UnityPoint Health Pekin
Department of Pathology
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Effective Date: 05/03/19
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Rh GROUPING (ANTI-D)

I. PRINCIPLE:

The test principle is hemagglutination. The antibodies in Seraclone® Anti-D (RH1) Blend bind to the D antigen on the red blood cells being tested and cause an antigen-antibody reaction visible as red blood cell agglutination.

II. CLINICAL SIGNIFICANCE:

The purpose of this procedure is to determine the Rh type of blood samples. The presence or absence of the D antigen is determined by testing the red blood cells with Anti-D. Agglutination indicates that the test cells are D-positive. No agglutination indicates that the test cells are D-negative, subject to a negative indirect antiglobulin test for weak D. Red cell possessing a weak D antigen may give a negative or perceptibly weaker-than-normal reaction in the direct agglutination phases of the test, but will normally yield definite agglutination at the antiglobulin phase. No agglutination at the antiglobin phase of the test indicates that a D antigen capable of detection is not present on the red cells.

III. SPECIMEN:

No special preparation of the patient is required prior to specimen collection. A pink top tube should be drawn by an aseptic technique. The specimen should be tested as soon as possible after collection. If delay in testing should occur, the specimen must be stored at 2° to 8°, and it should be tested within 10 days of collection. Citrated specimens (donor segments) should be stored at 1 to 6°C. Donor blood may be tested up to the expiration date. Blood specimens exhibiting gross hemolysis or contamination should not be used.

IV. INSTRUMENTATION/EQUIPMENT

A. 12 x 75 mm test tubes
B. Plastic transfer pipettes
C. Centrifuge
D. Automatic cell washer (if chosen for the Du testing)

V. QUALITY CONTROL

Quality control testing is performed daily on the Anti-D and the ABO+Rh control reagents with Immucor corQC kit.

VI. PROCEDURE:

A. Label two test tubes (12 x 75 mm), one D and one Rh control.
B. Label each tube with first and last initial of patient being tested. (Lengthen the minimum letters to differentiate the patients with the same initials, if necessary.)

C. Place 1 drop Anti-D Reagent and 1 drop Rh control into properly labeled test tubes (12 x 75 mm).
D. Add to each tube 1 drop of a freshly prepared 3-5% cell suspension of patient's blood in physiologic saline; or an equivalent amount of patient cells from whole blood adhering to the tip of an applicator stick (to facilitate reading a drop of physiologic saline may be added after centrifugation).
E. Shake to mix reagents.
F. Centrifuge for 20 seconds, or for optimum calibrated spin time, at 3400 rpm (approximately 1000 ref).
G. Gently resuspend cells completely and examine immediately for macroscopic agglutination. Grade and record results of D testing. Incomplete resuspension may counterfeit agglutination. If test reaction is negative, add 1 additional drop of Anti-D Reagent, shake to mix and centrifuge for 20 sec. Gently resuspend and examine for agglutination. If test is negative and weak D test is indicated (weak D tests are done on cord blood samples and positive feto-maternal hemorrhage rapid screens/fetal screens).
    1. Weak D Test (formerly D^u):
        a. Incubate test at 36-38°C for 15-30 minutes.
        b. Centrifuge for 20 seconds, or optimum calibrated spin time. If definite macroscopic agglutination is observed, the cells are D positive. Otherwise, proceed to next step.
        c. Wash tubes 3X with saline and decant completely (automatic cell washer may be used).
        d. Add 2 drops of antihuman serum (Anti-IgG Coombs) to each tube, mix and centrifuge for 20 seconds, or optimum calibrated spin time, at 3400 rpm (1000 ref).
        e. Gently resuspend cells completely and examine macroscopically for agglutination. Negative reactions may be examined with an agglutination viewer, however microscopic examination is not recommended.
        f. Add one drop of Coombscell-E control to all tubes with negative results and centrifuge for 20 seconds, or optimum calibrated spin time, at 3400 rpm (1000 ref). If cells are agglutinated, test result is valid, if not, testing must be repeated.
        g. Cap the original specimen and store in refrigerator for 14 days.
H. ABORH orders from ER:
    1. If patient is Rh negative, call ER for diagnosis of possible pregnancy.
    2. If patient is pregnant, ask if Rhogam will be needed. If so, instruct ER to order ANTRHG. (Cancel or credit ABORH)

3. Ask for gestational age, if pregnant. If more than 13 weeks, order and perform fetal screen testing: FS – Sun Quest order code. (See procedure UPPK BB-380).

VII. REPORTING RESULTS

A. Agglutination with Anti-D report as Rh positive.
B. No agglutination with Anti-D, agglutination after weak D test – report as Rh positive (weak D comment).
C. No agglutination with Anti-D, no agglutination after weak D test – report as Rh negative.
D. If control is positive, the test cells should be washed several times in saline and retested. If the control again gives a positive reaction, a valid interpretation of the results obtained with the Anti-D cannot be made.
E. Add on the Rh control test in Sun Quest with the little 'c' key on the keyboard to all ABO+Rh typings.
F. Add on the Du and Du control in Sun Quest to all typings that require the Du procedure (cord bloods) by using the ` key (key to the left of the 1 key) on the keyboard. Rhogam testing does not require the Du testing and will be resulted as Not Performed (5 key) for the reactions and Not Required for the Interpretation (;NRQ) unless already automatically hidden by the computer system.
G. Enter reaction results in computer. Enter interpretations in computer as positive or negative.

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. If the immediate reaction with Anti-D (RH1) Blend is negative, a test for weak D or partial D must be performed on cord blood samples and positive feto-maternal hemorrhage rapid screens/fetal screens.
C. Insufficient or inappropriate washing can lead to false negative or false positive reactions. Small amounts of residual patient sera/plasma can neutralize the Anti-Human Globulin.
D. Some conditions that may cause false positive results are:
    1. Contamination of 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-D (RH1) Blend Seraclone® Human Monoclonal Blend, 186249/10, Rev. 08/2014.

POLICY CREATION :		***Date***
Author:	***Sharrol Brisbin, MT (ASCP)***	***02/04/2003***
Medical Director:	***Kathryn Kramer, MD***	***02/04/2003***

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REVISION HISTORY (began tracking 2011)			
Rev	Description of Change	Author	Effective Date
01	Deleted weak D testing on females of childbearing age, added weak D only performed on cord bloods +positive fetal screens.	Jenny turner	02/26/19
02	Added instructions for how to report out the Du in SQ not performs anymore.	Jenny Turner	05/03/19

Reviewed by:

Jennifer Turner	5-6-19				