CROSSMATCHING & EMERGENCY CROSSMATCHING

SaBB Veterinary Clinic and Laboratory Dubai 045 911 007 www.smallanimalbloodbank.com

Crossmatching

The lack of comprehensive knowledge of canine and feline blood types means that cross matching is necessary to detect serious antibody led incompatibilities. Crossmathing detects (in vitro) presence of significant levels of antibodies against erythrocyte antigens capable of inducing haemagglutination or haemolysis.

Since cats have naturally occurring alloantibodies and may experience a severe reaction to their first transfusion, a blood cross match (BCM) should be performed prior to any blood transfusion.
Some literature indicates that dogs, lacking naturally occurring alloantibodies, may be transfused without a BCM prior to their first transfusion. Although immediate transfusion reactions involving first transfusions in canines are rare, we recommend crossmatching before any transfusion if the situation permits, especially in cases involving autoimmune problems and where background information may not be complete or does not include prior transfusions or blood typing. All dogs that have received transfusions more than 4 - 7 days previously must be cross matched before receiving any additional transfusions. This is especially important when the blood types of the donor and recipient were or are unknown.

1) **Major** cross-match detects antibodies in the recipient's plasma against the donor's red blood cells.
2) **Minor** cross-match detects antibodies in the donor's plasma against the recipient's erythrocytes.

An incompatible major crossmatch:

- ➔ manifested by agglutination (clumping) or lysis of the erythrocytes.
- ➔ Potentially fatal acute haemolytic reaction
  - Do NOT proceed

An incompatible minor crossmatch:

- ➔ Less relevant due to donor plasma dilution in recipient plasma
- ➔ Minor reactions can occur and a minor crossmatch can help prevent these
- ➔ If autoagglutination is seen on the minor crossmatch, there are two options:
  - Avoid using the donor blood and find a different donor.
  - Proceed with caution washing the red blood cells prior to administration.

Compatible Crossmatch:

- ➔ Absence of clumping
- ➔ formation of rouleaux

It is best practice to both type and cross match for any transfusion. Cross-matching is mandatory if:

- The animal had a previous transfusion more than four days previous (even if the same donor)

- Unknown transfusion history
- The animal is a breeding animal. This is to avoid increased risk of neonatal isoerythrolysis
- Major crossmatching should always be performed prior to transfusion in cats
- A whole-blood transfusion requires both a major and minor crossmatch, whereas transfusion of pRBCs only requires a major crossmatch.

Animals can still have reactions even with a compatible cross-match because of reactions from white blood cells, platelets and other proteins.

Procedure of Crossmatching

Procedure:

Ideally, crossmatching should be performed using a commercially available test kit. If the result is questionable a manual crossmatch may be performed. **An autocontrol with recipient red cells and plasma is included because some recipients have immune mediated hemolysis with autoagglutination interfering with BCM.** Any hemolysis and/or agglutination in the major or minor BCM but not in the control indicates an incompatibility and the need to choose a new donor. If control is weakly positive and test sample is strong, results may be valid; if both are equal, no conclusions as to compatibility can be drawn.

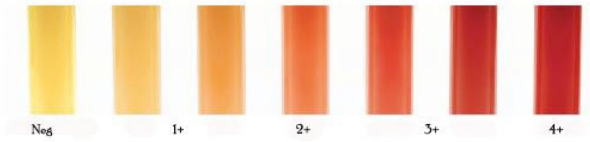
NB Some laboratories recommend incubating canine blood for 30 minutes. If time and equipment permit, it is also recommended that tubes be prepared and incubated at 4 degrees and 37 degrees C.

Major Crossmatch:

Collect one serum and one EDTA blood sample from the donor and recipient (at least 1 ml in each sample) or take one segment from the unit (but use a tube without anticoagulant if taken from the segment).

1) Centrifuge tube(s) for 5 min (1000x or 3400 rpm). Save the serum and discard the plasma from the EDTA tube
2) Prepare a 3%–5% suspension of washed donor RBCs in saline in a plain tube.
   - Pipette 0.1 ml of the pRBCs into 3 ml of 0.9% NaCl. (1 drop of pRBCs to 20 drops 0.9% NaCl)
   - Centrifuge the sample for 1 min, remove the supernatant, and resuspend the RBCs in 3 ml of 0.9% NaCl.
   - Repeat this three or four times to wash donor cells. Following the last wash, there should be a 3%–5% suspension (3 ml total).
3) Label plain tubes to make the following admixtures.
   a. *Major Crossmatch*: 2 drops patient's serum with 1 drop donor's RBC suspension
   b. *Minor Crossmatch:* 1 drop patient's RBC suspension with 2 drops donor's serum
   c. *Control*:1 drop patient's RBC suspension and 1 drop patient's serum
4) Mix gently and incubate for 15 min. at room temperature or if possible at 37°C).
5) Centrifuge the serum for 30 sec at low speed.
6) Check the supernatant for hemolysis
7) Gently resuspend button of cells by tapping tube with a finger and examine for macroscopic agglutination.
8) If no agglutination is present, let the sample sit for 5 min at room temperature.
9) If macroscopic agglutination is not observed, transfer a small amount onto a glass slide and examine for microscopic agglutination. Rouleaux is not an indication of incompatibility. To distinguish agglutination from rouleaux formation, centrifuge the tubes again for 15 seconds, remove serum, and add 2 drops of saline. Then centrifuge the tubes again and re-examine the RBC suspension.
10) Check the sample for evidence of macroagglutination or microscopic agglutination.

