TRANSFUSION MEDICINE

INSTRUCTIONS FOR USE

BLOOD GROUPING REAGENT
ORTHO™ Sera Anti-Le^{a} (Anti-LE1)
ORTHO™ Sera Anti-Le^{b} (Anti-LE2)

REF 6904497
6904498

Intended Use

For *in vitro* diagnostic use only
For Direct Agglutination Test by Column Agglutination Technology

The Anti-Le^{a} reagent is for the qualitative *in vitro* detection of human Le^{a} positive red blood cells by the direct agglutination test.
The Anti-Le^{b} reagent is for the qualitative *in vitro* detection of human Le^{b} positive red blood cells by the direct agglutination test.

Summary and Explanation

Monoclonal Anti-Le^{a} and Anti-Le^{b} blood grouping reagents enable red blood cells to be classified as one of four phenotypes: Le(a-b+), Le(a+b-), Le(a-b-), Le(a+b+). The latter phenotype, Le(a+b+), is extremely rare.
Agglutination of red cells with either of these reagents indicates the presence of the appropriate antigen on the red cell surface. Lewis antigens are also present in serum and other bodily fluids. Cord cells do not express Lewis antigens in sufficient quantity to be agglutinated by these reagents and therefore will group as Le(a-b-). An infant's true Lewis status does not normally become apparent until the age of 2 years (approx).[1]

Principles of Procedure

When used by the recommended technique, this reagent will cause agglutination (clumping) of red blood cells carrying the Le^{a} or Le^{b} antigen. Lack of agglutination of the red blood cells demonstrates the absence of the Le^{a} or Le^{b} antigen.

Reagents

Anti-Le^{a} is supplied as one reagent.

- 1 vial containing 3 mL of mouse monoclonal antibody of type IgM (cell line LEA1) containing 0.1% (w/v) sodium azide.

Anti- Le^{b} is supplied as one reagent.

- 1 vial containing 3 mL of mouse monoclonal antibody of type IgM (cell line LEB1) containing 0.1% (w/v) sodium azide.

No preparation of the reagent is required. Use directly from the vial.

Storage Requirements

Store at 2–8 °C.
May be at room temperature (15–30 °C) while in use. Replace cap when not in use.

Specimen Collection

No special preparation of the patient/donor is required prior to specimen collection. Specimens should be collected by aseptic technique with an anticoagulant. The specimen should be tested as soon as possible after collection. If testing is delayed, the specimen should be stored at 2–8 °C. Blood specimens exhibiting gross hemolysis or contamination should not be used. Do not use collection tubes that contain plasma/cell separation media. Samples collected in EDTA should be tested within seven days from collection. Donor blood stored in citrate anticoagulant may be tested until the expiry date of the donation.

Precautions

Do not use if turbid.
Do not dilute.
Do not use beyond the expiry date.
The Anti-Le[a] reagent contains 0.1% (w/v) sodium azide.
The Anti-Le[b] reagent contains 0.1% (w/v) sodium azide.
Handle and dispose of reagents as potentially infectious.
This reagent is for *in vitro* diagnostic use only.

Caution:	**Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded into sink, flush with a large volume of water to prevent azide buildup. (R22 - Harmful if Swallowed).**

Caution:	**Source material from which this product is derived was found non-reactive for HBsAg, Anti-HIV 1/2 and Anti-HCV. No known test methods can offer complete assurance that products derived from human blood will not transmit infectious disease. Appropriate care should be taken in the use and disposal of this product. Source materials may include human components and antibody producing cells that are used in the manufacture of polyclonal and monoclonal products.**

Procedure

General Information

This reagent has been standardized for use by the technique described below. When using methods other than those described in this IFU, the procedures provided by the manufacturer of those methods must be followed. Approved validation procedures must be performed. We advise that regulatory agencies be consulted to determine specific validation requirements. When using automated instruments, follow the procedures that are contained in the operator's manual provided by the device manufacturer.

