Epiclone™ Anti-S, Anti-s and Anti-P1

Human Monoclonal IgM Phenotyping Reagents

WARNING:
Contains
Sodium Azide 0.1%,
Harmful if swallowed.

IVD

Caution:
Handle as if capable
of transmitting
infection

immulab

METHOD SUMMARY

	Title	Tube	Tube	CAT^	MTP
Reagent	Anti-S	Anti-S and Anti-s	Anti-P1	Anti-S, Anti-s and Anti-P1	Anti-S
Validated Methods	Yes	Yes	Yes	Yes	Yes
Reagent Volume	1	1	1		1
Cell Volume	1	1	1		1
Cell Concentration	40-50%	3-5%	3-5%	0.8% / 3%	3-5%
Incubation Time	2 mins	Immediate Spin	Immediate Spin		15-20 mins
Temperature	Room Temp	Room Temp	Room Temp		Room Temp
Spin (Speed/Time)	N/A	High for 20 secs	Low for 20 secs		Low for 40 secs
Other		Anti-S only: Incubate all Negatives or Weakly Positives at Room Temp for 5 mins			

^ Validated for use in Ortho-Clinical Diagnostics BioVue™ Neutral cards and BioRad™ LISS Coombs cards (Anti-S) and NaCl, Enzyme Test and Cold Agglutinins cards (Anti-s and Anti-P1).

REAGENT DESCRIPTION

Epiclone™ Anti-S, Anti-s and Anti P1 monoclonal phenotyping reagents are prepared from monoclonal IgM antibodies. When used by the recommended methods these reagents will cause agglutination of red blood cells carrying the specific S, s or P1 antigens. The reagents contain Bovine Albumin, macromolecular potentiators and Sodium Azide as a preservative. These reagents have been optimised for use without further dilution or additions. The clones used to produce these reagents are: Epiclone™ Anti-S is MS-94 (IgM monoclonal) Epiclone™ Anti-s is P3BER (IgM monoclonal) and Epiclone™ Anti-P1 is P3NIL100 (IgM monoclonal).

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 those cells, whilst a negative reaction signifies the absence of the corresponding antigen. Red cells expressing the S antigen will agglutinate in the presence of the corresponding specific antibody in Epiclone™ Anti-S. Red cells expressing the s antigen will agglutinate in the presence of the corresponding specific antibody in Epiclone™ Anti-s. Red cells expressing the P1 antigen will agglutinate in the presence of the corresponding specific antibody in Epiclone™ Anti-P1.

BACKGROUND

MNS Blood Group System

Walsh and Montgomery first reported the S antigen in 1947 and it was realised that the gene producing S was linked to the allelic genes producing M and N antigens. In 1951 Levine ***et al.*** discovered the s antigen and recognised it as being allelic to S.

Whilst the M and N antigens are carried on the 131 amino acid Glycophorin A sialoglycoprotein, S and s are carried on the smaller 72 amino acid Glycoprotein B molecule. Rh_{null} red cells have greatly reduced Ss antigen expression. The MNS antigens are well developed at birth and appear on foetal red cells at around 12 weeks gestation.

While Papain and Ficin readily destroy M, N and s antigens, S is more resistant. Other proteolytic enzymes such as chymotrypsin can destroy S activity, however the amount of degradation may depend on the strength of the enzyme solution, length of treatment and enzyme to cell ratio. Trypsin, DTT, AET, chloroquine or acid treatment does not destroy S activity. Weak concentrations of sodium hypochlorite or common household bleach selectively destroy S reactivity.

Anti-S and anti-s have both been reported as causing transfusion reactions and severe and fatal Haemolytic Disease of the Foetus and Newborn (HDFN). Both are usually immune-stimulated and they are normally non-complement binding IgG antibodies, although IgM anti-S has been reported. Anti-S and anti-s usually react at 37°C in Anti-Human Globulin (AHG) but most are optimally reactive at temperatures between 10°C and 22°C. Anti-S is more commonly encountered and anti-s is considered a relatively rare antibody.

S/s Gene Frequency

As the S and s antigens are allelic, all red cells from Caucasians, except the very rare Rh_{null} type, should react with one or both of these reagents. However, approximately 1.5% of African Americans will be negative for both S and s antigen.

