**Blood Grouping Reagent**

▲ **immuClone® Anti-B IgM**

**For Manual Tube, Slide, Microplate and Automated Microplate Tests (Qualitative)**

MOLDOVA EUROPEANĂ

IVD In Vitro Diagnostic Medical Device

Consult Instructions for Use

1°C 10°C Temperature limitation Store at +1 to +10°C

Discard if markedly turbid

**GHS06 Toxic**
**Danger**
**Preservative: =0.1% Sodium Azide**

IMMUCOR 237-3

**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.

| | |
|---|---|
| Blood Grouping Reagent | Blood Grouping Reagent |
| For Manual Tube, Slide, Microplate and Automated Microplate Tests (Qualitative) | For Manual Tube, Slide, Microplate and Automated Microplate Tests (Qualitative) |
| Murine Monoclonal | Murine Monoclonal |
| Clone | Clone |

## Intended Purpose:

▲ immuClone® Anti-B IgM is an in vitro diagnostic Blood Grouping Reagent used to detect the 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.[1] On the basis of observed reactions, Landsteiner divided the bloods of his colleagues into three distinct phenotypes; A, B and O.[2] Decastello and Sturli described the fourth phenotype of this system, AB, in 1902.[3]

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.[4-7]

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[8]. Transfusion of ABO-incompatible blood components/products results in acute hemolytic reaction followed by disseminated intravascular coagulation and acute kidney injury. Currently in developed world, ABO-incompatible transfusions and transfusion-related acute lung injury are the leading transfusion-related causes of death[9, 10]. 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 significant[9].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[11-13].

With the widespread prevention of rhesus alloimmunization, ABO Hemolytic Disease of the Fetus and 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.[8, 14-16]

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

The approximate frequencies of ABO antigens:[8]

| 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 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 17 and 18.

The device is designed to be used as in vitro diagnostic 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[19]. Specific for automated use of this reagent, training programs are provided as part of customer implementation of those instrument systems.

## Reagents:

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

▲ Anti-B is derived from clone LB-2 and is coloured with Napthol Yellow.

▲

Antibodies are diluted in a buffered saline solution containing bovine 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-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 murine donor or the cell line used to produce these reagents has been tested and found to be negative for Mouse Antibody Production (MAP) viruses.

CAUTIONS: — CAUTIONS:

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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 28th 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-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).

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## Procedure:

**Materials Provided**

▲ immuClone® Anti-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 15mM) 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* (e.g., 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 "v2.0" / 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 "v2.0" / Echo Lumena:**

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

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* 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.

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**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.

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*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 oval area approximately 20 x 40 mm. On plastic slides follow the manufacture'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 manufacture's inset. 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-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: [20]

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 ▲ B may produce reactions that are weaker than those obtained with ▲ 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.

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immuClone® Anti-B IgM does not recognize the acquired B antigen.

▲

The red cells of people with some disease states may give falsely positive or falsely negative reactions with ▲ anti-B.[10] 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 $A_1$ and B cells in serum (reverse) grouping tests. They can also produce nonspecific agglutination in cell (forward) tests with ▲ Anti-B ▲ 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 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-B IgM ▲ is 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.[21] 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-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-B IgM ▲ is tested by insert methods against a panel of antigen–positive red cells to ensure suitable reactivity. 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 Services at (+49) 6074 8420-50 or via e-mail: tech.support.eu@immucor.com.

| Technique | immuClone® Anti-B IgM | | | |
|---|---|---|---|---|
| | n | Diagnostic Sensitivity | n | Diagnostic Specificity |
| Tube | 120/120 | 100.00 | 380/380 | 100.00 |
| Slide | 120/120 | 100.00 | 380/380 | 100.00 |
| Automated Microplate(1) | 37/37 | 100% | 272/272 | 100% |
| Automated Microplate(2) | 93/93 | 100% | 412/412 | 100.00 |

(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.

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All performance characteristics related to the automated use and provided in this IFU were obtained using the Galileo NEO (Product Code 0064600) and NEO Iris (Product Code 0064598) instruments. The performance characteristics related to the automated use of the reagent on the Galileo Echo (Immucor, Inc. Product Code 0087000R) or Echo Lumena (Immucor, Inc. Product Code 0086998) can be found in the 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-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-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-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-B IgM using automated microplate method on the NEO v2.0/Iris system. The generated data were obtained from a clinical performance study with a total sample size per assay n = 814 using 32.4% clinical samples for the NEO v2.0/Iris 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 system using horizontal and vertical assays.

