Epiclone™ Anti-C, Anti-E, Anti-c, Anti-e and Anti-C^w

Human Monoclonal IgM Phenotyping Reagents

FOR TILE, TUBE, CAT AND MICROPLATE METHODS

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

IVD

Caution:
Handle as if capable of transmitting infection

immulab

METHOD SUMMARY

	Tile	Tube	CAT^	MTP
Reagent	Anti-C, -E, -c, -e, -C^w	Anti-C, -E, -c, -e, -C^w	Anti-C, -E, -c, -e, -C^w	Anti-C, -E, -c, -e, -C^w
Validated Methods	Yes	Yes	Yes	Yes
Reagent Volume	1	1		1
Cell Volume	1	1		1
Cell Concentration	40-50%	3-5%	0.8% / 3%	3-5%
Incubation Time	2 mins	Immediate Spin		15-20 mins
Temperature	Room Temp	Room Temp		Room Temp
Spin (Speed/Time)	N/A	High for 20 secs		Low for 40 secs
Other		Incubate Negatives or Weak Positives 37°C for 5 mins		

^ Validated for use in Ortho-Clinical Diagnostics BioVue™ Neutral and BioRad™ NaCl, Enzyme Test and Cold Agglutinins cards.

REAGENT DESCRIPTION

Epiclone™ Anti-C, Epiclone™ Anti-E, Epiclone™ Anti-c, Epiclone™ Anti-e and Epiclone™ Anti-C^w monoclonal phenotyping reagents are prepared from human monoclonal IgM producing hybridomas. When used by the recommended methods these reagents will cause agglutination of the red cells carrying the specific C, E, c, e and/or C^w antigens. The reagents contain Bovine Albumin, macromolecular potentiators and Sodium Azide as a preservative. Each reagent has been optimised for use without further dilution or additions. The clones used to produce these reagents are: Epiclone™ Anti-C is MS-24 (IgM monoclonal), Epiclone™ Anti-E is MS-80/MS-258 (IgM monoclonal blend), Epiclone™ Anti-c is MS-33 (IgM monoclonal), Epiclone™ Anti-e is MS-16/MS-21/MS-63 (IgM monoclonal blend) and Epiclone™ Anti-C^w is MS-110 (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 C, E, c, e or C^w antigens will agglutinate in the presence of the corresponding specific antibody in Epiclone™ Anti-C, Epiclone™ Anti-E, Epiclone™ Anti-c, Epiclone™ Anti-e and/or Epiclone™ Anti-C^w.

BACKGROUND

Blood Group System

The Rh system is the most complex of all human blood group systems, comprising over 50 antigens. The most clinically important antigen in this group is the Rh1 or RhD antigen. Antisera to many of the antigens in the Rh System are extremely rare, and Rh phenotyping is generally restricted to testing for the common alleles at the D, C and E loci.

The testing of red cells with Anti-D, Anti-C, Anti-E, Anti-c and Anti-e enables the determination of the Rh phenotype (and, based on published frequencies, the probable Rh genotype). These five basic Rh antigens and their specific antibodies are most important in pre-transfusion testing and the prediction of Haemolytic Disease of the Foetus and Newborn (HDFN).

Callender and Race first described the C^w (Willis) antigen of the Rh system and showed it to be an allele of the Cc locus. There is now evidence that the C^w antigen may either be heterogeneous or a low-incidence antigen not specifically linked to C, as some C^w positive cells differ in their reactivity to various Anti-C sera.

Gene Frequency

The Rh phenotype or probable genotype of a blood sample can be determined by testing the red cells against the five antisera specific for the common alleles of the D, C and E loci.

The table shows the reactions obtained with such antisera, and gives the phenotype, probable genotype and frequencies in the Australian blood donor population. It should be noted that frequencies vary considerably in Negroid, Asian and the Australian Aboriginal populations.

Common Rh Phenotypes and Genotypes

	Reactions with Anti-					Phenotype	Probable Genotype	Shorthand Symbol	Approximate % Frequency in Australia	Other Possible Genotypes
	D	C	E	c	e					
RhD Positive	+	+	0	+	+	DCce	DCe/ce	R_1r	35.3	DCe/Dce Dce/Ce
	+	+	0	0	+	DCe	DCe/DCe	R_1R_1	17.3	DCe/Ce
	+	+	+	+	+	DCEce	DCe/DEc	R_1R_2	13.5	DCe/Ec DEc/Ce DCE/ce DCE/Dce Dce/CE
	+	0	+	+	+	DEce	DEc/ce	R_2r	12.3	DEc/Dce Dce/Ec
	+	0	+	+	0	DEc	DEc/DEc	R_2R_2	2.3	DEc/Ec
	+	0	0	+	+	Dce	Dce/ce	R_0r	1.7	Dce/Dce
RhD Negative	0	0	0	+	+	ce	ce/ce	rr	16.4	
	0	+	0	+	+	Cce	Ce/ce	r'r	0.4	
	0	0	+	+	+	Ece	Ec/ce	r''r	0.7	

Notes: Cells giving a positive reaction with Epiclone™ Anti-C may be further subdivided by testing with Epiclone™ Anti-C^w.
Other Rh genotypes may be found, but all have a frequency of < 0.2%.

