

# Blood Grouping Reagent

## immuClone® Anti-A,B IgM

For Manual Tube, Slide, Microplate and Automated Microplate Tests

(Qualitative)

IVD In Vitro Diagnostic Medical Device



Consult Instructions for Use

Temperature limitation Store at  
+1 to +10°C

Discard if markedly turbid



GHS06 Toxic

Danger

Preservative: =0.1% Sodium Azide

IMMUCOR 236-2



GHS09

Hazardous to the aquatic environment

H300+H400+H410



IMMUCOR Med. Diagnostik GmbH  
Robert-Bosch-Strasse 32  
63303 Dreieich, GERMANY

CAUTIONS: DO NOT PIPETTE BY MOUTH. ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. THIS PRODUCT HAS COMPONENTS (DROPPER BULBS) THAT CONTAIN DRY NATURAL RUBBER.

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Murine Monoclonal

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Clone

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### Intended Purpose:

▲ immuClone® Anti-A,B IgM is an in vitro diagnostic Blood Grouping Reagent used to detect the A and B erythrocyte antigen from donors and recipients by direct hemagglutination test for the purpose of a blood transfusion to ensure the safety and compatibility between the patient and the blood component selected for transfusion. For Manual Tube, Slide, Microplate and Automated Microplate Tests (qualitative).

### Summary:

In 1900, Landsteiner observed that the red cells of some of his colleagues were agglutinated by the sera of some of the others.<sup>1</sup> On the basis of observed reactions, Landsteiner divided the bloods of his colleagues into three distinct phenotypes; A, B and O.<sup>2</sup> Decastello and Sturli described the fourth phenotype of this system, AB, in 1902.<sup>3</sup>

The ABO groups of most adults can be determined directly in agglutination tests with Anti-A and Anti-B typing reagents derived from either human serum or supernate of hybridoma cells. Monoclonal antibodies derived from cultured hybridoma cell lines can be used to prepare well-defined, potent, pure Blood Grouping Reagents. That monoclonal antibodies can be used reliably for ABO grouping tests has been shown by several groups of investigators.<sup>4,7</sup>

An ABO incompatible blood transfusion can be fatal, due to the highly immunogenic nature of the A and B antigens, and the corresponding strongly hemolytic antibodies. Transfusion of ABO-incompatible blood components/products results in acute hemolytic reaction followed by disseminated intravascular coagulation, shock, acute and renal failure up to death.<sup>8</sup> Currently in developed world, ABO-incompatible transfusions and transfusion-related acute lung injury are the leading transfusion-related causes of death.<sup>9,10</sup> Anti-A or anti-B antibodies in donor plasma may destroy blood group A, B or AB red blood cells after transfusion (minor ABO incompatibility). Anti-A and anti-B antibodies in plasma can give rise to serious, even fatal, transfusion reactions, therefore, transfusion of ABO-compatible plasma is of clinical significance.<sup>9</sup> Detection and identification of A and B antigens plays a key role in transfusion medicine and is required for transfusion safety. ABO blood group antigens are also expressed on the surface of platelets, possibly allowing ABO compatibility to affect platelet transfusion outcomes. Studies have shown ABO-incompatible platelets are associated with increased transfusion reaction rates.<sup>11-14</sup>

With the widespread prevention of rhesus alloimmunization, ABO Hemolytic diseases of the newborn (HDFN) is the most common hemolytic consequence of maternofetal blood group incompatibility and occurs almost exclusively among A or B blood group infant born to O blood group mothers. This is because the anti-A and anti-B formed in group O individuals tend to be of the IgG type (and therefore can cross the placenta), whereas the anti-A and anti-B found in the serum of group B and A individuals, respectively, tends to be of the IgM type.<sup>8,14-17</sup>

The approximate frequencies of ABO antigens:<sup>8</sup>

Phenotype	Caucasians	Blacks	Asians
A1	33%	19%	27%
A2	10%	8%	rare
B	9%	20%	25%
O	44%	49%	43%
A1B	3%	3%	5%
A2B	1%	1%	rare

### Principle:

▲ The tests used with this monoclonal Blood Grouping Reagent is based on the principle of hemagglutination. When the insert procedure is followed, agglutination of red cells following incubation with immuClone® Anti-A,B IgM (positive result) indicates the presence of the corresponding antigen. Absence of agglutination indicates a negative test result and, within the accepted limitations of the test procedure, indicates the absence of the corresponding antigen on the test red cells. Discrepancies between cell and serum grouping must be resolved before the blood group is recorded. The resolution of typing discrepancies is discussed in references 18 and 19. immuClone® Anti-A,B IgM does detect Ax.