The expected results and frequencies of the phenotypes in the Australian blood donor population are given below:

Reactions obtained with:		Phenotype	Frequency
Anti-S	Anti-s		
0	+	S-s+	0.4797
+	+	S+s+	0.4261
+	0	S+s-	0.0942
0	0	S-s-	<0.01

P Blood Group System

Landsteiner and Levine discovered the P blood group system in 1927. As M, N and O had already been described they assigned the next letter in the alphabet, P. The P1 antigen is the sole antigen that comprises the P blood group system. It is a carbohydrate antigen and the antigenic determinant has been identified as a trisaccharide. P1-positive individuals are designated the P1 phenotype, and P1-negative individuals are designated the P2 phenotype. Other similar antigens have been shown to be under separate genetic control and are thus not included in this system. Homozygous expression of the P1 gene results in strong expression of the P1 antigen. In Caucasian populations approximately 80% of people are P1-positive.

Alloanti-P1 is usually an IgM antibody reactive at room temperature or lower. Most examples do not agglutinate red cells at 25°C or above, and are thus not usually considered clinically significant. The two reported cases of anti-P1 associated with transfusion reactions have both involved anti-P1 antibodies capable of agglutinating red cells at 37°C.

SPECIMEN COLLECTION AND PREPARATION

Blood samples should be withdrawn aseptically with or 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 7 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.

Samples collected in Citrate may be tested up to 42 days from the date of withdrawal provided storage has been at 2° to 8°C. Cells may also be stored in Celpresol™ at 2° to 8°C for up to 42 days.

RECOMMENDED METHODS

Tile Method for Anti-S

1. Prepare a 40-50% suspension of test red blood cells in autologous plasma, buffered or unbuffered isotonic saline.
2. Add 1 drop of Epiclone™ Anti-S phenotyping reagent to a clean, labelled glass or plastic tile.
3. Add 1 drop of the suspension of test red cells.
4. Mix the reagent and cells over an area of about 2cm in diameter by gently and continuously rocking the tile. Maintain the temperature at room temperature.
5. Examine for agglutination at 2 minutes. Record results. Do not confuse any drying of the mixture with agglutination.

Tube Method for Anti-S and Anti-s

1. Prepare a 3-5% suspension of test red cells in buffered or unbuffered isotonic saline, or in Celpresol™.
2. Add 1 drop of Epiclone™ Anti-S or Anti-s phenotyping reagent to an appropriately labelled glass test tube (10x75mm or 12x75mm).
3. Add 1 drop of the suspension of test red cells.
4. Mix and centrifuge at high speed (1000rcf) for 20 seconds*.
5. Gently agitate the tube to dislodge the red cells and examine for agglutination. Record results.
6. **Anti-S only:** Incubate all Negative or Weakly Positive tests at room temperature for 5 minutes and repeat steps 4 and 5.

   This may enhance the reaction strength in typing rare phenotypes. Prolonged incubation of tests for up to 60 minutes at room temperature has no adverse effect on reactions.

Note: *Or centrifuge at a speed and time appropriate for the centrifuge in use.

Tube Method for Anti-P1
1. Prepare a 3-5% suspension of test red cells in buffered or unbuffered isotonic saline, or in Celpresol™.
2. Add 1 drop of Epiclone™ Anti-P1 phenotyping reagent to an appropriately labelled glass test tube (10x75mm or 12x75mm).
3. Add 1 drop of the suspension of test red cells.
4. Mix and centrifuge at low speed (500rcf) for 20 seconds*.
5. Gently agitate the tube to dislodge the red cells and examine for agglutination. Record results.

Note: *Or centrifuge at a speed and time appropriate for the centrifuge in use.

Column Agglutination Technology (CAT) 0.8% Method (BioRad™ only) for Anti-S
1. Prepare a 0.8% suspension of test red cells in Celpresol™ LISS.
2. Label a BioRad™ LISS/Coombs card.
   Add 25µL of Epiclone™ Anti-S phenotyping reagent.
3. Add 50µL of the suspension of 0.8% test red cells (to be phenotyped).
4. Incubate at 37°C according to the manufacturer's instructions.
5. Centrifuge according to the manufacturer's instructions.
6. Read according to the manufacturer's instructions.

Column Agglutination Technology (CAT) 0.8% Method (BioVue™ only) for Anti-S – Immediate Spin (IS)
1. Prepare a 0.8% suspension of test red cells in Celpresol™ LISS.
2. Label a BioVue™ Neutral card.
3. Add 40µL of Epiclone™ Anti-S.
4. Add 50µL of the suspension of 0.8% test red cells (to be phenotyped).
5. Centrifuge according to the manufacturer's instructions.
6. Read according to the manufacturer's instructions.