Hemolysis



4+	**No RBC clump, Liquid is red**	**POSITIVE**
3+	**Small RBC pellet left after centrifugation** **Liquid is red** **(seen with 2+ agglutination)**	**POSITIVE**
2+	**Medium RBC pellet left after centrifugation** **Liquid is red** **(seen in 2+ agglutination)**	**POSITIVE**
1+	**Large RBC pellet left** **Liquid has a normal colour or soft pink**	**NEGATIVE**

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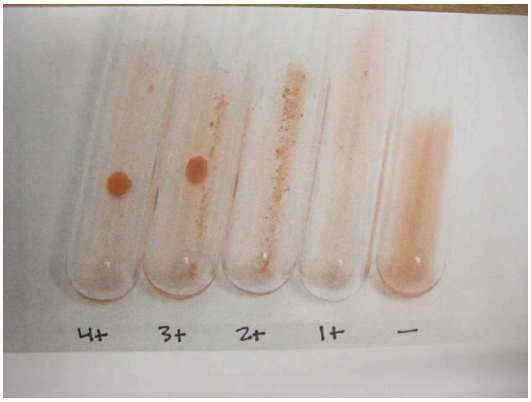
Macroagglutination



4+	**ONE large clump of RBC, no free cells**	**POSITIVE**
3+	**ONE large clumo and some small clumps**	**POSITIVE**
2+	**Many medium size clumps and some free cells**	**POSITIVE**
1+	**Many tiny clumps and lots of free cells**	**NEGATIVE**
NEG	**Only free cells**	**NEGATIVE**

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Emergency Crossmatching

Slide crossmatch

➔ <u>It's important to recognize that the slide crossmatch technique will fail to identify hemolytic reactions and other types of reactions, making it less beneficial than a commercially available crossmatch test.</u>

- **Major** crossmatch: two drops of recipient plasma are mixed with one drop of donor blood and examined under a microscope for agglutination.
- **Minor** crossmatch, similar test except using two drops of donor plasma and one drop of recipient blood.
- Examine Macroscopically (rolling tubes between you fingers) and Microscopically (10x and 40x with cover slip)

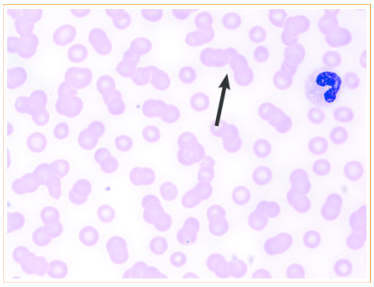
ROULEAUX DOES NOT MEAN AGGLUTINATION!

➔ Rouleaux should disperse as saline is added to the slide, and true agglutination will remain.

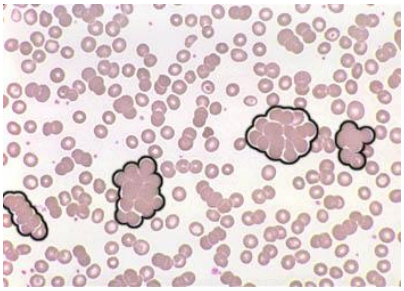
Feline emergency crossmatching

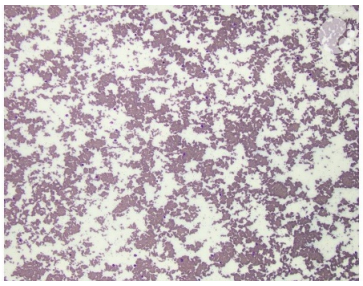
1. Draw 0.5 to 1 mL of blood from the donor and recipient cats in two serum tubes
 - Label the tubes appropriately.
2. Allow tubes to clot.
3. Spin in centrifuge (3000 rpm) for 5 to 10 minutes.
4. Decant and collect the serum into new red top tubes.
5. Apply two drops of recipient serum and one drop of donor red blood cells (RBCs) on a glass slide (major crossmatch).
6. Apply two drops of recipient serum and one drop of recipient RBCs on a glass slide (patient control).
7. Apply two drops of donor serum and one drop of donor RBCs on a glass slide (donor control).
6. Wait 1 to 5 minutes.
7. Examine the slides for microscopic clumping of RBCs (not Rouleaux!)

Adapted from the International Society of Feline Medicine



Rouleaux (stacked coins)





Agglutination (grape like clusters/clumps)