The device is designed to be used as blood grouping reagent in a professional environment. It is intended for professional use for the testing of patient and donor blood specimens. Professional users are any personnel who are qualified to perform IVD examinations through special education and training.<sup>20</sup> Specific for automated use of this reagent, training programs are provided as part of customer implementation of those instrument systems.

### Reagents:

immuClone® ▲ Anti-A,B IgM murine monoclonal Blood Grouping Reagents are to be used as supplied without further dilution or additions.

▲ Anti-A,B is a blend of antibodies from clones Birma-1, ES4 and ES15, no dye is added to this reagent.

Antibodies are diluted in a buffered saline solution containing bovine serum albumin (without stabilizers), ethylenediamine tetraacetate (EDTA), and ingredients to facilitate the resuspension of red cell buttons following centrifugation. The Bovine Albumin Solution is sourced from donor animals of United States origin that have been inspected and certified by US Veterinary Service inspectors to be disease free. This ruminant-based product is deemed to have low-TSE (Transmissible Spongiform Encephalopathy) risk. Sodium azide (=0.1% final concentration) has been added to each reagent as a preservative. The reagents have an approximate pH of 7.0.

These reagents are to be used as supplied without further dilution or additions.

The concentration of active ingredients is indicated with the titer. Potency titrations should be equal or exceed any existing relevant international reference standard sera (WHO minimum standard: Anti-A 1:8 and Anti-B 1:4). The lot specific titer is documented on the respective Certificate of Analysis.

### Precautions:

For in vitro diagnostic use by trained professionals only.



GHS06

Signal Word: Toxic



GHS09

Signal Word: Hazardous to the aquatic environment

Danger: This reagent contains =0.1% sodium azide, H300: Fatal if swallowed; H400: Very toxic to aquatic life; H410: Very toxic to aquatic life with long lasting effects

Sodium azide may react with lead and copper plumbing to form explosive compounds. If discarded into the sink, flush with a large volume of water to prevent azide build-up. Store at 1-10°C when not in use. Do not freeze or expose to elevated temperatures.

Discard if markedly turbid

Discard if markedly turbid

Avoid contaminating this product during use. Contamination will adversely affect a product's performance during its shelf life. Markedly hemolysed or bacterially contaminated samples should not be tested with this reagent. Marked turbidity may indicate reagent deterioration or contamination. Do not use contaminated reagents. Do not use leaking vials. Do not use unlabeled vials. Do not use if the information on the label is not complete.

Handle and dispose of reagent as if potentially infectious. The human donor or the cell line used to produce these reagents has been tested and found to be negative for

Anti-HIV, Anti-HCV, HBsAg and Mouse Antibody Production (MAP) viruses. No known tests can guarantee that any product derived from human blood is free from infectious agents.

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Do not use beyond the expiration date. The format for the expiry date is CCYY-MM-DD, i.e. the date 28<sup>th</sup> May 2008 would be expressed as 2008-05-28.

Allow reagent to equilibrate to ambient room temperature (~ 18 – 25 °C) prior to use.

**Specimen Collection:**

Draw a blood specimen using an acceptable phlebotomy technique.

Samples should be tested as soon as possible after collection. Do not use samples drawn into tubes with neutral gel separators. False positive results may occur with such samples.



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. Failure to store the specimens at the correct temperature (+2°C to +8°C), for example, storage at higher temperature or repeated freezing and thawing may result in false positive or false negative results.

In manual and automated testing using immuClone® Anti-A,B IgM, samples drawn into EDTA and citrate-based anticoagulant group (e.g., CPDA) can be used.

