Blood Grouping Reagent
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immuClone® (1) Anti-K IgM
immuClone® (1) Anti-K Galileo IgM
For Manual Tube, Slide, Microplate and Automated Microplate Tests

- IVD In Vitro Diagnostic Medical Device
- Consult Instructions for Use
- +2 °C +8 °C Temperature limitation
- Discard if markedly turbid

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.

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

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BLOOD GROUPING REAGENT

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For Manual Tube, Slide, Microplate and Automated Microplate Tests

IMMUCOR®

Blood Grouping Reagent	Blood Grouping Reagent
For Manual Tube, Slide, Microplate and Automated Microplate Test (Qualitative)	For Manual Tube, Slide, Microplate and Automated Microplate Test (Qualitative)
Human Monoclonal	Human Monoclonal
Clone	Clone

Intended Purpose:

▲ immuClone® (1) Anti-K IgM and immuClone® (1) Anti-K Galileo IgM are in vitro diagnostic Blood Group Reagents used to detect the K (Kell) 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). immuClone® (1) Anti-K IgM is intended for Manual Tube, Slide and Microplate Tests (qualitative). immuClone® (1) Anti-K Galileo IgM is intended for Automated Microplate Tests (qualitative).

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Summary:

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Since the discovery of the K antigen (K1 or Kell) by Coombs[1] in 1946 and its antithetical partner, k (K2 or Cellano) by Levine in 1949, the Kell system classification has been expanded to include 25 phenotypes[2]. Anti-K (Anti-K1) and Anti-k (Anti-K2) can cause severe hemolytic transfusion reactions (HTR) and haemolytic disease of the newborn (HDFN)[3].

A common HTR is alloimmunization and occurs as immune response against foreign red blood cell antigens potentially leading to hemolysis in transfused patients. Especially in multi-transfused thalassemia patients Kell-autoimmunization causes significant health problems[4].

The most common Kell antigens that cause HDFN are Kell (K1) and Cellano (K2). The K1 antigen is found on red blood cells (RBC) of 9% of the population and is associated with 5% risk of an affected fetus in Kell-alloimmunized pregnancies[5]. As K-immunization is often associated with RBC transfusions of mothers' history it can be prevented by selecting K-negative units for transfusion to women of childbearing age[6]. Determining noninvasive the blood group of the fetus can enable early monitoring and treatments[7].

The frequencies of the K and k antigens vary in different populations[8]:

Phenotype	Caucasians	Blacks
K-k+	91%	98%
K+k-	0.2%	Rare
K+k+	8.8%	2%

Principle:

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The tests used with these monoclonal Blood Grouping Reagents are based on the principle of hemagglutination. When the insert procedure is followed, agglutination of red cells following incubation with immuClone® (1) Anti-K IgM and Galileo (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.

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[9]. Specific for automated use of this reagent, training programs are provided as part of customer implementation of those instrument systems.

Reagents:

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immuClone® (1) Anti-K IgM and Galileo is prepared from monoclonal, human IgM antibodies (MS-56). Antibodies are diluted in a buffered saline solution containing bovine albumin, and macromolecular chemical potentiators. 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.▲

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

The concentration of the active ingredient is indicated with the titer. The minimum titer for immuClone® (1) Anti-K IgM and Galileo is defined at 1:16 using a heterozygous cell. The lot specific titer is documented on the respective Certificate of Analysis.

Precautions:

For in vitro diagnostic use by trained professionals only.

Sodium azide (< 0.1%) has been added as a preservative to these reagents.

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 2-8°C when not in use. Do not freeze or expose to elevated temperatures.

Discard if markedly turbid — Discard if markedly turbid

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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 if a precipitate, fibrin gel or particles are present. 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, EBV 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 expiry 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.

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.

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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.

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In manual and automated testing using immuClone® (1) Anti-K IgM and Galileo, samples drawn into EDTA and citrate-based anticoagulant group (e.g., CPDA) can be used.

Blood samples 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® (1) Anti-K IgM and immuClone® (1) Anti-K Galileo IgM antisera 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. 10x75mm or 12x75 mm test tubes and a test tube rack
3. Serological centrifuge*
4. Interval timer

Microplate methods (manual):

1. Transfer pipettes or pipetting system* (e.g., ABS Precis, Hamilton Microlab AT, Packard Multiprobe 104/204)
2. Microplates*
3. Centrifuge* (e.g., Sorval 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)

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Slide method:

1. Glass or plastic slides
2. Wax marker (optional)
3. Applicator sticks
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 "v2.0" (Product Code 0064600) or NEO Iris (Product Code 0064598)

Automated Microplate method using the Galileo Echo "v2.0" / 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 "v2.0" (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 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 produced 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.

