UnityPoint Health Pekin
Department of Pathology
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Effective Date: 10/18/18
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ABO Reverse Grouping

Test Code: ABORH

I. PRINCIPLE

The test principle is a hemagglutination test. The antigens of the Reagent Red Blood Cells react with the respective antibodies in the serum or plasma to be tested. The existence or lack of Anti-A and/or Anti-B antibodies must correspond with the existence or lack of A and/or B antigens on the Reagent Red Blood Cells.

II. CLINICAL SIGNIFICANCE

Bio-Rad Reagent Red Blood Cells A^1 & B are used to test for the presence or absence of the corresponding antibodies in reverse grouping for the ABO system. Routine pretransfusion studies always include tests for the ABO antigens and reverse grouping.

III. SPECIMEN

A. Plasma from a pink top (EDTA) tube collected following general blood sampling guidelines is acceptable. The specimen should be tested as soon as possible after collection.
B. If testing is delayed blood specimens should be stored at 2° to 8°C or the plasma can be separated from the red blood cells and frozen.
C. Stored samples should be allowed to reach room temperature prior to testing.
D. Blood specimens exhibiting gross hemolysis or contamination are not acceptable.
E. EDTA samples older than 10 days can be tested, however antibody reactivity has been shown to decrease in older samples.

IV. REAGENT

A. Biotestcell® A_1 & B pooled cells are human reagent red blood cells, ready to use, for plasma or serum grouping. They are available suspended 3.0-3.4% in modified Alsevers solution and can be used immediately following careful resuspension.
    1. Preservative:
        a. 0.01% Neomycin
        b. 0.033% Chloramphenicol
        c. 5ppm Amphotericin B
    2. Precautions:
        a. For In-vitro diagnostic use.

b. Store at 2 to 8° C.
c. Do not use beyond the expiration date.
d. Do not use damaged vials.
e. Do not use if markedly hemolyzed or discolored.
f. Handle and dispose of reagents as potentially infectious.
g. Caution: Do not pipette by mouth. The absence of all viruses has not been determined.
h. Caution: All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when tested with FDA licensed EIA/ELISA tests. NAT testing was not performed. No known test method can offer assurance that products derived from human blood will not transmit infectious agents.
i. Caution: This product contains natural rubber latex which may cause allergic reactions.

V. INSTRUMENTATION/EQUIPMENT

A. Test Tubes (12 x 75 mm)
B. Dispo Pipettes
C. Centrifuge (Dade Immufuge II)

VI. PROCEDURE:

A. Label 2 tubes A cells, B cells
B. Label each tube with first and last initial of patient being tested. (Lengthen the minimum letters to differentiate patients with the same initials, if necessary.)
C. Add 1 drop of each Biotestcell A¹ & B® suspension to properly labeled tube. (Resuspend reagent red blood cells prior to use and allow to reach room temperature.)
D. Place 2 drops of test patient plasma in each tube. Shake to mix.
E. Centrifuge for 20 seconds or at the optimum calibrated spin time, at 3400 rpm (approximately 1000 rcf).
F. Gently resuspend cells completely and examine macroscopically immediately for agglutination. Negative reactions may be examined with an agglutination viewer, however, microscopic examination is not recommended. Grade and record results.

VII. REPORTING RESULTS/INTERPRETATION

A. Agglutination (positive reaction) +
B. No agglutination (negative reaction) =

Reactions with test plasma:

Reagent Cell Group A_1	Reagent Cell Group B	Blood Group	% Frequency Caucasians
+	+	O	45
-	+	A	40
+	-	B	11
-	-	AB	4

C. Correlate with forward type, if no discrepancies report result.

VIII. PROCEDURAL NOTES/PROBLEM-SOLVING TIPS

A. If forward and reverse typing looks like a possible A^2 or A^2B patient, the patient's sample must be sent to UPH-Methodist for verification with Anti-A^1Lectin. If we do not have enough of the specimen left to send, the patient must be called back in for the collection of another pink top tube. Order an ABO and RH typing at Methodist. When results come back, place a comment in LIS that type was confirmed at UPH-Methodist.

B. In very rare cases weak reactions (reaction strength under 3+) or hemolysis may occur.

C. Since serum characteristics may react at different strengths, incubation for 15-30 minutes at room temperature may be performed.

D. Generally, newborns and young babies do not show test reaction due to missing isoagglutinins. Isoagglutinins may also be absent in elderly patients.

E. The reactivity of the product may decrease during the dating period and there for should not be used after the expiration date.

F. Not for use in detection or identification of unexpected antibodies.

G. Not recommended to be used instead of antiglobulin crossmatch for the detection of unexpected antibodies.

H. Discrepant results may occur if:

1. The unexpected antibody anti-A_1 is present in a blood group A_2 or A_2B individual (frequency approximately 1% to 2% in A_2 bloods, 22% to 25% in A_2B bloods).
2. Unexpected antibodies, such as an anti-Lewis, anti-P_1, and anti-M, etc., are present. Confirm by testing plasma sample with antibody screening cells. Then identify antibodies by using an antibody identification panel.
3. Plasma contains cold autoagglutinins (such as anti-I or anti-H) having sufficient activity at room temperature to produce agglutination. Such reactions can be clarified by testing the plasma with autologous cells.
4. Neonatal serums are used since these may contain IgG anti-A and/or anti-B passively acquired from maternal blood.

I. In general, the ABO forward results are more reliable than the reverse results.
J. The NIH standards require that the forward and reverse typing be in agreement. If they are not:
   1. Check and make sure that the plasma and cells are from the same patient.
   2. Repeat both procedures with careful technique and proper controls (wash patient's cells 3X).
   3. Check the reactivity of anti-A and anti-B plasma with known A and B cells.
   4. Test group A and AB patients for subgroups of A.

IX. REFERENCES

A. Bio-Rad Medical Diagnostics GmbH, Dreieich, Germany, Reagent Red Blood Cells Biotestcell® A_1 & B, 186183/09, Rev. 08/2014..
B. AABB Technical Manual, Bethesda, Maryland 20817, 19th Edition, 2017.

POLICY CREATION :	***Date***
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