Column Agglutination Technology (CAT) 0.8% Method (BioVue™ and BioRad™) for Anti-s and Anti-P1 – Immediate Spin (IS)
1. Prepare a 0.8% suspension of test red cells in Celpresol™ LISS.
2. Label a BioVue™ Neutral card or a BioRad™ NaCl, Enzyme Test and Cold Agglutinins card.
3. BioVue™: Add 40µL of Epiclone™ Anti-s or Epiclone™ Anti-P1 phenotyping reagent.
   BioRad™: Add 25µL of Epiclone™ Anti-s or Epiclone™ Anti-P1 phenotyping reagent.
4. Add 50µL of the suspension of 0.8% test red cells (to be phenotyped).
5. Centrifuge according to the manufacturer's instructions.
6. Read according to the manufacturer's instructions.

Column Agglutination Technology (CAT) 3% Method (BioVue™ only) for Anti-S, Anti-s and Anti-P1 – Immediate Spin (IS)
1. Prepare a 3% suspension of test red cells in Celpresol™.
2. Label a BioVue™ Neutral card.
3. Add 40µL of Epiclone™ Anti-S, Epiclone™ Anti-s or Epiclone™ Anti-P1 phenotyping reagent.
4. Add 10µL of the suspension of 3% test red cells (to be phenotyped).
5. Add 40µL of Celpresol™.
6. Centrifuge according to the manufacturer's instructions.
7. Read according to the manufacturer's instructions.

Microplate Method for Anti-S
Epiclone™ Anti-S is validated for the following microtitre plate method. Due to variation in methods and equipment, microtitre plate users should validate this reagent using their method.
1. Prepare a 3-5% suspension of test red cells in buffered or unbuffered isotonic saline containing 1% Bovine Serum Albumin.
2. Add 1 volume of Epiclone™ Anti-S phenotyping reagent to the appropriate test wells.
3. Add an equal volume of the suspension of test red cells to the appropriate test well.
4. Mix the contents of each well using manual means or a microplate shaker. The time required to achieve this will depend on the speed and orbit of the shaker.
5. Incubate the microplate at room temperature for 15-20 minutes.
6. Centrifuge at low speed (100rcf) for 40 seconds*.
7. Resuspend the red cells using a microplate shaker for an optimal time and agitation speed.
8. Read the tests macroscopically or with an automated reader.

Note: *Or centrifuge at a speed and time appropriate for the centrifuge in use.

INTERPRETATION OF RESULTS
Agglutination of the test red cells constitutes a positive result and indicates the presence of the appropriate antigen.
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. In the case of P1 testing a sample demonstrating normal expression of the P1 antigen i.e. not strong.

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

LIMITATIONS OF PROCEDURE
False results may occur due to:
1. Incorrect technique.
2. Presence of gross rouleaux.
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 Direct Antiglobulin Test (DAT).
6. Other deviation from the recommended test methods.
7. Incorrect cell concentrations.
8. Enzyme techniques are not recommended as they may denature the S and s antigens.

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. No chronic health effects known.
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 appropriate source free of Bovine Spongiform Encephalopathy (BSE).

REFERENCES

1. United Kingdom National Blood Service. Guidelines for the Blood Transfusion Services in the United Kingdom. 7th Ed. 2005 (or current edition).
2. Issitt PD. Serology and genetics of the Rhesus blood group system. Montgomery Scientific Publications. Cincinnati, Ohio. 1979; 228-31.
3. Harmening DM. Modern Blood Banking and Transfusion Practices. 5th Ed. FA Davis Company. Philadelphia 2005 (or current edition).
4. Daniels G. Human Blood Groups. 2nd Ed. Blackwell Science. Carlton, Victoria 2002 (or current edition).
5. Ortho BioVue™ System Neutral instructions for use leaflet.
6. BioRad™ LISS/Coombs instructions for use leaflet.
7. BioRad™ NaCl, Enzyme Test and Cold Agglutinins instructions for use leaflet.

	Consult instructions for use	IVD	*In vitro* diagnostic medical device	REF	Catalogue number		Temperature limitation		Manufacturer

	WARNING: Health Hazard

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