Blood drawn into EDTA can be tested up to 10 days. Blood drawn into a citrate-based anticoagulant can be tested up to period specified in the instructions for use of the anticoagulant (e.g., up to 35 days for blood drawn into CPDA).

It should be considered that samples expressing Ax phenotype demonstrate lower antigen stability during storage due to the lower number of the A antigen present on the red cell surface. Blood samples expressing Ax phenotype drawn into EDTA and citrate-based anticoagulants can be tested up to 5 days.



**Procedure:**

**Materials Provided**

▲ immuClone® Anti-A,B IgM reagent, in vials ready for use (with dropper for manual use).

**Additional Materials Required:**

**All manual methods:**

1. Donor or patient red cells
2. Marking pens
3. Isotonic saline or phosphate-buffered (approximately 15 mM) isotonic saline, pH 6.5-7.5

**Tube method:**

1. Transfer pipettes
2. 10x75 mm or 12x75 mm test tubes and a test tube rack
3. Serological centrifuge\*
4. Interval timer

**Microplate method (manual):**

1. Transfer pipettes or pipetting system\* (e.g., ABS Precis, Hamilton Microlab AT, Packard Multiprobe 104/204)
2. Microplates\*
3. Centrifuge\* (eg, Sorvall T6000, IEC Centra-8, Jouan C422, Hettich 30F, Heraeus Labofuge 400) with rotor and carriers capable of accommodating rigid 96-well plates
4. Mechanical microplate shaker\* (e.g., Titramax 3101) (optional)
5. Microplate reader\* (e.g., I-STAR) (optional)

**Slide method:**

1. Glass or plastic slides
2. Wax marker (optional)
3. Applicator sticks (for glass slides)
4. Stopwatch or timer
5. Transfer pipettes

**Automated Microplate method using the Galileo NEO / NEO Iris:**

1. Microplates (barcoded) Galileo (Immucor Med. Diagnostik GmbH Product Code 0066050)
2. Galileo diluent (Immucor Med. Diagnostik GmbH Product Codes 0066055, 0066058)
3. Galileo System Liquid Concentrate (Immucor Med. Diagnostik GmbH Product Code 0066056)
4. Stirball 2 Vial Set (50/Vial) (Immucor, Inc. Product Code 0006226)
5. Galileo NEO (Immucor Med. Diagnostik GmbH Product Code 0064600) or NEO Iris (Immucor Med. Diagnostik GmbH Product Code 0064598)

**Automated Microplate method using the Galileo Echo / Echo Lumena:**

1. CMT Plates (Immucor, Inc. Product Code 0089000)
2. Specimen Diluent (Immucor, Inc. Product Codes 0066052, 0066053)
3. Galileo System Liquid Concentrate (Immucor Med. Diagnostik GmbH Product Code 0066056)
4. Stirball 2 Vial Set (50/Vial) (Immucor, Inc. Product Code 0006226)
5. Galileo Echo (Immucor, Inc. Product Code 0087000R) or Echo Lumena (Immucor, Inc. Product Code 0086998)

\* It is the user's responsibility to validate an accessory device for its intended use.

**Test Methods:**

**A. TUBE TEST:**

1. Label 1 test tube for each blood grouping reagent to be tested.
2. Add 1 drop (approximately 50 µl) of each blood grouping reagent to the appropriately labeled tube.
3. Using a transfer pipette add 1 drop (approximately 50 µl) of a 2-5% suspension of red cells prepared in saline, plasma or serum to each tube. (Cells may be washed prior to their resuspension in saline). Mix the contents of each tube thoroughly and centrifuge.\*
4. Gently agitate each tube to resuspend the red cells buttons. Examine for agglutination.
5. 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 cells, yet allows easy resuspension of antigen-negative red cells. The centrifugal force applied should be the minimum required to produce a clear supernatant and a clearly delineated red cell button that can be easily resuspended.

No single speed or time can be recommended for all types of available centrifuges or test applications. Centrifuges should be calibrated individually to determine the optimal time and speed required to achieve the desired results.



