UnityPoint Health Pekin
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Date Reviewed/ Date Revised: New Procedure

BIO-RAD Rh PHENOTYPING

I. PRINCIPLE

Bio-Rad Seraclone Rhesus Blood Group Reagents are used to test for the presence or absence of the Rhesus antigens C, c, D, E, and e. Routine pretransfusion studies always include tests for the D antigen. Other Rhesus reagents like Bio-Rad Seraclone Anti-C (RH2), Seraclone Anti-c (RH4), Seraclone Anti-E (RH3), and Seraclone Anti-e (RH5) are used principally in the resolution of antibody problems or family studies. The test principle is hemagglutination. The antibodies in Seraclone Blood Grouping Reagents bind to the corresponding antigen on red blood cells and cause an antigen-antibody reaction visible as red blood cell agglutination.

II. CLINICAL SIGNIFICANCE

UnityPoint Health-Pekin Laboratory will utilize this procedure to perform Rh phenotyping.

III. SPECIMEN

Fresh samples of EDTA (pink top tube) anticoagulated whole blood collected following general blood sampling guidelines are acceptable. The specimen is to be tested as soon as possible after collection. If testing is delayed, specimens should be stored at 2-8°C. Blood specimens exhibiting hemolysis or contamination should not be used. Specimens collected in EDTA may be tested within ten days from collection. Donor blood stored in citrate anticoagulant may be tested until the expiration date of the donor unit.

IV. REAGENT

A. Bio-Rad Seraclone Blood Grouping Reagents contain the reactive components human monoclonal antibodies of the immuglobulin class IgM. They are derived from cell culture supernatant and demonstrate the consistent specificity and reproducibility characteristic for monoclonal antibodies. Seraclone Anti-E (RH3) does react with E^w.

1. Anti-C- The antibodies are diluted in an isotonic saline solution containing bovine albumin.
2. Anti-c- The antibodies are diluted in a buffered saline solution containing macromolecular potentiator.
3. Anti-E and Anti-e- Antibodies are diluted in a buffered protein solution containing macromolecular potentiators.
4. All have a 0.1% Sodium azide preservative.

B. Precautions:

1. For in-vitro diagnostic use.

2. Store at 2 to 8°C.
3. Do not use beyond the expiration date.
4. Do not use if turbid.
5. Handle of dispose of reagents as potentially infectious.
6. Caution: Do not pipette by mouth. The absence of all viruses has not been determined.
7. Caution: This product contains natural rubber latex which may cause allergic reactions.
8. Warning: Contains sodium azide which may react with lead or copper plumbing to form explosive azides. If discarded in the sink, flush with large amounts of water to prevent the buildup of explosive metal azides.
9. The bovine albumin used for the production of this reagent is sourced from donor animals of U.S. origin that have been inspected and certified by U.S. Veterinary Service inspectors to be disease free.

V. QUALITY CONTROL

The reactivity of all Blood Grouping Reagents is to be confirmed by testing with known positive and negative red blood cells on each day of use. To confirm the reactivity or specificity of Bio-Rad Monoclonal Rh Blood Grouping Reagents, each is to be tested with antigen-positive and antigen negative red blood cells, and the Seraclone Control ABO & Rh. Each reagent is satisfactory for use if it reacts only with antigen-positive red blood cells.

VI. PROCEDURE:

A. Label each tube with the letter of the antigen being tested.
B. Label with + or = control, ABO Rh control, donor number or initials of patient being tested.
C. Prepare a 3 to 5% suspension of red blood cells to be tested in saline.
D. Place one drop of reagent into each appropriately labeled tube.
E. Add one drop (approx. 40-50 µL) of red blood cell suspension into the tube and mix.
F. Incubate at room temperature (15-30°C) for 5-10 minutes.
G. Centrifuge for:
    1. 20 seconds at 800 to 1000 x g,
    2. or at a time and speed appropriate for the centrifuge calibration.
H. Gently dislodge red blood cell button and observe for macroscopic agglutination. Negative reactions are to be examined with an agglutination viewer.
I. Record results.

VII. REPORTING RESULTS

A. Following centrifugation, all tubes are to be read immediately and results interpreted without delay. Time delays may cause a dissociation of the antigen-antibody complexes resulting to false negative or more often weak positive reactions.
B. Agglutination of the red blood cells is a positive result and indicates the presence of the corresponding antigen.
C. No agglutination is a negative result and indicates the absence of the corresponding antigen.
D. An agglutination viewer may facilitate the reading of tube tests (as recommended by the AABB Technical Manual).
E. Frequencies in the population are listed in the "Summary" section of the package insert.

VIII. LIMITATIONS:

A. Samples with a positive direct antiglobulin test, cold agglutinins, or rouleaux formation may show false positive results in testing with monoclonal antibodies. Results on these samples must be interpreted with caution. False positive results or reaction suspected to be due to cold agglutinins should be resolved according to in-house procedures.
B. The Anti-C clone MS24 gives only a weak positive reaction if the C and E antigens are located on the same chromosome.
C. Some conditions that may cause false positive results are:
    1. Contamination of the sample or reagents
    2. Autoantibodies
    3. Improper storage or preparation of red blood cells
    4. Antibodies to antibiotics or other reagents
    5. Cold antibodies

IX. REFERENCES

A. Bio-Rad Medical Diagnostics GmbH, Dreieich, Germany, Blood Grouping Reagent, Anti-C, Anti-c, Anti-E, Anti-e Seraclone Human Monoclonal, 186251/09, Revised 08/2014.

POLICY CREATION :	*Date*
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Rev	Description of Change	Author	Effective Date

Reviewed by

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