C•A•B•B
Canadian Animal Blood Bank

CANADIAN ANIMAL BLOOD BANK
2055 Notre Dame Ave. Room AB 71
Winnipeg, MB R3H 0J9
(204) 632-2586

CROSSMATCH TECHNIQUE

A crossmatch is performed to detect serological incompatibility.

DOGS

Crossmatching is recommended before transfusion, although dogs usually lack naturally occurring alloantibodies. All dogs that have received red cells more than four days previously must be crossmatched with a fresh sample of serum before receiving any more red cells.

CATS

Cats have naturally occurring alloantibodies and a crossmatch should **always** be performed prior to transfusion.

Equipment and Reagents

5 glass tubes[1]
Test tube rack(s)
Pasteur pipettes (plastic or glass)
Incubator or waterbath at 37º C[3]
Centrifuge
Phosphate Buffered Saline (PBS)[2]
Scissors
Thermometer

Procedure

1. Prepare 37°C waterbath. If using a styrene box, this may take 30 minutes to achieve equilibrium to hold the temperature for the 30 minute incubation time.

2. Collect blood from the RECIPIENT in a tube containing no anticoagulant or serum separator.

3. Centrifuge the specimen at 1000 g for at least 5 minutes to separate the serum from the red cells.

4. Remove the serum with a pipette into a clean, labelled test tube.

5. Remove some of the free red cells at the bottom of the original blood collection tube into a clean labelled test tube.

6. Prepare DONOR red cells to be crossmatched as follows:

   a) Cut off a segment at the heat seal from a unit or red cells concentrate and place into a test tube labelled with the unit number.

   b) Cut off one end of the red cell segment. Invert the segment so that the open end is in the test tube.

   c) Cut off the other end of the segment. The red cells should empty into the test tube.

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[1] 10mm x 75mm glass tubes (or equivalent) – enough tubes for 1 crossmatch, add 2 more tubes for each additional crossmatch unit

[2] Although 0.9% saline can be used, PBS reacts better with canine and feline red cells.

[3] A waterbath can be made with a styrene container with a ceramic mug (heat sink), will hold a test tube rack and filled with 2 - 3 inches of tap water at 37º C.

d) Wash the recipient and donor red cells with saline.
- Fill the test tube with saline.
- Centrifuge tube at 1000 g for 1minute.
- Decant the supernatant.
- Repeat 2 more times.
- If the supernatant shows haemolysis, continue washing the cells until the supernatant is clear and colourless.

e) Make a 5% suspension of the red cells in the same tube.
(1 drop of red cells + 20 drops saline)

7. Prepare tubes as follows for EACH unit to be crossmatched:

Label tube with:	**CANINE** 37º C Phase
Auto Control	4 drops **RECIPIENT** serum + 1 drop of 5% **RECIPIENT** red cell suspension (see 5.e)
Unit Number to be crossmatched	4 drops **RECIPIENT** serum + 1 drop of 5% **DONOR** red cell suspension

Label tube with:	**FELINE ONLY** Room Temp Phase
Auto Control	4 drops **RECIPIENT** serum + 1 drop **RECIPIENT** red cells suspension
Donor to be crossmatched	4 drops **RECIPIENT** serum + 1 drop **DONOR** red cell suspension
Minor Crossmatch of Donor Unit	4 drops **DONOR** serum + 1 drop **RECIPIENT** red cell suspension
Donor Control	4 drops **DONOR** serum + 1 drop **DONOR** red cell suspension

8. Mix all tubes gently.
9. Incubate the **CANINE** set of tubes at 37° C for 30 minutes. (Check the temperature of the incubator).

- **PROCEED DIRECTLY TO STEP 10 FOR FELINE TUBES!**

10. Centrifuge all tubes for 15 seconds at 1000 g.
11. Examine the supernatant for haemolysis.
12. **GENTLY** suspend the cell button by rocking the tube and examine for macroscopic agglutination.

Interpretation

Compatibility is indicated when there is no haemolysis or agglutination in any test tube, either macroscopically or microscopically. Test result is NEGATIVE.

Incompatibility is indicated by any haemolysis and/or agglutination in the red cells crossmatch tubes but not in the control tubes. A new donor must be selected. Test result is POSITIVE. If the control cells are weakly positive and the test sample is a strong positive, the crossmatch is **incompatible**.

If the control cells and the test cells are equally positive, **no conclusions as to compatibility can be drawn.**

Notes

A compatible crossmatch does not prevent sensitization or a delayed transfusion reaction. It simply indicates that at the present time there are no significant antibodies against the red cells.