**B. MICROPLATE TEST:**

1. Label the microplates to be used in testing.
2. Add 1 drop (approximately 50 µl) of each reagent under test to labeled or identified wells.
3. Prepare a 2-4% approximate suspension of red cells in saline (cells may be washed prior to their resuspension in saline).
4. Using a transfer pipette add 1 drop (approximately 50 µl) of each red cell suspension to the appropriate wells.
5. Mix the contents of each well thoroughly by tapping the plate manually or by using a mechanical microplate shaker.\*
6. Centrifuge the plate at 100-250 x g for 40-60 seconds, or for an appropriate time and speed to produce positive results with antigen-positive red cells and negative results with antigen-negative red cells\*\*.
7. Agitate the plate to resuspend each cell button by manually tapping the plate or placing the plate on a plate agitator. Examine each well for agglutination. If desired, a mirror or reader may be used to examine the reaction in each well.
8. Record results.



\*Suggested times for mechanical shaker: 1) Mixing: 10-30 seconds on a medium agitation setting. 2) Resuspension: 10-30 seconds on a medium setting or a time and speed appropriate for the shaker used, that allows complete resuspension of the entire cell button without destroying positive reactions.

\*\*Suggested centrifugation time: 40-60 seconds at 100-250 x g or a time, appropriate for the centrifuge used, that produces the strongest reaction of antibody with antigen-positive cells, yet allows easy resuspension of antigen-negative red cells. The centrifugal force applied should be the minimum required to produce a clear supernatant and a clearly delineated red cell button that can be easily resuspended.

No single speed or time can be recommended for all types of available centrifuges or test applications. Centrifuges should be calibrated individually to determine the optimal time and speed required to achieve the desired results.

**C. SLIDE TEST:**

1. Label slide to be used in testing

2. Place one drop (approximately 50 µl) of each blood grouping reagent to be tested on separate clean glass or plastic slide. Do not place the slides on a heated illuminated surface.
3. Add one drop (approximately 50 µl) of whole blood (or 35-45% suspension of red cells in saline or group-compatible plasma or serum) from the sample to each reagent on glass or plastic slide using a transfer pipette or applicator stick.
4. Mix the blood and reagent. On glass slides, use a separate clean applicator stick to mix each reagent/cell mixture over and over area approximately 20 x 40 mm. On plastic slides follow the manufacturer's insert.
5. Observe for macroscopic agglutination. On glass slides this is achieved by slow rotation over a period up to a maximum of 2 minutes. On plastic slides follow the manufacturer's insert. Do not place slides on a heated illuminated surface.
6. Record results.

#### D. AUTOMATED MICROPLATE METHOD:

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

#### Stability of the Reaction:

Following centrifugation, all tube 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. Slide tests should be completed within the time period specified to avoid the possibility that a negative result may be incorrectly interpreted as positive due to drying of the reagents. Microplate tests should be interpreted immediately following resuspension to avoid erroneous test results due to settling of red cells or dissociation of red cell agglutinates.

#### Quality Control:

To confirm the reactivity of immuClone® ▲ Anti-A,B IgM, it is recommended that these reagents be tested each day of use with antigen positive and negative cells, such as Referencells A1, A2, B and O (Product code: 0002338). For QC frequency minimum requirements refer to national guidelines. These reagents can be considered to be satisfactory if the antigen-positive cells are agglutinated.

#### Interpretation of Results<sup>21</sup>:

Positive Test: agglutination of red cells.  
 Negative Test: no agglutination of red cells.

▲

#### Limitations:

Falsely positive or falsely negative test results can occur from bacterial or chemical contamination of test materials, inadequate incubation time or temperature, improper centrifugation, improper storage of materials, or omission of test reagents. Under-centrifugation or over-centrifugation may result in the occurrence of numerous false-negative or false-positives.

Certain subgroups of A and B may produce reactions that are weaker than those obtained with A or B cells of most random donors. Depending on the subgroup involved, some may appear non-reactive in direct agglutination tube, microtitration plate or slide tests.

▲

The red cells of people with some disease states may give falsely positive or falsely negative reactions with anti-A or anti-B.<sup>9</sup> Some cord blood specimens may give weakened reactions with these reagents. Cord cells contaminated with Wharton's jelly may give falsely positive reactions.

