**METHOD SUMMARY**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Tile Method</th>
<th>Tube Method</th>
<th>Tube/IAT Method</th>
<th>CAT Method</th>
<th>MTP Method</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Validated Methods</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Reagent Volume</strong></td>
<td>1</td>
<td>1</td>
<td>1-2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Cell Volume</strong></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Cell Concentration</strong></td>
<td>35-50%</td>
<td>3-5%</td>
<td>3-5%</td>
<td>0.8%</td>
<td>3-5%</td>
</tr>
<tr>
<td><strong>Incubation Time</strong></td>
<td>2 mins</td>
<td>Immediate Spin</td>
<td>30 mins</td>
<td>15-20 mins</td>
<td></td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td>Room Temp</td>
<td>Room Temp</td>
<td>37°C</td>
<td>Room Temp</td>
<td></td>
</tr>
<tr>
<td><strong>Spin (Speed/Time)</strong></td>
<td>High for 20 secs</td>
<td>Low, 20 secs</td>
<td>Low, 40 secs</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Validated for use in Ortho-Clinical Diagnostics BioVue™ Neutral cards (Anti-K) and AHG cards (Anti-k and Anti-Kp). The clone used to produce Epiclone™ Anti-K is MS-56 (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. However, the agglutination reaction signifies the absence of the corresponding antigen.

**BACKGROUND**

**Blood Group System**

The Kell system was discovered by Coombs et al. in 1946 and its antigen, Kell, was identified in 1947 by Levine et al. A new antigen, Kpa, was described in 1990 by Nalefski et al. (Kell-Kpa). The Kell antigen is a red cell membrane glycoprotein, and to date, over 30 Kell antigens have been defined within the system.

**Antigen/ Antibody Characteristics**

Antigens of the Kell system are expressed on red cells, as well as on tissues of the bone marrow and fetal liver, but are not expressed on brain, kidney, or adult liver tissues. Kell system antigens are well expressed on feto-placental tissues and are fully developed at birth. Kell and Kpa antigens are resistant to treatment by the enzymes ficin, papain, trypsin, and sialidase and by chloroquine. They are sensitive however to treatment by DI and acid.

Most antibodies of the Kell system are immune in nature, reacting well by indirect antiglobulin test (IAT) and have been implicated in transfusion reactions. They generally do not bind complement. They may cause haemolytic disease of the Fetus and Newborn (HDN) and are capable of crossing the placenta and they are well developed in utero.

**Reactions obtained with:**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Australian Blood Donor</th>
<th>Caucasians</th>
<th>Negroid Populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>+</td>
<td>N/A</td>
<td>K+k</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>1.0000</td>
<td>1.0000</td>
</tr>
<tr>
<td>K-k</td>
<td>0.9100</td>
<td>0.9091</td>
<td>0.9800</td>
</tr>
<tr>
<td>K-k</td>
<td>0.9773</td>
<td>0.9770</td>
<td>1.0000</td>
</tr>
<tr>
<td>Kpa</td>
<td>0.0227^</td>
<td>rare</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

^ The frequency of Kp(a+) in K+ positive persons is only about half that in the general population.

**SPECDON 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. Samples collected in Citaltine 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 Cellprep™ at 2° to 8°C for up to 42 days.

**RECOMMENDED METHODS**

**Epiclone™- Anti-K**

**Tile Method**

1. Prepare a 35-50% suspension of test red cells in autologous plasma, buffered or unbuffered isotonic saline.
2. Add 1 drop of Epiclone™ Anti-K phenotyping reagent to a clean, labelled glass test tube (10x75mm or 12x70mm). Mix and centrifuge at high speed (1000rcf) for 20 seconds^.
3. Gently agitate the tube to dislodge the red cells and examine for agglutination.
4. Incubate all negative or weakly positive tests at 37°C for 5 minutes and repeat steps 4 and 5.

**Tube Method**

1. Prepare a 3-5% suspension of test red cells in buffered or unbuffered isotonic saline, or in Cellprep™.
2. Add 1 drop of Epiclone™ Anti-K phenotyping reagent to an appropriately labelled glass test tube (10x75mm or 12x70mm).
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 tube at room temperature.
5. Examine for agglutination at 2 minutes. Record results. Do not confuse any drying of the mixture with agglutination.

**Phenotyping Reagents**

**Cell Alignment Technology (CAT)**

1. Prepare a 3-5% suspension of test red cells in buffered or unbuffered isotonic saline, or in Cellprep™.
2. Add 1 drop of Epiclone™ Anti-K phenotyping reagent to an appropriately labelled glass test tube (10x75mm or 12x70mm).
3. Add 1 drop of the suspension of test red cells.
4. Mix and centrifuge at high speed (1000r/min) 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.