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**Analytical Performance**

**B. Accuracy**

Accuracy of immuClone® Anti-B IgM was confirmed by performing a Comparability Study. The objective of the study protocol (Comparability Study immuClone® Anti-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 shown that the reagent under test and the comparator reagent performed equivalent and did not differ qualitatively when results obtained with the same samples have been compared. Grading results of tested reagents did not change more than +/- 2 when comparing to each other. Calculated specificity and sensitivity met the defined criteria of "100% of the test results obtained with reagent under test shall not differ in qualitative result from the reference reagent", "Results will not differ +/- 2 or more reaction grade when compared to the reference reagent" and "The specificity and sensitivity of IVD under test should be ≥95% when results obtained with CE-marked comparator reagent are considered as reference". In addition, there was no discrepancy observed between results obtained with reagent under test and results obtained with CE-marked comparator reagent during the study. Therefore, the acceptance criteria as required for the Study Protocol Comparability Study immuClone® Anti-B IgM have been completely met.

**C. Precision**

Repeatability and Reproducibility were confirmed by testing three (3) samples in quadruplicate by three Verification Technologists on five nonconsecutive days over eleven days using the positive and negative cells. Results demonstrated that the acceptance criteria of "The immuClone® Anti-B IgM reagent 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-B (Series 3 and immuClone®) reagents" were met.

Reproducibility of immuClone® Anti-B IgM was confirmed by testing ten (10) replicates using two (2) antigen-positive and three (3) 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-B IgM. The testing confirmed sensitivity and specificity of 100 % for all lots.

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**E. Robustness**

Robustness of immuClone® Anti-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 method. In addition, testing was carried out with variable red cell concentrations while retaining all other parameters at nominal. 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". Thus, performance was found to be acceptable in terms of robustness. For automated method using immuClone® Anti-B IgM the parameters of the approved automated assays are fixed and cannot be modified.

**F. Cut-off values**

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

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

The cut-offs as set for immuClone® Anti-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 assays utilizing the immuClone® Anti-B IgM reagent on the NEO v2.0/Iris System (methods under test) in comparison to the automated predicate ABOD LONG assay utilizing the immuClone® Anti-B IgM 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-B IgM as described in the table below:

| Assays | Grade | Lower Limit > | Upper Limit <= |
|---|---|---|---|
| ABDCHECK2 | 0 | 0 | 30 |
| ABORH2 | ? | 30 | 76 |
| ABODFULL2<br>ABODFULLH<br>ABOD LONG<br>ABDLONG2<br>ABDLONG2K<br>ABDLONG3<br>ABDLONG4<br>ABDLONGK<br>ABD6_I<br>ABD6CDE_I<br>ABD6K_I<br>BABY_BG<br>AB_CTR2 | 1+(not reported)* | N/A | N/A |
| | 2+(not reported)* | N/A | N/A |
| | 3+(not reported)* | N/A | N/A |
| | 4+ | 76 | 100 |

*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.

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

The cut-offs as set for immuClone® Anti-B IgM reagent within the assay file are defined with regards to clear distribution of negative and positive results obtained utilizing the immuClone® Anti-B IgM reagent on the Echo v2.0/Lumena System.

The analysis of 504 samples with regards to negative and positive results distribution on the Echo platform confirmed the cut off values for immuClone® Anti-B IgM as described in the table below:

| Assay | Grade | Lower Limit > | Upper Limit <= |
|---|---|---|---|
| ABD_Type | 0 | 0 | 2 |
| ABOD Full | ? | 3 | 28 |
| ABOD Full Screen | 1+ | 29 | 30 |
| ABO1D Full | 2+ | 31 | 45 |
| ABO1D Full Screen | 3+ | 46 | 62 |
| ABOD Long Screen<br>ABO1D Long<br>ABO1D Long Screen<br>Neonate<br>ABOD Check2<br>ABOD Check2 Screen | 4+ | 63 | 100 |

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-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-B IgM under real-time stability testing.

Short-term on-board stability was performed by testing ten (10) samples at least three (3) different time points 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-B IgM was left for 72 hours on board. Moreover, ten (10) specimen were tested with one (1) aged lot of immuClone® Anti-B IgM close to the end of its shelf-life. There was no discrepancy between the results obtained with aged lot and the fresh lot.

Long-term on-board stability was performed by testing ten (10) samples twice per week over a period of five (5) weeks, which gives a total number of fifty (50) samples tested using automated microplate test method. Moreover, fifty (50) samples were tested with an aged lot of immuClone® Anti-B IgM close to the end of their shelf-life and compared to a fresh lot. There was no discrepancy between the results obtained with the aged lot and the fresh lot.

## Summary of Safety and Performance:

The Summary of Safety and Performance of this device is available via the Customer Center (www.immucor.com). Once available the Summary of Safety and Performance will be available via the EUDAMED database.

