Bound One-Sided CI)	98.9%
	Equivocal	0	0	0	Negative % Agreement	99.1%
					NPA (95% Lower Bound One-Sided CI)	98.7%

Results are for North America Market assays. NPA lower 95% CI did not meet 99% due to negative sample (N) size tested.

B Cells N=5044		Galileo Neo				
		Positive	Negative	Equivocal		
Galileo Echo	Positive	4185	27	0	Positive % Agreement	99.6%
	Negative	16	815	1	PPA (95% Lower Bound One-Sided CI)	99.4%
	Equivocal	0	0	0	Negative % Agreement	96.8%
					NPA (95% Lower Bound One-Sided CI)	95.6%

Results are for North America Market assays. Galileo Echo testing performed with software v2.1. NPA lower 95% CI did not meet 99% due to 27 false-positive results. ABO serum (reverse) grouping results that are not concordant with ABO cell (forward) grouping results result in NTD (No Type Determine) interpretation.

B Cells N=5322		Galileo Neo				
		Positive	Negative	Equivocal		
Echo Lumena	Positive	4066	12	0	Positive % Agreement	99.5%
	Negative	22	881	1	PPA (95% Lower Bound One-Sided CI)	99.3%
	Equivocal	0	0	0	Negative % Agreement	98.7%
					NPA (95% Lower Bound One-Sided CI)	97.8%

Results are for North America Market assays. NPA lower 95% CI did not meet 99% due to negative sample (N) size tested.

Performance Characteristics on NEO Iris:

Method comparison studies were performed at three external clinical sites, including transfusion services and donor centers. Immucor, Inc., as the manufacturer, was a fourth site. Specimens were tested on NEO Iris and Galileo Neo. Test results were evaluated for agreement between analyzers. Specimens with equivocal test results were retested and repeat equivocal or discordant results were further tested by manual methods. Combined results from all sites are summarized in the following tables:

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

A1 Cells N=2944		Galileo Neo / Manual			
		Positive	Negative		
NEO Iris	Positive	1795	0	Positive % Agreement	99.9%
				PPA (95% Lower Bound One-Sided CI)	99.7%
	Negative	1	1148	Negative % Agreement	100.0%
				NPA (95% Lower Bound One-Sided CI)	99.7%

Results are for North America Market assays.

B Cells N=2944		Galileo Neo / Manual			
		Positive	Negative		
NEO Iris	Positive	2520	1	Positive % Agreement	99.9%
				PPA (95% Lower Bound One-Sided CI)	99.8%
	Negative	1	422	Negative % Agreement	99.8%
				NPA (95% Lower Bound One-Sided CI)	98.9%

Results are for North America Market assays.

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

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2. Race R, Sanger R. Blood groups in man, 6th ed. Oxford: Blackwell Scientific, 1975.
3. Fung MK, Eder A, Spitalnik SL, Westoff CM. eds. Technical manual. 19th ed. Bethesda MD: AABB, 2017.

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