The frequency of C^w varies between populations. Generally in Caucasians the frequency is 2.6% but is very rare in populations of Asian or African descent and can be as high as 8% in some European populations.

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

1. Prepare a 40-50% suspension of test red blood cells in autologus or compatible plasma, serum, buffered or unbuffered isotonic saline, or in Celpresol™.
2. Add 1 drop of the applicable Epiclone™ Anti-C, Epiclone™ Anti-E, Epiclone™ Anti-c, Epiclone™ Anti-e or Epiclone™ Anti-C^w 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.
5. Examine for aggultination at 2 minutes. Record results. Do not confuse any drying of the mixture with agglutination.

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-C, Epiclone™ Anti-E, Epiclone™ Anti-c, Epiclone™ Anti-e or Epiclone™ Anti-C^w 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. Incubate all negative or weakly positive tests at 37°C 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 37°C has no adverse effect on reactions.

Note: *Or centrifuge at a speed and time appropriate for 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™ LISS.
2. Label a BioVue™ Neutral card or a BioRad™ NaCl, Enzyme Test and Cold Agglutinins card.

3. BioVue™: Add 40µL of Epiclone™ Anti-C, Epiclone™ Anti-E, Epiclone™ Anti-c, Epiclone™ Anti-e or Epiclone™ Anti-C^w phenotyping reagent.
   BioRad™: Add 25µL of Epiclone™ Anti-C, Epiclone™ Anti-E, Epiclone™ Anti-c, Epiclone™ Anti-e or Epiclone™ Anti-C^w 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™ Neutral card.
3. Add 40µL of Epiclone™ Anti-C, Epiclone™ Anti-E, Epiclone™ Anti-c, Epiclone™ Anti-e or Epiclone™ Anti-C^w 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
Epiclone™ Anti-C, Epiclone™ Anti-E, Epiclone™ Anti-c, Epiclone™ Anti-e and Epiclone™ Anti-C^w is validated for the following microplate method, however due to variation in methods and equipment, microplate users should validate these reagents using their methods.
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 the applicable Epiclone™ Anti-C, Epiclone™ Anti-E, Epiclone™ Anti-c, Epiclone™ Anti-e or Epiclone™ Anti-C^w phenotyping reagent to the appropriate test wells.
3. Add an equal volume of the suspension of test red cells to the appropriate test wells.
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 microplates at room temperature for 15-20 minutes.
6. Centrifuge at low speed (100rcf) for 40 seconds*.
7. Resuspend the red cells using the microplate shaker for an optimal time and agitation speed.
8. Read tests macroscopically or with an automated reader.

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

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, red cells or expired reagents.
8. Many human monoclonal IgM anti-Rh antibodies have been shown to possess anti-I/i cold agglutinin activity, particularly with cord cells or enzyme treated cells. This may become apparent if tests are incubated below the recommended temperature.

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. Epiclone™ Anti-C, Epiclone™ Anti-E, Epiclone™ Anti-c, and Epiclone™ Anti-C^w should be clear; turbidity may indicate bacterial contamination. The reagent should not be used if a precipitate or particles are present.
5. Epiclone™ Anti-e should be a clear to slightly opaque particle-free liquid.
6. The bovine material used is from an approved source free of Bovine Spongiform Encephalopathy (BSE).

REFERENCES

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4. Habibi B, Andre J, Fouilllade MT, Lopez M, Salmon C. An unusual Rh phenotype indicating heterogeneity of the C^w antigen. Vox Sang 1976; 31: 103-8.
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6. Lawler SD, van Loghem JJ. The Rh antigen C^w causing haemolytic disease of the newborn. Lancet 1947; 2: 545-6.
7. Chown B, Lewis M. The occurrence of Rh haemagglutin of specificity anti-C^w in the absence of known stimulation: suggestions as to cause. Vox Sang 1954; 4 (Old Series): 41-5.
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9. White WD, Issitt CH, McGuire D. Evaluation of the use of albumin controls in Rh phenotyping: Transfusion 1974; 14: 67-71.
10. Harmening DM. Modern Blood Banking and Transfusion Practices. 5th Ed. FA Davis Company. Philadelphia 2005 (or current edition).
11. Ortho BioVue™ System Neutral instructions for use.
12. BioRad™ NaCl, Enzyme Test and Cold Agglutinins instructions for use.

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

	WARNING: Health Hazard


Immulab Pty Ltd
11 Dalmore Drive, Scoresby Victoria 3179 Australia
ABN: 77 623 052 095 P: 1300 369 559