Human Polyclonal Phenotyping Reagents

FOR TUBE AND CAT IAT METHODS

Duffy (Fy) antigen. Seqirus Anti-Fya and Anti-Fyb phenotyping reagents have been tested with Fy(a+b+) containing the appropriate antibodies active by the Indirect Antiglobulin Test (IAT). When used by the Tube Method – Indirect Antiglobulin Test (IAT), red cell samples to ensure adequate potency. Specificity is assured by testing each reagent against a panel of red cells known to be negative for the appropriate antigens. The reagents contain Gamma-globulin and bovine albumin as a preservative. The reagents have been optimised for use without further dilution.

**METHOD DESCRIPTION**

Seqirus Anti-Fya and Anti-Fyb polyclonal phenotyping reagents are prepared from human serum containing the appropriate antibodies active by the Indirect Antiglobulin Test (IAT). When used by the Tube Method – Indirect Antiglobulin Test (IAT), red cell samples to ensure adequate potency. Specificity is assured by testing each reagent against a panel of red cells known to be negative for the appropriate antigens. The reagents contain Gamma-globulin and bovine albumin as a preservative. The reagents have been optimised for use without further dilution.

**REAGENT DESCRIPTION**

Seqirus Anti-Fya and Anti-Fyb polyclonal phenotyping reagents are prepared from human serum containing the appropriate antibodies active by the Indirect Antiglobulin Test (IAT). When used by the Tube Method – Indirect Antiglobulin Test (IAT), red cell samples to ensure adequate potency. Specificity is assured by testing each reagent against a panel of red cells known to be negative for the appropriate antigens. The reagents contain Gamma-globulin and bovine albumin as a preservative. The reagents have been optimised for use without further dilution.

**STORAGE CONDITIONS**

Store at 2° to 8°C (Refrigerate. Do Not Freeze).

**PRINCIPLE OF THE TEST**

The agglutination of red cells by a specific reagent indicates the presence of the corresponding antigen on the red cell. If a negative reaction signifies the absence of the corresponding antigen. Test cells expressing the Fy(a+b+) and Fy(a-b+) antigens have been detected on foetal red cells as early as 6-7 weeks gestation and are fully developed at birth. This contributes to the potential for HDFN. Duffy antigens are not expressed on granulocytes, monocytes, mononuclear cells, red cells, platelets, lymphocytes, megakaryocytes and kidney, but not on the liver or placenta.

**Enzymes, particularly papain, inhibit anti-Fya and anti-Fyb reactions by destroying the Fy(a+b+) and Fy(a-b+) antigens.** Fy(a+b+) and Fy(a-b+) antigens are sensitive to most other proteolytic enzymes, except Trypsin.

Most anti-Fya antibodies are of the IgG class and are mostly developed as a result of chimeric transfusion. They may occur alone but are generally found in mixtures of antibodies Anti-Fy is relatively rare, is of the IgG class and is mostly developed from pregnancy and after transfusion. It is usually found in mixtures with other antibodies. Antibodies of the Duffy system rarely blend complement and therefore cause mild to severe immediate or delayed transfusion reactions.

**Gene Frequency**

Although the red cells of most Caucasians react with either or both of the Anti-Fya and Anti-Fyb reagents, Sanger, Faus and Jack found that nearly 70% of Negroes were of the Fy(a-b-) phenotype. It was later found that the rare Fy(a-b+) trait in white people, is caused by a different genetic control mechanism than that causing Fy(a+b-) in Negroes. In 1976 Weller et al. found that Fy(a-b-) individuals (particularly Negroes) were resistant to infection by the malarial parasite Plasmodium vivax.

The frequencies of phenotypes in the Duffy system vary in different populations. The expected results and frequencies of the phenotypes are given below:

<table>
<thead>
<tr>
<th>Reaction obtained when Anti-Fya, Anti-Fyb are present</th>
<th>Approximate frequency in:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Fya, Anti-Fyb (phenotyping reagents)</td>
<td>Australian Bl, African Bl,</td>
</tr>
<tr>
<td>Fy(a+b-)</td>
<td></td>
</tr>
<tr>
<td>Fy(a-b+)</td>
<td></td>
</tr>
<tr>
<td>Fy(a-b+)</td>
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<td>0</td>
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**SPECIMEN COLLECTION AND PREPARATION**

Blood samples should be withdrawn aseptically and without the addition of anticoagulants. Tests should be performed as soon as possible after collection of the sample. If testing the blood samples is delayed, samples should be stored between 2° to 8°C. Samples collected into EDTA or Heparin may be tested up to 14 days from the date of withdrawal provided storage has been at 2° to 8°C. Clotted samples may be tested up to 14 days from the date of withdrawal provided storage has been at 2° to 8°C. Cells may also be stored at Celpresol™ at 2° to 8°C for up to 42 days.

