UnityPoint Health Pekin
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TRM.31234, TRM.31400, TRM.40210, TRM.40600

ANTIBODY SCREEN

INDIRECT COOMBS

Test Code: ABSCR AND TYSC

I. PRINCIPLE

The principle is a hemagglutination test or solid phase test test. Antigens on the Reagent Red Blood Cells react with the corresponding antibodies in the serum or plasma directly or after addition of Anti-Human Globulin. In a tube test agglutination will occur. In solid phase test Solidscreen® II a uniform layer of red blood cells on the micro test plate wells will occur.

II. CLINICAL SIGNIFICANCE

The detection of clinically significant antibodies is an important component of pre-transfusion and donor testing. This is to ensure that the donor red blood cells chosen for transfusion are those that will not cause harm to the recipient and will have optimum survival once transfused.

III. SPECIMEN

A. Fresh samples of EDTA anticoagulated whole blood (pink top) collected following general blood sampling guidelines are acceptable.
B. The specimen should be tested as soon as possible after collection.
C. If testing is delayed, specimens should be stored at 2 to 8°C or the plasma can be separated from the red blood cells and frozen.
D. Stored samples should be allowed to reach room temperature prior to testing.
E. Blood samples exhibiting gross hemolysis or contamination should not be used.
F. Do not use specimens collected with gel separators.
G. Samples may be used for up to three days after collection. (The day of sample draw is day zero.) Exception: Outpatients that have not been pregnant or transfused within the last three months are good for seven days.

IV. REAGENT

A. Biotestcell® 1, 2, and 3 are Reagent Red Blood Cells with polyvalent antigens of two or three single blood donors in separate vials for the detection of red blood cell antibodies. Biotestcell® 1, 2, and 3 contain the following antigens: D, C, E, c, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, M, N, S, s, Lea, Leb, P^1, Xg^a. They are suspended 3.0 to 3.4% in a modified Alsevers solution and can be used immediately following careful resuspension. Preservative: 0.01% Neomycin, 0.033% Chloramphenicol, 5ppm Amphotericin B.

B. Precautions
   1. For in vitro diagnostic use.
   2. Store at 2 to 8°C.
   3. Do not use beyond expiration date.
   4. Do not use damaged vials.
   5. Do not use if markedly hemolyzed or discolored.
   6. Handle and dispose of reagents as potentially infectious.
   7. Caution: Do not pipette by mouth. The absence of all viruses has not been determined.
   8. Caution: This product contains natural rubber latex which may cause allergic reactions.

V. INSTRUMENTATION/EQUIPMENT

A. 12 x 75 mm Disposable Glass Tubes
B. Plastic Transfer Pipettes
C. Serofuge
D. Heat Block
E. Automatic Cell Washer

VI. QUALITY CONTROL

A. Controls are performed daily on Biotestcells® 1, 2, and 3 with the Immucor corQC kit.

VII. PROCEDURE

A. Place 1 drop of appropriate cell suspension in each properly labeled 12 x 75 mm tube (Cell 1, Cell 2, and Cell 3).
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 2 drops of the test plasma to each tube.
D. Centrifuge for 20 seconds at 800 to 1000 x g, or at a time and speed appropriate for the centrifuge calibration.
E. Gently resuspend cells and examine macroscopically for agglutination or hemolysis. (See recommended grading system – Policy BB 0619.)
F. Add 2 drops of LISS to each tube. Gently shake to mix.
G. Incubate tubes at 36°-38°C for 10 minutes.
H. Centrifuge for 20 seconds at 800 to 1000 x g, or at a time and speed appropriate for the centrifuge calibration.
I. Gently resuspend cells and examine macroscopically for agglutination or hemolysis. (See recommended grading system – Policy BB 0619.)
J. Wash tubes with saline 3X (an automatic cell washer may be used). Make sure the supernatant is decanted completely after the last wash.
K. Add 2 drops of IgG Coombs serum and gently shake to mix.

L. Centrifuge for 20 seconds at 800 to 1000 x g, or at a time and speed appropriate for the centrifuge calibration.
M. Gently resuspend cells and examine macro and microscopically for agglutination or hemolysis.
N. Add 1 drop of Coombscell-E control cells to all tubes with negative results. Centrifuge for 20 seconds at 800 to 1000 x g, or for a time and speed appropriate for the centrifuge calibration. Gently shake to dislodge the cell button and observe for macroscopic agglutination. If the cells are agglutinated, the negative result is valid.