Red cells that have a positive direct antiglobulin test (DAT) may produce false positive results. The use of immuClone® Rh-Hr Control reagent (Product Code 0006720, 0006721, 0066006, 0066083) is recommended for detection of such potentially false positive results. It is recommended that a tube test rather than a slide test be used to test these types of samples.

The ABO system is the only blood group system known where individuals, older than 6 months of age, consistently and predictably produce antibodies to antigens they lack. Serum grouping tests, employing red cells of known ABO groups, are used to confirm the results of cell typing procedures. However, discrepancies may occur between serum and cell grouping if the specimen under test possesses unexpected antigens or agglutinins, or if the specimen lacks expected antigens or agglutinins. See reference 10 for a more detailed discussion of ABO grouping discrepancies. Any discrepancies that occur should be resolved before an ABO group is assigned.

Do not use murine monoclonal reagents in indirect antiglobulin tests using antihuman globulin reagents.

Autoagglutinins reactive at room temperature are a potential source of error in ABO grouping tests. The presence of these antibodies cannot be predicted. When sufficiently strong they can cause the nonspecific agglutination of reagent A1 and B cells in serum (reverse) grouping tests. They can also produce nonspecific agglutination in cell (forward) tests with ▲ immuClone® Anti-A,B IgM when unwashed, plasma-suspended or serum-suspended cells are used. It is for this reason that both forward and reverse grouping tests are performed and the results are compared before

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ABO interpretations are made. All ABO tests should be read carefully. Discrepancies between forward and reverse results should be investigated thoroughly before an ABO group is assigned, regardless of the strength of the reactions obtained in any cell or serum test. The strong reactions obtained in forward tests cannot be assumed to be more correct than weaker reactions seen in reverse tests with the same sample and vice-versa. Some autoagglutinins reactive at room temperature react best when the test environment is below pH 6.5. immuClone® ▲ Anti-A,B IgM are prepared in a diluent at approximately pH 7.0. Nonspecific agglutination produced by autoagglutinins can range in strength from weak to strong. When unwashed red cells are used and an ABO discrepancy persists on repeat testing, evaluating the cells with other blood grouping reagents (prepared at pH 7.0) or testing the serum or plasma with additional reagent blood cells may be indicated.

With reference to the microplate method, new, unused plastic microplates are capable of passively adsorbing cells and serum proteins to their surfaces. This nonspecific adsorption can lead to erroneous test results.<sup>22</sup> Each batch of microplates should be evaluated in the user's system prior to acceptance for routine use. Where necessary, microplates can be treated prior to use to block nonspecific adsorption. Bovine albumin (1-2%) or 1% gelatin can be used as a blocking agent. Incubate the solution in the wells for 10 minutes at 18-30°C. Plates should then be thoroughly rinsed (approximately 10 times) in distilled or deionized water. Decant the water from the wells as thoroughly as possible following each rinse. Allow plates to dry before their use in testing.

Deviation from the Recommended Instructions for Use may result in less-than-optimal product performance. Slide test procedures may not be sufficiently sensitive for reliable detection of weakened antigen expression.

#### Incidents related to the device:

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

#### Specific Performance Characteristics:

immuClone® ▲ Anti-A,B IgM meets the requirements of the Common Specifications (EU) 2022/227 for products as described in IVDR 2017/746 Article 9 for Class D in vitro Diagnostic Medical Devices. The reagents show same or better performance characteristics compared to established and approved devices.

Prior to release, each lot of immuClone® ▲ Anti-A,B IgM is tested by insert methods against a panel of antigen-positive red cells to ensure suitable reactivity. immuClone® Anti-A,B IgM does detect Ax. Part of lot release testing includes testing each lot with 3 examples of Ax cells. The performance of this product is dependent on adhering to the recommended methods found in this insert. Additional information regarding specificity testing performed at the time of the manufacture or as performed subsequent to product release may be furnished upon request by consulting Immucor's Technical Service at (+49) 6074 8420-50 or via e-mail: [tech.support.eu@immucor.com](mailto:tech.support.eu@immucor.com).