## Bibliography:


1. Landsteiner K. Zur Kenntnis der antifermentativen, lytischen und agglutinierenden Wirkungen des Blutserums und der Lymphe, Zbl Bakt 1990;27:357.
2. Landsteiner K. Über Agglutinationserscheinungen normalen menschlichen Blutes. Wien klin Wschr 1901;14:1132.
3. Decastello A von, Sturli A. Über die Isoagglutinine im Serum gesunder und kranker Menschen. Muchen med Wchnschr. 1902; 26:1090.
4. Voak D, Sacks S, Alderson T et al. Monoclonal anti-A from a hybrid- myeloma: evaluation as a blood grouping reagent. Vox Sang 1980:39:134.
5. Munro AC, Inglis G. Blue A et al. Mouse monoclonal anti-A and anti-B as routine blood grouping reagents: an evaluation. Med Lab Sci 1982;39:123.
6. Oak D, Lennox E, Sacks S et al. Monoclonal anti-A and anti-B: development as cost effective reagents. Med Lab Sci 1982;39:109.
7. Messeter L, Brodin T, Chester MA et al. Mouse monoclonal antibodies with anti-A, anti-B and anti-A,B specificities: some superior to human polyclonal ABO reagents. Vox Sang 1984;46:185.
8. Dean L. Blood Groups and Red Cell Antigens [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2005. Chapter 5, The ABO blood group. Available from: https://www.ncbi.nlm.nih.gov/books/NBK2267/.
9. Solheim BG. Universal pathogen-reduced plasma in elective open-heart surgery and liver resection. Clin Med Res. 2006 Sep;4(3):209-17. doi: 10.3121/cmr.4.3.209. PMID: 16988101; PMCID: PMC1570484.
10. Namikawa A, Shibuya Y, Ouchi H, Takahashi H, Furuto Y. A case of ABO-incompatible blood transfusion treated by plasma exchange therapy and continuous hemodiafiltration. CEN Case Rep. 2018 May;7(1):114-120. doi: 10.1007/s13730-018-0307-4. Epub 2018 Jan 31. PMID: 29383577; PMCID: PMC5886938.
11. Shachner TR, Clark CT. Acute Hemolytic Transfusion Reaction in Group B Recipient Associated with Group A Apheresis Platelet Donor: Case Report and Literature Review. Case Rep Med. 2018 Jun 24;2018:8259531. doi: 10.1155/2018/8259531. PMID: 30034477; PMCID: PMC6035818.
12. Malvik N, Leon J, Schlueter AJ, Wu C, Knudson CM. ABO-incompatible platelets are associated with increased transfusion reaction rates. Transfusion. 2020 Feb;60(2):285-293. doi: 10.1111/trf.15655. Epub 2020 Jan 8. PMID: 31912889; PMCID: PMC7769037.
13. Curtis BR, Edwards JT, Hessner MJ, Klein JP, Aster RH. Blood group A and B antigens are strongly expressed on platelets of some individuals. Blood. 2000 Aug 15;96(4):1574-81. PMID: 10942408.
14. Bel Hadj I, Boukhris R, Khalsi F, Namouchi M, Bougmiza I, Tinsa F, Hamouda S, Boussetta K. ABO hemolytic disease of newborn : Does newborn's blood group a risk factor? Tunis Med. 2019 Mar;97(3):455-460. PMID: 31729720.
15. Akanmu AS, Oyedeji OA, Adeyemo TA, Ogbenna AA. Estimating the Risk of ABO Hemolytic Disease of the Newborn in Lagos. J Blood Transfus. 2015:2015:560738. doi: 10.1155/2015/560738. Epub 2015 Sep 17. PMID: 26491605; PMCID: PMC4600530.
16. Rieneck K, Egeberg Hother C, Clausen FB, Jakobsen MA, Bergholt T, Hellmuth E, Grønbeck L, Dziegiel MH. Next Generation Sequencing-Based Fetal ABO Blood Group Prediction by Analysis of Cell-Free DNA from Maternal Plasma. Transfus Med Hemother. 2020 Feb;47(1):45-53. doi: 10.1159/000505464. Epub 2020 Jan 16. PMID: 32110193; PMCID: PMC7036558.
17. Race RR, Sanger R. Blood groups in man. 6th ed. Oxford: Blackwell Scientific, 1975: 9-56.
18. Nance ST, Serology of the ABH and Lewis blood group systems. In : Wallace ME, Gibbs FL, eds. Blood group systems: ABH and Lewis. Arlington VA: American Association of Blood Banks, 1986:57-81.
19. EN ISO 18113-1:2011 - In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 1: Terms, definitions and general requirements.
20. Brecher ME, ed. Technical manual. 14th ed. Bethesda MD: American Association of Blood Banks, 2002.
21. Crawford MN, Gottman FE, Gottman CA, Microplate system for routine use in blood bank laboratories. Transfusion 1970;10:258.


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