The direct antiglobulin test will detect complement component C3 bound to red blood cells in vivo in serological conditions where the presence of autoantibodies, antibodies as a result of a transfusion reaction and hemolytic disease of the fetus and newborn. **PRINCIPLE OF THE TEST**

The Anti-Human Globulin Anti-C3d will cause the agglutination of red blood cells coated with human C3d and/or C3b complement components. No agglutination will be observed with red blood cells that are not coated with C3b or C3d.

**REAGENT DESCRIPTION**

The main component of this reagent is a murine monoclonal antibody to C3d (clone number 3G8). The formulation contains bovine serum albumin, 0.1% (w/v) sodium azide and Tween 80. **NOTE:** The volume delivered by the reagent bottle dropper is approximately 40 µL. Care should be taken to ensure that appropriately sized cell to serum ratios are maintained in all test systems.

**STORAGE**

The reagent should be stored at 2–8 °C.

**WARNINGS AND PRECAUTIONS**

For in vitro diagnostic use only. Products should be used by qualified personnel. Do not use beyond expiration date. Do not use if turbid. Do not dilute.

The format of the expiration date is expressed as YYYY-MM-DD (Year-Month-Day).

This reagent contains 0.1% (w/v) sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded into a sink, flush with a large volume of water to prevent azide buildup. This reagent is of animal origin; therefore care must be taken during use and disposal as there is a potential infection risk.

**SPECIMEN COLLECTION AND PREPARATION**

Specimens should be collected by a standard collection technique. The specimen should be tested as soon as possible after collection. If testing is delayed, the specimen should be stored at refrigerated temperatures. Do not use blood specimens that exhibit contamination. Extreme care should be taken if hemolyzed samples must be tested. Clotted samples, or those collected in EDTA, should be tested within fourteen days from collection. Donor blood may be tested until the expiration date of the donation.

For the Direct Antiglobulin Test it is recommended that testing is performed within 45 hours for blood drawn into EDTA. Blood collected into other anticoagulants may be used (ACD, CPD, CPDA-1, CP2D, CP2D-A53). Clotted specimens should be tested prior to refrigeration to avoid in vitro sensitization with complement.

**MATERIALS**

**Material provided**

- Anti-Human Globulin Anti-C3d

**Materials required but not provided**

- Isotonic saline
- Donor or patient red blood cells

**C3 sensitized red blood cells**

- 10 x 75 mm or 12 x 75 mm glass test tubes
- Centrifuge
- Timer
- Optical aid (optional)

**PROCEDURE**

**NOTE:** This reagent has been standardized for use by the technique described below and therefore its suitability for use by other techniques cannot be guaranteed. When a test is required to be incubated for a specific time period, a timer should be used.

**Direct Antiglobulin Test**

1. Prepare a 2-4% suspension of red blood cells in isotonic saline.
2. Add 1 drop of the 2-4% suspension of red blood cells to a glass test tube.
3. Wash the test 3-4 times with a large excess of isotonic saline. (e.g. 4 mL of saline per 12 (or 10) x 75 mm glass test tube.)

**NOTE:**

(i) allow adequate spin time to sediment the red blood cells.
(ii) make sure that the residual saline is removed at the end of each wash.
4. Add 2 drops of Anti-Human Globulin Anti-C3d to each test tube.
5. Mix the contents of the test tube well and centrifuge. The Anti-C3 reactivity can be enhanced by incubation at 21 °C ± 3 °C for 5-6 minutes prior to centrifugation.

**NOTE:** Suggested centrifugation: 900-1000 g (approx. 3400 rpm) for 10 seconds or a time and speed appropriate for the centrifuge used that produces the strongest reaction of positive tests, yet allows easy re-suspension of negative tests.

6. After centrifugation, gently shake the test tube to dislodge the cell button from the bottom and immediately observe macroscopically for agglutination. Negative reactions may be examined with an optical aid.