Materials Provided

ORTHO™ Sera Anti-Le[a]
ORTHO™ Sera Anti-Le[b]

Additional Materials Required but not Provided

- Isotonic saline
- Reagent red blood cells suitable for the control of Anti-Le[a]
- Reagent red blood cells suitable for the control of Anti-Le[b]
- Reverse Diluent Ortho BioVue® System Cassettes
- Micropipetters for delivery of 10 μL, 40 μL and 50 μL
- Ortho BioVue® System Centrifuge

Test Procedure

Direct Agglutination Test

1. Prepare a 0.8% or 3-5% red cell suspension from patient or donor cells, using isotonic saline.
2. Allow the cassette and reagent to come to room temperature before use.
3. Label the cassette appropriately with a sample identifier.
4. Add 40 μL of the reagent antisera to the appropriate reaction chamber(s) of the opened cassette. **Do not touch the pipette to the side of the reaction chamber. If this occurs, change pipette tip before proceeding to the next chamber.**
5. Add 50 μL of 0.8% red cell suspension or add 10 μL of 3-5% red cell suspension to the appropriate reaction chamber(s) of the cassette.
6. Observe that the contents of the reaction chamber(s) are combined. If necessary tap gently.

   NOTE: Assure that the reagents remain in the reaction chamber. There should be no mixing of reactants with reagents in the column prior to centrifugation.
7. Immediately centrifuge the cassette using the Ortho BioVue® System Centrifuge.
8. Read the front and back of the individual columns for agglutination upon test completion.
9. Record the reaction strength.

Interpretation of Results

Agglutination = positive test result
No agglutination = negative test result

Stability of Results

Test results should be read, interpreted and recorded upon completion of centrifugation.

Quality Control

Quality Control (QC) of reagents is required. Quality Control should be performed on each lot of reagent on each day of use according to standard operating procedures.

Limitations of the Procedure

1. The expression of certain red blood cell antigens may diminish in strength during storage, particularly in EDTA samples. Better results will be obtained with fresh samples.
2. Suppressed or weak expression of blood group antigens may give rise to false-negative reactions.
3. Anomalous results may be caused by the following:
   - Fibrin or particulate matter
   - Red blood cells sticking to the sides of the reaction chamber
   - DAT positive red cells
   - Do not use cassettes that appear damaged (i.e., break in foil seal or break, crack or bubble in the column), exhibit drying (i.e., liquid level is at or below the top of the glass beads) or exhibit discoloration (due to bacterial contamination, which can cause false reactions).
   - Loss of fluid in the cassette column may cause (weak) false positive results.
   - J reactions may occasionally be observed with high red cell concentrations. J reactions may also be observed if during centrifugation the cassettes are not seated properly in the holder or not allowed to spin at a 90° angle.

     Note: A J reaction consists of cells forming a button at the bottom of the bead column or microtube when either end of the cell button goes up the side of the column.
     The cell button may be disrupted. A J reaction may represent a weakly positive reaction.
4. Tests with these or other anomalous results should be repeated.
5. Erroneous results could occur if final reactions are not read upon completion of centrifugation.
6. Mixed cell populations may be encountered as a result of, for example, transfusion, fetal maternal hemorrhage, or transplantation. Consult patient history when results of this nature are encountered before assigning an antigen type.

Performance Characteristics

Expected Results*

In performance evaluation studies, samples were tested with ORTHO™ Sera Anti-Le^a Mouse Monoclonal IgM and ORTHO™ Sera Anti-Le^b Mouse Monoclonal IgM by Ortho BioVue® System Column Agglutination Technology (CAT) as follows:

Reagent	Number Tested**	CAT Red Cell Suspension	Concordance***	Positive Samples in Performance Evaluation	
				N	Frequency (%)
Anti-Le^a	296	0.8%	100%	69	23
	100	3-5%			
Anti-Le^b	298	0.8%	99%	191	64
	100	3-5%			

* Data on file at Alba Bioscience Limited.
** Total number of distinct samples tested with Anti-Le^a is 296, as 100 samples were tested using both 0.8% and 3-5% red cell suspensions. Total number of distinct samples tested with Anti-Le^b is 298, as 100 samples were tested using both 0.8% and 3-5% red cell suspensions.
*** Concordance indicates agreement between the corresponding reagents only and does not indicate which reagents gave the correct results.

Results were evaluated against comparable CE marked products using the appropriate methods for the comparators.