VIII. REPORTING RESULTS

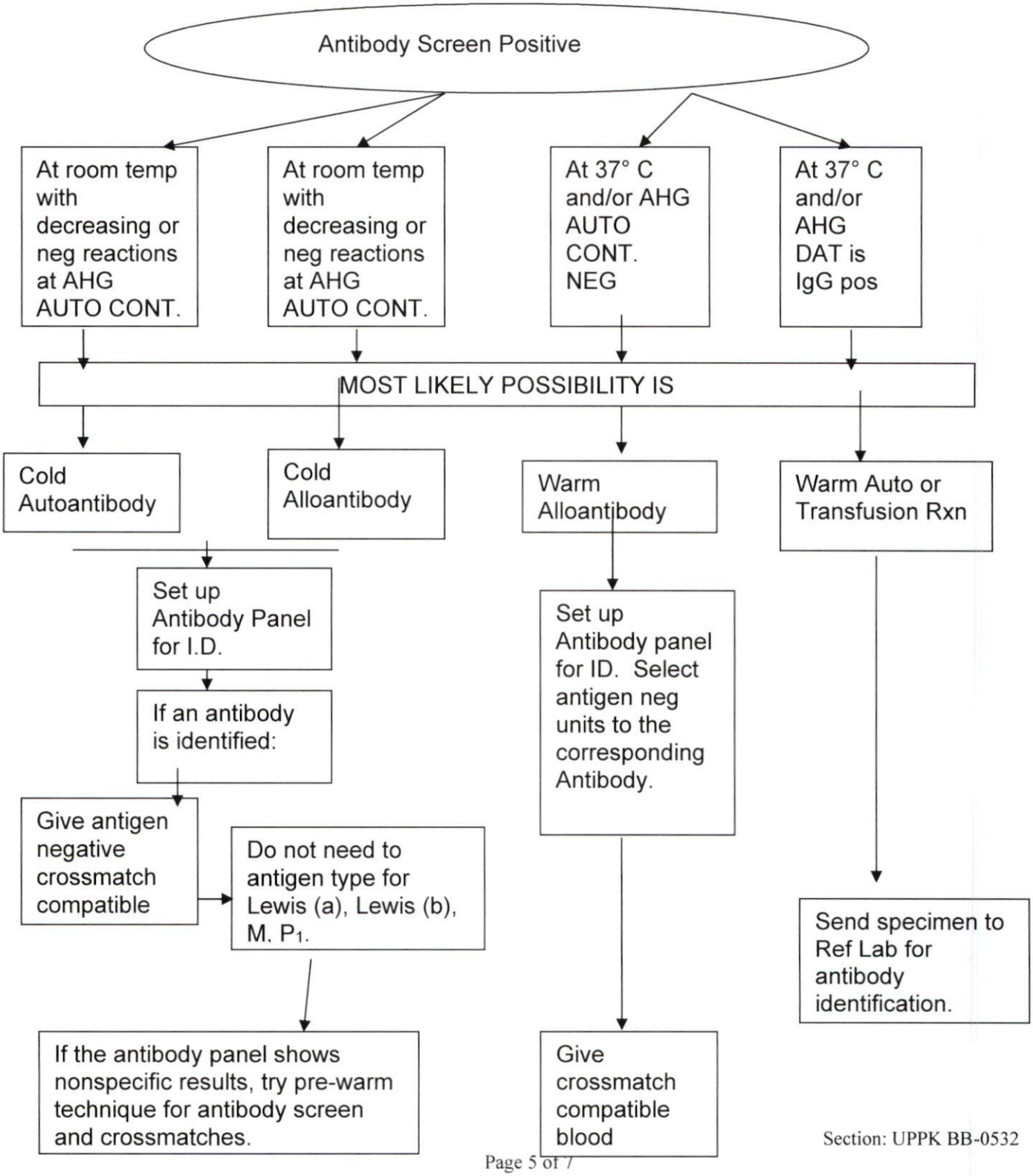
A. Positive – cells agglutinated or hemolyzed.
   1. Proceed with antibody identification. Perform an auto control when setting up a panel.
   2. Antigen type all donor units for antibodies identified.
B. Negative – no agglutination or hemolysis.
   1. If patient has a history of an antibody, all donor units must be antigen typed for previously identified antibodies prior to transfusion. It is not necessary to antigen type for IgM antibodies which include Lewis antigens Le(a) and Le(b), P_1, and M.. Perform a Coombs crossmatch to find compatible units. For anti-A_1, Coombs crossmatch type O units.
C. Enter reaction results in computer immediately after reading each tube. Enter interpretation as positive or negative.
D. The positive and negative reactions should be compared to the Biotestcell® antigen pattern and read accordingly.
E. An agglutination viewer may facilitate the reading of the tube tests (as recommended by the AABB Technical Manual).
F. Agglutination and/or hemolysis in any of the tubes at any phase of the test procedure prior to the addition of Coombs control cells indicates the presence of unexpected antibodies directed against the known antigens present on the screening cells.

IX. PROCEDURAL NOTES/PROBLEM-SOLVING TIPS

A. Low frequency antigens may not always be present on Biotestcell® 1, 2, and 3. Therefore, negative reactions with the screening Reagent Red Blood Cells do not always indicate the absence of unexpected antibodies.
B. Because some antibodies show dosage effect, the antigen density on the Reagent Red Blood Cells needs to be considered when evaluating the test results (homozygous or heterozygous hereditary disposition). A heterozygous expression of the antigen may result in non-detection of weak antibodies depending on the test method used.

C. In very rare cases HLA-antigens within the product may lead to false positive reactions.
D. The reactivity of the product may decrease during the dating period and therefore should not be used after the expiration date.
E. Negative reactions will be obtained if the sample contains antibodies present in concentrations too low to be detected by the test method employed. No test method is capable of detecting all red cell antibodies.

Flowchart for the Resolution of Antibody Problems



XVIII. REFERENCES

A. Bio-Rad Medical Diagnostics GmbH, Dreieich, Germany, Reagent Red Blood Cells, Biotestcell® 1 & 2, Biotestcell® 3, 186184/16, Rev. 08/2016.
B. Bio-Rad Medical Diagnostics GmbH, Dreieich, Germany, MLB 2, Modified LISS Solution, 187734/17, Rev. 05/2017.
C. AABB Technical Manual, Bethesda, Maryland, 19th Edition, 2017.

POLICY CREATION :		***Date***
Author:	***Sharrol Brisbin, MT (ASCP)***	***05/01/1990***
Medical Director:	***Sheikh, MA, MD***	***05/01/1990***

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REVISION HISTORY (began tracking 2011)			
Rev	Description of Change	Author	Effective Date
11/25/18	Added Sunquest resulting requirements.	Jenny Turner	11/25/18
09/14/19	Added on pink top EDTA for specimen, Added new serofuge, changed centrifugation wording to match package inserts, Deleted Auto Control with Ab Screens, Added Auto Control to Ab Panel.	Jenny Turner	9/14/19

Reviewed by:

Lead	Date	Coordinator/ Manager	Date	Medical Director	Date