Technique	immuClone® Anti-A,B IgM			
	n	Diagnostic Sensitivity	n	Diagnostic Specificity
Tube	263/263	100%	237/237	100%
Slide	263/263	100%	237/237	100%
Automated Microplate <sup>(1)</sup>	140/140	100%	169/169	100%
Automated Microplate <sup>(2)</sup>	129/129	100%	130/130	100%

- (1) These data were obtained using the Galileo NEO "v2.0" (Product Code 0064600) and NEO Iris instruments (Product Code 0064598) instruments. The results generated are also applicable for manual microplate method since the employed method – hemagglutination and the principle is the same. In addition, the automated method also employs microplates as the carrier for the sample processing.
- (2) These data were obtained using the Galileo Echo "v2.0"/Lumena System (Echo Lumena Product Code 0086998). The results are also generated are also applicable for manual microplate method since the employed method – hemagglutination and the principle is the same. In addition, the automated method also employs microplates as the carrier for the sample processing.

Note: All performance characteristics related to the automated use and provided in this IFU were obtained using the Galileo NEO "v2.0" (Product Code 0064600) and NEO Iris (Product Code 0064598) instruments.

The performance characteristics related to the use of the reagent on the Galileo Echo "v2.0" (Immucor, Inc. Product Code 0087000R) and Echo Lumena (Immucor, Inc. Product Code 0086998) can be found in the respective Operator Manual of the Instrument.

**A. Clinical Performance (Diagnostic Sensitivity, Specificity, PPV, NPV and Likelihood Ratio)**

Diagnostic Sensitivity and Specificity of 100% have been obtained for immuClone® Anti-A,B IgM within a clinical performance study using manual tube and slide test method in comparison with a state-of-the-art CE-marked comparator device. In this study, 500 samples have been tested with both, immuClone® Anti-A,B IgM and CE-marked comparator device using 21.6% clinical and 2.4% neonatal samples. The study design fulfilled the requirements of the Common Specifications (EU) 2022/1107. The following acceptance criteria were met: "The percent agreement between subject and comparator method in random samples shall be ≥99%". The positive predictive value (PPV) and negative predictive value (NPV) have been determined showed 100% with a likelihood ratio of ∞. Thus, immuClone® Anti-A,B IgM performs equivalent to the state-of-the-art CE-marked comparator reagent.

100% Diagnostic Sensitivity and Specificity have been established for immuClone® Anti-A,B IgM using automated microplate method on the NEO v2.0/Iris and Echo v2.0/Lumena system. The generated data were obtained from a clinical performance study with a total sample size n = 563 using 32.4% clinical samples for the NEO v2.0/Iris system and 43.3% clinical samples for the Echo v2.0/Lumena system. The acceptance criteria were set as following:

- Concordance: Result interpretations for phenotyping reactions by the System shall be at least 99% (PE) overall concordant, 99% (PE) PPA concordant, and 99% (PE) NPA concordant to the expected result of the test well.
- The visual grading of wells shall be within +/- 1 between the visual grade and assays under test with the reagent when interpreting hemagglutination images (90% concordance point estimate).

The acceptance criteria were met and PPV and NPV of 100% have been determined and a likelihood ratio of ∞. Thus, 100% Concordance was shown between subject and the CE-marked device when run automated on NEO v2.0/Iris and Echo v2.0/Lumena system using horizontal and vertical assays.

**Analytical Performance**

**B. Accuracy**

Accuracy of immuClone® Anti-A,B IgM was confirmed by performing a Comparability Study. The objective of the study protocol (Comparability Study immuClone® Anti-A,B IgM) was to demonstrate the equivalency of the reagent under test with the relevant CE marked comparator reagent as required for Technical Documentation of In Vitro Diagnostic medical devices. The equivalency of results obtained with reagent under test and comparator reagent was demonstrated by testing 170 donor blood samples (including A1, A2, Ax, A1B, A2B, B, and O phenotypes) in triplicates with the reagent under test (510 tests in total) and the same 170 donor blood samples with comparator reagent using manual tube test method (direct agglutination). It was demonstrated that the reagent under test and the comparator reagent performed equivalent (100% Sensitivity and Specificity) and did not differ qualitatively when compared to the results obtained with the same samples when using respective comparator reagent. Grading results of tested reagents did not change more than +/- 2 when comparing to each other. Therefore, the acceptance criteria as required for the Study Protocol Comparability Study immuClone® Anti-A,B IgM have been completely met.