NOTE: Incubation for 5-60 minutes at 18-30°C may be necessary to enhance the reactivity of the blood grouping reagents with some of the rare phenotypes.

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.

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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 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 correct reactivity of immuClone® (1) Anti-K IgM and Galileo, it is recommended that these reagents be tested each day of use with antigen positive and antigen negative cells, such as Immucor corQC Extend (Product Code 0066297). For QC frequency minimum requirements refer to national guidelines. These reagents can be considered to be satisfactory if the antigen-positive cells are agglutinated and antigen negative cells are not agglutinated. In addition, the use of immuClone® Rh-Hr Control reagent (Immucor Med. Diagnostik GmbH, Product Codes 0006720, 0006721, 0066006, 0066083) is recommended for detection of potentially false positive results.

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Interpretation of Results[10]:

Positive Test (antigen detected): agglutination of red cells.

Negative Test (antigen not detected): 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. Many monoclonal human IgM anti-Rh antibodies have been shown to possess anti-I/i cold agglutinin activity, particularly with cord cells or enzyme tested cells[11]. This may become apparent if tests are incubated below the recommended temperature.

Red cells that have a positive direct antiglobulin test (DAT) or are from patients with autoimmune disease or protein abnormalities 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.

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[12]. 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.

Under-centrifugation or over-centrifugation may result in the occurrence of numerous false-negative or false positives.

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

Autoagglutinins reactive at room temperature are a potential source of error in phenotyping tests. The presence of these antibodies cannot be predicted. They can produce nonspecific agglutination when unwashed, plasma-suspended or serum – suspended cells are used[13]. For this reason, the use of immuClone® Rh-Hr Control reagent (Product Code 0006720, 0006721, 0066006, 0066083) is recommended for detection of such false positive results.

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Rare antigen variants have been discovered such as a mutated allele described as *KEL 2* which can cause a weakened Kell antigen expression. Those rare antigen variants can have variable reactivity with some monoclonal Anti-K reagents. Reactivity with these cells cannot be guaranteed[14,15].

Deviation from the Recommended Directions 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. User-defined modifications to test procedures may require validation.

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:

The results obtained show that ▲ immuClone® (1) Anti-K IgM and immuClone® (1) Anti-K Galileo IgM are safe and effective products for the determination of the presence of ▲ K antigen.

Prior to release, each lot of immuClone® (1) Anti-K IgM and immuClone® (1) Anti-K Galileo IgM antiserum is tested by insert methods against a panel of appropriate antigen-positive and antigen-negative red cells to ensure appropriate reactivity and specificity. The performance of these products 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.

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Technique	immuClone® (1) Anti-K IgM and Galileo			
	n	Diagnostic Sensitivity	n	Diagnostic Specificity
Tube	16/16	100%	87/87	100%
Slide	16/16	100%	87/87	100%
Automated Microplate(1)	644/644	100%	1139/1139	100%
Automated Microplate(2)	65/65	100%	228/228	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 Echo Lumena (Product Code 0086998) instruments. The data are also applicable to the Galileo Echo "v2.0" system. 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.

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

Diagnostic Sensitivity and Specificity of 100% have been obtained for immuClone® (1) Anti-K 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, 103 samples have been tested with both, immuClone® (1) Anti-K IgM and CE-marked comparator device using 27.18% clinical and 4.85% 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® (1) Anti-K IgM performs equivalent to the state-of-the-art CE-marked comparator reagent.

100% Diagnostic Sensitivity and Specificity have been established for immuClone® (1) Anti-K Galileo IgM using automated microplate method on the NEO v2.0/Iris system. The generated data were obtained from a clinical performance study with a sample size n = 1783 using 33.8% clinical samples. 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 grading of wells shall be within +/-1 between the visual grade and NEO v2.0/Iris assays under test 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.