7. **Record results.**

To all negative tests add C3 sensitized reagent red blood cells.

a) Add 1 drop of C3 sensitized reagent red blood cells to each negative Anti-C3d test.

b) Mix the contents of the test tube well and centrifuge.

Suggested centrifugation: see note above.

c) Suggested centrifugation: see note above.

d) Refer to Step 6 above for instructions.

e) Any test which does not show a positive reaction should be considered invalid and repeated.

**STABILITY OF REACTION**

Test results should be read and interpreted immediately after centrifugation. Delays may cause dissociation of antigen-antibody complexes resulting in weak positive or false negative reactions.

**INTERPRETATION OF RESULTS**

Agglutination of the test red blood cells indicates a positive test result with detectable C3d or C3b present on the surface of the red blood cells.

No agglutination of the test red blood cells indicates a negative test result with no detectable C3d or C3b present on the surface of the red blood cells.

**QUALITY CONTROL**

Quality control of reagents is essential and should be performed on each day of use and in accordance with local, state and federal regulations.

Anti-C3 reactivity can be confirmed by testing the Anti-Human Globulin reagent with C3 coated red blood cells.

Any reagent red blood cell with a negative direct antiglobulin test may be used as a negative control, if desired.

**LIMITATIONS**

**NOTE:** Any saline present after the completion of the wash portion may dilute the Anti-Human Globulin Anti-C3d reagent beyond its optimal working concentration. Therefore, it is important to ensure that the maximum amount of wash solution is removed after each centrifugation step.

Gently re-suspend tube tests before reading. Excessive agitation may disrupt weak agglutination and produce false negative results.

Excessive centrifugation can lead to difficulty in resuspending the cell button, while inadequate centrifugation may result in agglutinates that are easily dispersed.

False positive or false negative results can occur due to contamination of test materials, improper reaction temperature, improper storage of materials, omission of test reagents and certain disease states.

**SPECIFIC PERFORMANCE CHARACTERISTICS**

**Comparator Study Results**

During comparator studies (data on file at Alba Bioscience Limited), blood samples were tested with ALBAclone® Anti-Human Globulin Anti-C3d (Murine Monoclonal) as follows:

<table>
<thead>
<tr>
<th>Anti-C3d</th>
<th>Comparator Reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>135</td>
<td>1404</td>
</tr>
</tbody>
</table>

**Positive Percent Agreement**

100.00 ± 0.98

**Negative Percent Agreement**

98.94 ± 0.99

Indicates agreement between the ALBAclone® Anti-Human Globulin Anti-C3d and comparator reagents only and does not indicate which reagent gave the correct result(s).

In performance evaluation studies, 1539 samples were tested with Anti-Human Globulin Anti-C3d (Murine Monoclonal). The positive percent agreement at the one-sided 95% exact lower confidence limit was 0.99 for agglutination tests based on a comparison of interpreted results. The negative percent agreement at the one-sided 95% exact lower confidence limit was 0.998 for agglutination tests based on a comparison of interpreted results. The positive percent agreement did not meet the acceptance criteria of 0.999 at the one sided 95% lower confidence limit.

Five discrepancies between the trial reagent and the comparator reagent associated with the NPA were observed, all were associated with weak reactivity. One could not be resolved as no investigation was performed by the trial site at the time of the discrepancy; four were possible test errors resulting from weak reactions with the trial and the comparator reagents.

Results were evaluated against comparable FDA approved products using the appropriate methods for the comparators.
Precision Study Results

As part of the performance evaluation, precision and lot to lot studies were performed using multiple operators, days and runs to confirm repeatability and reproducibility of test results in the same run, day and with the same operator and between runs, days and operators. The study took account of variables such as days of the week, times of day and supplementary reagents used in testing.

There were no discordant results; all expected positive test outcomes generated unequivocal positive reactions and expected negative test outcomes generated unequivocal negative reactions.

Prior to release, each lot of Anti-Human Globulin Anti-C3d is tested using FDA recommended methods against IgG and complement coated red blood cells to ensure suitable reactivity.

BIBLIOGRAPHY


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