**C. Precision**

Repeatability and Reproducibility of immuClone® Anti-A,B IgM for manual use (manual tube, slide, microplate) were confirmed by testing three (3) samples in quadruplicate by three (3) Verification Technologists on five nonconsecutive days over eleven days using the positive and negative cells. Results demonstrated that the acceptance criteria of "The Anti-A,B (Series 1 and immuClone®) reagents shall demonstrate repeatable and reproducible results for the five (5) nonconsecutive days of testing over the maximum fourteen (14)-day test period", "Expected positive samples will result consistently positive, and expected negative samples will result consistently negative throughout the study" and "A 100% point estimate shall be achieved for overall concordance, PPA, and NPA with the Anti-A,B (Series 1 and immuClone®) reagents" were met.

Reproducibility of immuClone® Anti-A,B IgM was confirmed by testing ten (10) replicates using three (3) antigen-positive and two (2) antigen-negative red cells with automated microplate testing. All expected-positive samples tested in replicates generated positive results, and all expected-negative samples tested in replicates generated negative results

**D. Lot-to-lot consistency**

Lot to lot verification was performed by testing three (3) different lots of immuClone® Anti-A,B IgM in tube, slide and manual microplate test methods. The testing confirmed sensitivity and specificity of 100 % for all lots.

**E. Robustness**

Robustness of immuClone® Anti-A,B IgM was demonstrated by testing the lower limit and then the upper limit for all test parameters (Red cell concentration, Red cell suspension volume, reagent volume, incubation time and incubation temperature, centrifugation time and speed) using manual tube, slide and microplate test methods. In addition, testing was carried out with variable red cell concentrations while retaining

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all other parameters at nominal. In total, five (5) test scenarios will be performed on each sample, for each method type. The results demonstrate robustness by meeting the acceptance criteria of "Results with each antigen positive cell must produce a positive result", "Results with each antigen negative cell must produce a negative result". For automated method using immuClone® Anti-A,B IgM the parameters of the approved automated assays are fixed and cannot be modified.

**F. Cut-off values**

Cut-off values of immuClone® Anti-A,B IgM for the use on the NEO platform

The cut-offs as set for immuClone® Anti-A,B IgM within the assays' Aurora DMS ATF files are defined with regards to clear distribution of negative and positive results obtained with the automated assay ABDLONG2K utilizing the immuClone® Anti-A,B IgM reagent on the NEO v2.0/Iris System (methods under test) in comparison to the automated predicate ABDLONG assay utilizing a CE-marked comparator device on the same system (reference method) while testing all samples.

The analysis of 309 tests with regards to negative and positive results distribution on the NEO Iris confirmed the cut-off values for immuClone® Anti-A,B IgM as described in the table below:

Assay	Grade	Lower Limit >	Upper Limit <=
ABODFULL	0	0	30
ABDLONG	2	30	76
ABDLONG2K	1+(not reported)	N/A	N/A
OCABD6 I			
OCABDLNG2	2+(not reported)	N/A	N/A
ABDLONG2			
BABY BG	3+(not reported)	N/A	N/A
ABDFULL2			
ABOD12 I	4+	76	100
BABY BG2			
ABODBB I			

Note: The 1+, 2+ and 3+ grades are not reported as they expand the equivocal range. This allows a follow up testing of equivocal results to determine mixed field reactions or weak expression of the antigen.

Note: The listed cut-off values represent the assays that are released at the time of preparing this report.

Note: The reagent immuClone® Anti-A,B IgM is used within different assays that run on the NEO Iris instrument (i.e. ABODFULL, ABDLONG). The difference of these assays are variations related to combination of reagents other than immuClone® Anti-A,B IgM. Therefore, the same cut offs can be used for the immuClone® Anti-A,B IgM on all assays.