**RECOMMENDED METHODS**

**Table Method – Indirect Antiglobulin Test (IAT)**

1. Prepare a 3-5% suspension of test red cells in buffered or unbuffered isotonic saline, or in Celpresol™.
2. Add 1 or 2 drops of the applicable Seqirus Anti-Fya or Anti-Fyb phenotyping reagent to an appropriately labelled, clean glass test tube (15x75mm or 12x75mm).
3. Add 1 drop of the suspension of test red cells.
4. Mix well and incubate at 37°C for 30 minutes.
5. Spin (Speed/Time) 1-2
6. Reagent Volume 1.0
7. Incubation Time 30 mins
8. Temperature 37°C
9. Spin (Speed/Time) Low for 15-20 secs
10. Store at 2° to 8°C (Refrigerate. Do Not Freeze).

**NOTE:**

- *On centrifugation at a speed and time appropriate for the centrifuge in use.*
- Seqirus recommends the use of buffered isotonic saline solutions (pH 7.0 to 7.2).
1. Prepare a 3% suspension of test red cells in Celpresol Column Agglutination Technology (CAT) 3% Method (BioVue Mark II™). Add 40µL of the applicable Seqirus Anti-Fy

2. Label a BioVue™ card or a BioRad™/DiaMed™ ID Micro Typing System ID-Card (Product Identification 004014) with the test sample.

3. Add 40µL of the applicable Seqirus Anti-Fy

4. Add 50 µL of the suspension of 0.8% test red cells (to be phenotyped).

5. Incubate at 37°C according to the manufacturer’s instructions.

6. Centrifuge according to the manufacturer’s instructions.

7. Read according to the manufacturer’s instructions.

COLUMN AGGLUTINATION TECHNOLOGY (CAT) 0.8% Method (BioVue™ and BioRad™/DiaMed™)

1. Prepare a 0.8% suspension of test red cells in Celpresol™.

2. Label a BioVue™ ID-Card.

3. Add 40µL of the applicable Seqirus Anti-Fy

4. Add 10µL of the suspension of 3% test red cells (to be phenotyped).

5. Add 10µL of Seqirus Rapid Antibody Medium (RAM).

6. Add 0.8% test red cells to the RHM reagent.

7. Read according to the manufacturer’s instructions.

8. Read according to the manufacturer’s instructions.

RECOMMENDATIONS

Agglutination of the test red cells constitutes a positive result and indicates the presence of the appropriate antigen (subject to the cells giving a negative Direct Antiglobulin Test (DAT)). No agglutination of the test red cells indicates the absence of the relevant antigen.

CONTROLS

The use of controls is essential in the performance of all blood grouping tests. Control samples should be tested in parallel with the test sample.

Positive Control – red cells known to be heterozygous for the antigen as appropriate for the phenotyping reagent in use (Fy(a+b)).

Negative Control – red cells known to lack the antigen as appropriate for the phenotyping reagent in use.

Following the interpretation of each test, one volume of undiluted cells (e.g. Seqirus Anti-Fy Control Card 3%) should be added to all negative tests and the reagents re-examined to ensure that agglutination occurs. This control is based on the principle that a truly negative test, the Anti-human Globulin reagent will have remained unsuppressed after exposure to the particular washed cell suspension and therefore agglutinate the AHG Control Cells. Its use safeguards against error caused by imperfect techniques, poor washing and inadequately reactive antibodies in reagents.

Cautions: non-agglutinated cells with a positive DAT will invalidate any positive result obtained.

LIMITATIONS OF PROCEDURE

After results may occur due to:

1. Presence of haemolytic substances in the test sample.
2. Presence of gross haemolysis.
3. Use of aged blood samples, reagents or supplementary materials.
4. Contaminated blood samples, reagents or supplementary materials.
5. Red cells that have a positive DAT.
6. Other deviations from the recommended test methods.
7. Incorrect cell concentrations.
8. Use of inappropriate enzyme techniques.

PRECAUTIONS

1. For in vitro diagnostic use only.
2. The material from which this product was derived was found to be non-reactive for specified markers for HIV 1 and 2, Hepatitis B and C, HTLV and Syphilis by currently approved methods. However no known method can assure that products derived from human blood will not transmit infectious agents.
3. Contains Sodium Azide 0.1% as a preservative. Products containing Sodium Azide can react with acids or oxidisers, harmful if swallowed. May be harmful if inhaled. May cause irritation to skin and eyes. Its chronic health effects are unknown.
4. This product should be clear; turbidity may indicate bacterial contamination. The reagent should not be used if a precipitate or particles are present.
5. The bovine material used is from an approved source free of Bovine Spongiform Encephalopathy (BSE).

REFERENCES

10. Scientific Sub-committee of the Australian and New Zealand Society of Blood Transfusion Ltd Guidelines for Pretransfusion Laboratory Practice. 3rd Ed. 2007 (or current edition).
16. Ortho Biotech™ System Anti-human Globulin Anti-IgG,-C3d; polyspecific (Rabbit and Murine Monoclonal) (Green) (Product Codes 707300 and 707350) instructions for use leaflet.

WARNING:

Health Hazard

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