Epiclone™ Anti-Jk^a and Anti-Jk^b

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

	Tube	CAT^
Reagent	Anti-Jk^a, Jk^b	Anti-Jk^a, Jk^b
Validated Methods	Yes	Yes
Reagent Volume	1	
Cell Volume	1	
Cell Concentration	3-5%	0.8% / 3%
Incubation Time	Immediate Spin	Immediate Spin
Temperature	Room Temp	Room Temp
Spin (Speed/Time)	Low for 60 secs	

^ Validated for use in Ortho-Clinical Diagnostics BioVue™ AHG cards and BioRad™ LISS/ Coombs cards.

REAGENT DESCRIPTION
Epiclone™ Anti-Jk^a and Epiclone™ Anti-Jk^b monoclonal phenotyping reagents are prepared using human monoclonal IgM antibodies. When used by the recommended methods these reagents will cause agglutination of red blood cells carrying the specific Jk^a and/ or Jk^b antigen. The reagents contain Sodium arsenite and Sodium Azide as a preservative. Anti-Jk^a contains Bovine Albumin. Each reagent has been optimised for use without further dilution or additions. The clone used to produce each reagent: Epiclone™ Anti-Jk^a is P3HT7 (IgM monoclonal) and Epiclone™ Anti-Jk^b is P3.143 (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 Jk^a and Jk^b antigens will agglutinate in the presence of the corresponding specific antibody in Epiclone™ Anti-Jk^a and/or Epiclone™ Anti-Jk^b.

BACKGROUND
Blood Group System
In 1951, Allen, Diamond and Niedziela reported the first example of anti-Jk^a, whilst anti-Jk^b was discovered in 1953 by Plaut ***et al***. Anti-Jk^a and anti-Jk^b antibodies are capable of causing Haemolytic Disease of the Foetus and Newborn (HDFN) and transfusion reactions. Delayed transfusion reactions are frequently encountered due to Kidd system antibodies. Previously formed antibodies may be undetectable in the patient's serum a few months post stimulation. A further transfusion of antigen positive blood will result in a rapid boost of antibody level. Kidd antibodies exhibit dosage. They are often weak and are found in combination with other antibodies, which may make them difficult to identify.

Antigen Characteristics
Kidd antigens are detected on foetal red cells as early as 11 weeks for Jk^a and 7 weeks for Jk^b. They are well developed at birth, which contributes to the potential for HDFN. Kidd antigens are not altered by enzymes, ZZAP chloroquine diphosphate, AET, DTT, or acid reagents, which readily affect many other blood group antigens. They may not be very accessible on the red cell surface and this may explain why they do not always react well with Kidd antibodies. It is important to note that Kidd antibodies are often difficult to detect. They can activate complement and therefore often cause severe or fatal immediate or delayed transfusion reactions.

Gene Frequency
The Kidd system was expanded in 1959 when Pinkerton ***et al***. found an example of Jk(a-b-) red cells. It should be noted that the frequency of Jk(a-b-) is much higher in Polynesian and Micronesian races than in Europeans. Jk(a-b-) red cells have been shown to be resistant to lysis by 2M-urea solution. These Jk(a-b-) people often produce anti-Jk3, which will react with all Jk(a+) or Jk(b+) red cells but which is detecting an antigen distinct from either Jk^a or Jk^b.

The frequencies of phenotypes in the Kidd system vary in different populations. The expected results and frequencies of the phenotypes in the Australian blood donor population are given below:

Reactions obtained with:		Phenotype	Frequency
Anti-Jk^a	Anti-Jk^b		
+	+	Jk(a+b+)	0.4996
+	0	Jk(a+b-)	0.2660
0	+	Jk(a-b+)	0.2344
0	0	Jk(a-b-)	very rare*

*The Jk (a-b-) phenotype is more frequent in Polynesians including indigenous people of Pacific Island and New Zealand origin and therefore may be expected to be occasionally detected in Australia.

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
Tube Method
1. Prepare a 3-5% suspension of test red cells in buffered or unbuffered isotonic saline, or in Celpresol™.
2. Add 1 drop of the applicable Epiclone™ Anti-Jk^a or Epiclone™ Anti-Jk^b 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 well.
5. Centrifuge at low speed (500rcf) for 60 seconds*.
6. 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 (BioVue™ and BioRad™) – Immediate Spin (IS)
1. Prepare a 0.8% suspension of test red cells in Celpresol™.
2. Label a BioVue™ AHG card or a BioRad™ LISS/Coombs card.
3. BioVue™: Add 40µL of the applicable Epiclone™ Anti-Jk^a or Epiclone™ Anti-Jk^b phenotyping reagent.
   BioRad™: Add 25µL of the applicable Epiclone™ Anti-Jk^a or Epiclone™ Anti-Jk^b 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) – Immediate Spin (IS)
1. Prepare a 3% suspension of test red cells in Celpresol™.
2. Label a BioVue™ AHG card.

3. Add 40µL of the applicable Epiclone™ Anti-Jk[a] or Epiclone™ Anti-Jk[b] phenotyping reagent.
4. Add 10µL of the suspension of 3% test red cells (to be phenotyped).
5. Add 40µL of Immulab Rapid Antibody Medium (RAM) or Celpresol™
6. Centrifuge according to the manufacturer's instructions.
7. Read according to the manufacturer's instructions.

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.

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 treated red cells may give a falsely positive reaction with Anti-Jk[b].

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, Hepatitis B and C. 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 approved source free of Bovine Spongiform Encephalopathy (BSE).

REFERENCES

1. Allen FM, Diamond LK, Niedziela B. A new blood group antigen. Nature, London 1951; 167: 482.
2 Plaut G, Ikin EW, Mourant AE, Sanger R, Race RR. A new blood-group antibody, anti-Jk[b]. Nature, London 1953; 171: 431.
3. Matson GA, Swanson J, Tobin JD. Severe hemolytic disease of the newborn caused by anti- Jk[a]. Vox Sang 1959; 4: 144-7.
4. Kornstad L, Halvorsen K. Haemolytic disease of the newborn caused by anti-Jk[b]. Vox Sang 1958; 3: 94-9.
5. Kronenberg H, Kooptzoff O, Walsh RJ. Haemolytic transfusion reaction due to anti-Kidd. Aust Ann Med 1958; 7: 34-5.
6. Morgan P, Wheeler CB, Bossom EL. Delayed transfusion reaction attributed to anti-Jk[b]. Transfusion 1967; 7: 307-8.
7. Mollison PL, Newlands M. Unusual delayed haemolytic transfusion reaction characterised by the slow destruction of red cells. Vox Sang 1976 31: 54-7.
8. Harmening DM. Modern Blood Banking and Transfusion Practices. 5th Ed. FA Davis Company. Philadelphia 2005.
9. Roback JD. American Association of Blood Banks Technical Manual. 17th Ed. Bethesda, Maryland 2011 (or current edition).
10. Daniels G. Human Blood Groups. 2nd Ed. Blackwell Science. Carlton, Victoria 2002 (or current edition).
11. United Kingdom National Blood Service. Guidelines for the Blood Transfusion Services in the United Kingdom. 7th Ed. 2005 (or current edition).
12. BioRad™ LISS/Coombs instructions for use leaflet.
13. Ortho BioVue™ System Anti-Human Globulin Anti-IgG,-C3d; polyspecific (Rabbit and Murine Monoclonal) (Green) 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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