Cut-off off values of immuClone® Anti-A,B IgM for the use on the Echo platform

The cutoffs as set for immuClone® Anti-A,B IgM reagent within the EU ABOD Full and EU Neonate assay file are defined with regards to clear distribution of negative and positive results obtained with the automated EU ABOD Full and EU Neonate assays under test utilizing the immuClone® Anti-A,B IgM reagent on the Echo v2.0/Lumena System using the Lumenera camera (methods under test) in comparison to results generated with the Digi camera on the Echo v2.0/Lumena System.

The analysis of 520 samples with regards to negative and positive results distribution on the NEO Iris confirmed the cut off values for immuClone® Anti-A,B IgM as described in the table below:

Assay	Grade	Lower Limit >	Upper Limit <=
ABOD Full	0	0	2
ABOD Full Screen	2	3	28
ABO1D Full	1+	29	30
ABO1D Full Screen	2+	31	45
Neonate	3+	46	62
RfxABOD Full	4+	63	100
RfxABOD Full Scrn			
RfxABO1D Full			
RfxABO1D Full Scrn			
RfxNeonate			

Note: The listed cut-off values represent the assays that are released at the time of preparing this report.

**G. Carry-Over**

Carry-over studies were performed for representative assays on the automated blood grouping instruments. The reagent assay testing was performed using the ABDFULL assay by running a full plate with twelve (12) samples of A-positive. All samples were

assigned to the reagent assay and started. If reagent carryover was present, the Anti-B wells in the assay would have displayed positive or equivocal behavior. All twelve (12) samples resulted in A-positive with no equivocal or positive Anti-B wells. Thus, no reagent carryover was detected. All in all, no sample or reagent carryover was revealed during execution of all testings and demonstrated that assays performed on the automated systems are absent of sample and reagent carryover.

#### H. Interfering substances

Interfering Substance Studies were performed with hemolytic, lipemic and icteric samples with the following concentrations tested:

- Triglycerides up to 600 mg/dL
- Bilirubin up to 30 mg/dL
- Albumin up to 5.2 g/dL
- Cholesterol up to 400 mg/dL
- Hemoglobin up to 20 g/dL

The study confirmed reliable and correct results up to the stated concentrations with the tested samples. Results obtained during this study with automated microplate method are also applicable for all intended purposes of the reagents since the employed method – hemagglutination and the principle is the same for Tube, Slide, Microplate and Automated Microplate Test.

#### Stability

Real-time stability testing of immuClone® Anti-A,B IgM was performed by testing three (3) lots every three (3) months for at least 3-6 months post expiration using manual tube, slide and microplate method. For real-time, vials assigned were stored in 1-10°C storage until the scheduled day of testing. The results verified the shelf life of immuClone® Anti-A,B IgM under real-time stability testing.

Short-term on-board stability was performed by testing ten (10) samples at least 3 (three) different time point using automated microplate test method. Reagent vials were left open and at room temperature on the instrument. The result demonstrated the qualitative result of the tested specimens did not change from positive to negative and vice versa after the reagent immuClone® Anti-A,B IgM was left for 72 hours on board.

Long-term on-board stability was performed by testing 10 (ten) samples twice per week over a period of 5 (five) weeks, which gives a total number of fifty (50) samples tested using automated microplate test method. Moreover, one aged test vial of immuClone® Anti-A,B IgM, which was at the end of its shelf-life, has been tested in the same settings and with the same samples as regular tested reagent vials. There was no discrepancy between the results obtained with aged reagent vial and regular tested reagent vials. **This study demonstrated that immuClone® Anti-A,B IgM retains activity for five weeks of intermittent use on the NEO the instrument when left in the instrument using direct agglutination method over the whole period of claimed shelf life as confirmed by the testing of aged reagent vial. When not in use, the reagent was stored at temperatures indicated in the IFU.**

#### Summary of Safety and Performance:

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REF	Description
▲ 0066003; 0066082	▲ immuClone® Anti-A,B IgM



Insert Code 236-2\*

Rev 08/23

\*The previous version of this IFU is 536-7

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