**Notes:**

- O* centrifuge at a speed and time appropriate for the centrifuge in use.

**Column Agglutination Technology (CAT)**

1. Prepare a 0.8% suspension of test red cells in Cellprep™ LSS.
2. Label a BioVue™ Neutral card or a BioVue™DiaMed™ ID-Card NaCl enzyme test and cold agglutinins card.
5. + 50 µL of the suspension of 0.8% test red cells (to be phenotyped).
6. Centrifuge according to the manufacturer's instructions.
7. Read according to the manufacturer’s instructions.

**Column Agglutination Technology (CAT)**

1. Prepare a 3% suspension of test red cells in Cellprep™.
2. Label a BioVue™ Neutral card.
3. Add 40 µL of Epiclone™ Anti-K phenotyping reagent.
4. Add 10 µL of the suspension of 3% test red cells (to be phenotyped).
5. Add 40 µL of Cellprep™.
6. Centrifuge according to the manufacturer’s instructions.
7. Read according to the manufacturer’s instructions.
Microplate Method

Epicleone™ Anti-K is validated for the following microtitre plate method, however 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 solution containing 1% BSA.
2. Add 1 volume of Epiclone™ Anti-K phenotyping reagent to the appropriate test wells.
3. Add an equal volume of the suspension of test red cell to the appropriate test well.
4. Mix the contents of each well manually or using a microtitre shaker. The time required to achieve this will depend on the speed and orbit of the shaker.
5. Incubate the microtitre plate at room temperature for 15-20 minutes.
6. Centrifuge at low speed (1000g) for 40 seconds.
7. Resuspend the red cells using a microtitre shaker for an optimal time and agitation speed.
8. Read the tests macroscopically or with an automated reader.

RECOMMENDED METHODS – bioCSL Anti-k (Cellano) and bioCSL Anti-Kp

Tube 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 bioCSL Anti-k (Cellano) or bioCSL Anti-Kp phenotyping reagent to an appropriately labelled, clean glass test tube (10x15mm 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. Wash the cells with 4 changes of buffered or unbuffered isotonic saline, ensuring that the saline is decanted completely after each wash and that the cells are completely resuspended between washes.
6. To the 'dry' button of cells remaining after the fourth wash, add 1 or 2 drops of Epiclone™ AHG Poly reagent.
7. Mix well and centrifuge at low speed (500 rcf) for 15 to 20 seconds.
8. Gently agitate the tube to dislodge the red cells and examine for agglutination. Record results.
9. Add 1 drop of bioCSL AHG Control Cells 3% to all negative test tubes to validate the results.
10. Repeat steps 7 and 8.

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

bioCSL recommends the use of buffered isotonic saline solutions (pH 7.0 to 7.2).

Column Agglutination Technology (CAT) 0.8% Method (BioVue™ and BioHIV™/DiaMed™) – Indirect Antiglobulin Test (IAT)

1. Prepare a 0.8% suspension of test red cells in Celpresol™.
2. Label a BioVue™ AHG card or a BioHIV™/DiaMed™ IC-Card LISS/Combs card.
3. Add 50 µl of the suspension of 0.8% test red cells to be phenotyped.
4. BioVue™: Add 40 µl of bioCSL Anti-k (Cellano) or bioCSL Anti-Kp phenotyping reagent.
6. Incubate at 37°C according to the manufacturer’s instructions.
7. Centrifuge according to the manufacturer’s instructions.
8. Read according to the manufacturer’s instructions.

Column Agglutination Technology (CAT) 3% Method (BioVue™ only) – Indirect Antiglobulin Test (IAT)

1. Prepare a 3% suspension of test red cells in Celpresol™.
2. Label a BioVue™ AHG card.
3. Add 40µl of bioCSL Anti-k (Cellano) or bioCSL Anti-Kp phenotyping reagent.
4. Add 10µl of the suspension of 3% test red cells to be phenotyped.
5. Add 40µl of bioCSL Rapid Antibody Medium (RAM).
6. Incubate at 37°C according to the manufacturer’s instructions.
7. Centrifuge according to the manufacturer’s instructions.
8. 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 (subject to the cells giving a negative DAT). No agglutination of the test red cells indicates the absence of the relevant antigen. As the K and k antigens are allelic, all test cells, except the very rare Kk type, should react with one or both of these reagents.

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 e.g. use a Kk+ k-+ control to cell the Anti-k and Anti-Kp reagents.

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

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