Analytical Performance

B. Accuracy

Accuracy of immuClone® (1) Anti-K IgM was confirmed by performing a comparability study. The objective of the study protocol (Comparability Study immuClone® (1) Anti-K IgM) was to demonstrate the appropriate reactivity of the immuClone® (1) Anti-K IgM 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 50 donor blood samples in triplicates with the reagent under test (150 tests in total) and the same 50 donor blood samples with comparator reagent using manual tube test method. 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® (1) Anti-K IgM have been completely met.

C. Precision

Repeatability of immuClone® (1) Anti-K IgM was confirmed by testing six (6) replicates in parallel using two (2) antigen positive and two (2) antigen negative cells in tube, slide and manual microplate test methods. Results demonstrated that the acceptance criteria of "Replicate results with each antigen positive cell must produce a positive result and be within ± 1 reaction grade" and "*Replicate results with each antigen negative cell must produce an unequivocal negative result"* were met.

Repeatability of immuClone® (1) Anti-K Galileo IgM was confirmed by testing ten (10) replicates using three (3) antigen positive cells and two (2) antigen negative cells with automated microplate testing. Results demonstrated that the following acceptance criteria were met:

- Overall concordance will be ≥ 95% at the lower bound 95% confidence interval
- Positive percent agreement LCL ≥ 95%
- Negative percent agreement LCL ≥ 95%

All expected-positive samples tested in replicates generated positive results, and all expected-negative samples tested in replicates generated negative results.

Reproducibility of immuClone® (1) Anti-K IgM was confirmed by compiling from historical specificity testing with a minimum of six (6) occasions using four (4) antigen positive and (4) antigen negative red cells. The lot and reference data were used to determine reproducibility on different days, with different analysts using different reagents in tube (48 positive and 48 negative reactions), slide and manual microplate test (24 positive and 24 negative reactions) methods. Results demonstrated that the acceptance criteria Replicate results with each antigen positive cell must produce a positive result and be within ± 1 reaction grade" and "Replicate results with each antigen negative cell must produce an unequivocal negative result" were met.

Reproducibility of immuClone® (1) Anti-K Galileo IgM testing ten (10) replicates using three (3) antigen positive cells and two (2) antigen negative cells with automated microplate testing. Results demonstrated that the following acceptance criteria were met:

- Overall concordance will be ≥ 95% at the lower bound 95% confidence interval
- Positive percent agreement LCL ≥ 95%
- Negative percent agreement LCL ≥ 95%

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 six (6) different lots of immuClone® (1) Anti-K IgM in tube, slide and manual microplate test methods. The testing confirmed sensitivity and specificity of 100 % for all lots.

E. Whole system failure rate

The whole system failure rate of immuClone® (1) Anti-K Galileo IgM for Use on the automated systems is covered by the Precision studies performed on the instrument. The analysis compared the interpretations of the samples, as well as interpretations between-instrument, between-run and between-day variation. The acceptance criteria were met and the data obtained for repeatability is applicable to confirm whole system failure rate of immuClone® (1) Anti-K Galileo IgM on the instrument. There are no false negative results in repeat assays for positive specimen.

F. Robustness

Robustness of immuClone® (1) Anti-K IgM was demonstrated by testing the lower limit for all test parameters (Red cell concentration, red cell suspension volume, reagent volume, incubation time and incubation temperature and centrifugation time and speed) and then the upper limit for all test parameters in tube and manual microplate test methods. In addition, testing was performed with variable red cell ratio while retaining all other parameters at nominal. The results demonstrate robustness by meeting the acceptance criteria of „Replicate results with each antigen positive cell must produce a positive result and be within ± 1 reaction grade" and "Replicate results with each antigen negative cell must produce an unequivocal negative result". For automated method using immuClone® (1) Anti-K Galileo IgM the parameters of the approved automated assays are fixed and cannot be modified.

G. Cut-off values

Cut-off off values of immuClone® (1) Anti-K Galileo IgM for the use on the NEO platform

The cut offs as set for immuClone® (1) Anti-K Galileo IgM reagent within the assay files are defined with regards to clear distribution of negative and positive results obtained with the automated assays under test (PHENO16, RHFORMEL, Kell) utilizing the immuClone® (1) Anti-K Galileo IgM reagent on the NEO v2.0/Iris System in comparison to the automated predicate assays utilizing immuClone® (2) Anti-K Automated IgM reagent (PHENO16_4) on the NEO v2.0/Iris System while testing the same samples. The analysis of 897 samples with regards to negative and positive results distribution on the NEO Iris confirmed the cut-off values for immuClone® (1) Anti-K Galileo IgM as described in the table below:

Assay	Grade	Lower Limit >	Upper Limit <=
PHENO16 PHENO16_2	0	0	23
PHENO12 PHENO12_2 RHFORMEL	?	23	35
RHFORM_D RHFORM_Cw AG_K Kell	1+ (not reported)	35	35
	2+	35	50
	3+	50	80
	4+	80	99.9

Note: 1+ grades are not reported as it expands 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 document.

