UnityPoint Health Pekin
Department of Pathology
Pekin, IL 61554

Effective Date: 05/03/19
Date Reviewed/ Date Revised: 09/03/19

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. A lavender top tube may be used for an ABO/Rh confirmation. 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

V. REAGENTS

A. Seraclone® Anti-D (RH1) Blend
B. Seraclone® Control ABO+Rh

VI. QUALITY CONTROL

A. Quality control testing is performed daily on the Anti-D and the ABO+Rh control reagents with Immucor corQC kit.
B. A negative control will be performed on samples testing positive with Anti-A,

Anti-B, and Anti-D. Seraclone® Control ABO+Rh will be used for this.

VII. PROCEDURE

A. Label a test tube (12 x 75 mm) as Anti-D. ABO+Rh Control will only be performed if you have an AB Positive patient.
B. Label the tube with first and last initial of the patient being tested. (Lengthen the minimum letters to differentiate the patients with the same initials, if necessary.)
C. Place 1 drop Anti-D Reagent into the properly labeled test tube.
D. Add to the 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 gently to mix.
F. Centrifuge for 20 seconds, or for optimum calibrated spin time, at 3400 rpm (approximately 1000 ref).
G. Gently dislodge red cell button and observe for macroscopic agglutination. Negative reactions may be examined with an agglutination viewer, however microscopic examination is not recommended. Grade and record reaction result. If test reaction is negative, add 1 additional drop of Anti-D Reagent, shake to mix and centrifuge for 20 sec. Gently dislodge and examine macroscopically for agglutination. If the test reaction is still negative, a Weak D test only needs to be performed on women who have a positive Fetal Screen. If this occurs, specimen will be sent to Methodist for the weak D testing.
H. If patient is typing as an AB Positive, label a test tube (12 x 75) as ABO/Rh control.
I. Label the tube with the first and last initial of the patient being tested. (Lengthen the minimum letters to differentiate the patients with the same initials, if necessary.)
J. Place 1 drop of Seraclone® ABO/Rh Control Reagent into the properly labeled tube.
K. Add to the 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).
L. Shake gently to mix.
M. Centrifuge for 20 seconds, or for optimum calibrated spin time, at 3400 rpm (approximately 1000 ref).
N. Gently dislodge red cell button and observe for macroscopic agglutination. Negative reactions may be examined with an agglutination viewer, however microscopic reading is not recommended.
O. A valid ABO/Rh Control will give a negative result.

P. 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 send fetal screen testing to Methodist: FS (K^2 EDTA pink or lavendar top tube stored at 2-8°C). If Rhogam is needed, give one dose to start with. If an additional dose(s) is required after the results of the Fetal Screen are done, we will have the patient called back in.

VIII. REPORTING RESULTS

A. Agglutination with Anti-D report as Rh positive.
B. No agglutination with Anti-D, agglutination after weak D test (if applicable) – report as Rh positive (weak D comment).
C. No agglutination with Anti-D, no agglutination after weak D test (if applicable) – report as Rh negative.
D. For AB Positive patients, 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. Samples with autoimmune antibodies, cold agglutinins, or rouleaux formation may show false positive reactions in testing.
E. Add on the Rh control test in Sun Quest to only AB Positive patients with the little 'c' key on the keyboard.
F. Rhogam testing does not require the weak D 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.

IX. 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 patients with a positive feto-maternal hemorrhage rapid screens/fetal screens. If necessary, the weak D testing will be sent to Methodist.
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

X. REFERENCES

A. Bio-Rad Medical Diagnostics GmbH, Dreieich, Germany, Blood Grouping Reagent Ant-D (RH1) Blend Seraclone® Human Monoclonal Blend, 186249/10, Rev. 08/2014.

B. Bio-Rad Medical Diagnostics GmbH, Dreieich, Germany, Seraclone® Control ABO+Rh, 187701/07, Rev. 08/2014.

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

MEDICAL DIRECTOR		
DATE	NAME	SIGNATURE
9/03/19	Lori Rausa	
SECTION MEDICAL DIRECTOR		

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
03	Added Reagents. Changed Rh Control to only on AB Positive patients. Fetal Screens to Methodist. Weak D testing to Methodist if indicated. Added ABO/Rh Control pkg insert reference. Deleted cord bloods.	Jenny Turner	09/01/19

Lead	Date	Coordinator/ Manager	Date	Medical Director	Date