The testing included the following minimum numbers of samples: donor and patient samples, a minimum of 10% each; neonatal samples, a minimum of 2%; samples of ABO blood group A or B, minimum of 40%. The total proportion of group O samples tested with ORTHO™ Sera Anti-Le^{a} was 41%. The total proportion of group O samples tested with ORTHO™ Sera Anti-Le^{b} was 40%. The test outcomes were representative of typical antigen frequency, based on the UK population as the testing was performed at sites in the UK.[1]

Specific Performance Characteristics

ORTHO™ Sera Anti-Le^{a} Mouse Monoclonal IgM and ORTHO™ Sera Anti-Le^{b} Mouse Monoclonal IgM Blood Grouping Reagent have been tested manually using the Ortho BioVue® System and when used according to the recommended instructions for use, found to specifically agglutinate human red cells with the corresponding antigen.

The ORTHO™ Sera Anti-Le^{a} reagent reacts with cells expressing the Lea antigen.
The ORTHO™ Sera Anti-Le^{b} reagent reacts with cells expressing the Leb antigen.

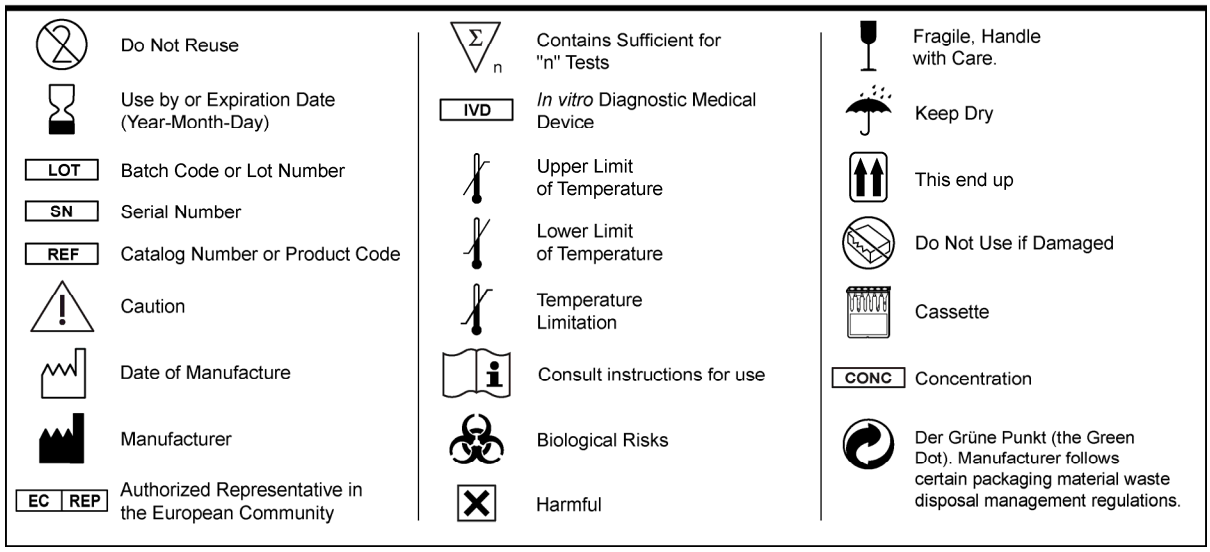
For additional information or technical support, contact Customer Technical Support.

BIBLIOGRAPHY

1. Daniels, G. Human Blood Groups, 2nd ed. Oxford; Malden MA: Blackwell Science, 2002.
2. Reid MA, Lomas-Francis C. The Blood Group Antigen Facts Book, 2nd ed. London, Academic Press, 2004.
3. Klein HG, Anstee DJ. Mollison's Blood Transfusion in Clinical Medicine, 11th ed. Oxford; Malden MA: Blackwell Publishing, 2005.
4. Roback JD, Combs MR, Grossman BJ, Hillyer CD, eds. Technical Manual, 17th ed. Bethesda, MD: AABB, 2011.

Glossary of Symbols

The following symbols may have been used in the labeling of this product.



Summary of Revisions

Date of Revision	Version	Section	Description of Technical Changes*
2013-05-23	1.0		Initial version of Instructions for Use.

* The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.

CE

Alba Bioscience Limited
Ellen's Glen Road, Edinburgh EH17 7QT, UK

Distributed by:

Ortho-Clinical Diagnostics, Inc.
1001 US Highway 202
Raritan, NJ 08869 USA

Ortho Clinical Diagnostics
PART OF THE Johnson & Johnson FAMILY OF COMPANIES