Cut-off off values of immuClone® (1) Anti-K Galileo IgM for the use on the Echo platform

The cut-offs as set for immuClone® (1) Anti-K Galileo IgM within the assay file are defined with regards to clear distribution of negative and positive results obtained with the automated Ag_CcEeK assay under test utilizing the immuClone® (1) Anti-K Galileo IgM reagent on the Echo v2.0/Lumena System (methods under test) in comparison to the automated predicate Ag_CcEeK2 assay utilizing the immuClone® (2) Anti-K Automated IgM reagent on the Echo v2.0/Lumena System (reference methods) while testing all samples. The analysis of 293 samples with regards to negative and positive results distribution on the Echo platform confirmed the cut off values for immuClone® (1) Anti-K Galileo IgM as described in the table below:

Assay	Grade	Lower Limit >	Upper Limit <=
Ag_Kell Auto_Kell Kell1u2 Ag_CcEeK Ag_CEceK	Negative	0	5
	Query	6	9
	1+	10	30
	2+	31	45
	3+	46	62
	4+	63	100

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

H. Carry-Over

Carry-over studies were performed for representative assays on the automated blood grouping instruments. The reagent assay testing was performed using the Ag_CcEe assay by running a full plate with twelve (12) samples of little-c positive, E-negative samples ten (10) times. All samples were assigned to the reagent assay and started. If reagent carryover was present, the Anti-E wells in the assay would have displayed positive or equivocal behavior. All twelve (12) samples resulted in little-c positive, E-negative results with no equivocal or positive Anti-E wells. In addition, ABDFULL testing was performed by running a full plate of twelve (12) A-positive samples. All samples were assigned to the ABDFULL assay and started. All twelve (12) samples resulted in A-positive results 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.

I. Interfering substances

Interfering Substance Studies were performed with hemolytic, lipemic and icteric samples. The studies confirmed reliable and correct results 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 manual Tube, Slide, Microplate and Automated Microplate Test.

Stability

Short-term on-board stability study of immuClone® (1) Anti-K Galileo IgM was performed by testing ten (10) samples at 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® (1) Anti-K Galileo IgM were left for 72 hours on board.

Long-term on-board stability study of immuClone® (1) Anti-K Galileo IgM was performed by testing ten (10) samples twice per week over a period of five (5) weeks using automate microplate testing. This study demonstrated that immuClone® (1) Anti-K Galileo IgM retains activity and provide expected, reliable results for five (5) weeks of intermittent use when tested. When not in use, the reagent was stored at temperatures indicated in the IFU.

A long-term in-use stability study was performed to demonstrate the long-term stability of the immuClone® (1) Anti-K Galileo IgM reagent after the first opening of the primary container. In this study, three (3) independent vials of the same lot of reagent have been tested with an automated method for specificity and manually for potency after the first opening of the primary container. The testing was performed every three (3) months over a time period of 36 months according to the instructions for use and the specificity and potency of reaction was determined. The study demonstrated the reliable use of the reagent after the first opening of the primary container until the end of the shelf-life.

Shipping stability and stress testing of immuClone® (1) Anti-K IgM was performed by testing nine (9) samples at nine (9) different time point over the entire shelf life (36 months) using manual tube test method. Reagent vials were left open and at room temperature on the instrument. An extreme condition (extreme heat) was simulated during transport, afterwards the product was incubated at 37°C for three days then storing at 2-8°C for the proposed shelf life of the product. The results demonstrated the immuClone® (1) Anti-K IgM is stable during transport stability conditions up to thirty-six (36) months.

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.

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REF	Description
▲	▲
0008016; 0008026	immuClone® (1) Anti-K IgM
0066020	immuClone® (1) Anti-K Galileo IgM

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

CE 0197

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