Expert Guide

Step by step guide to manual cross matching

pet blood bank uk

As the UK's trusted pet blood banking charity, we provide quick access to high quality products as well as expert advice and guidance when you need it most.

We hope this guide is useful.
If you have any further queries, please contact our team.

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When to cross match

It is best practice to cross match all dogs receiving transfusions, but you MUST cross match any dog who is known to have had a previous transfusion > 4-7days previously.

Cross matching is also recommended in:

- dogs with an unknown transfusion history
- history of previous transfusion reaction
- multiparous bitches
- some cases of IMHA

Cats have naturally occurring alloantibodies to 'foreign' red cell antigens, so should always be cross matched prior to transfusion.

Minor versus major cross matching

A cross match is utilised to avoid acute immune mediated transfusion reactions.

A major cross match allows you to pre-screen for the presence of existing recipient antibody to the red blood cell antigens of the donor. **Major: donor red blood cells vs. recipient plasma.**

A minor cross match allows you to detect the presence of existing antibody in the donor plasma against recipient red blood cell antigens. A minor cross match is more relevant for whole blood or plasma transfusions than for packed red cell transfusions where most of the donor plasma has been removed during processing. **Minor: recipient red blood cells vs. donor plasma.**

Positive reactions in a minor cross match are not likely to hold as much clinical significance compared to reactions detected in a major cross match.

The procedure described in this guide is for a major cross match but the same procedure applies to a minor cross match with the recipient/donor samples changed accordingly.

The ideal scenario

The aim is to try and find compatible blood to transfuse.

In the case of incompatibility, the best scenario occurs when the patient is not in need of urgent transfusion, as this allows for a serial cross match and the potential to find the most compatible blood to transfuse.

However, for patients who are profoundly anaemic and in urgent need of transfusion, a decision may have to be made to transfuse despite some incompatibility. While this decision is being made, the vet can modify patient care (e.g. oxygen supplementation, rest to decrease oxygen demand) until a decision has been reached and the transfusion can begin.

Equipment

- Centrifuge set at speed 3400rpm
- 2 EDTA tubes (1ml) to fit centrifuge
- 8 plain tubes (1ml) to fit centrifuge
- 1 x 5ml plain sample holder (could be urine tube if no 5ml plain blood tubes)
- A few single use dropper pipettes
- 5ml syringe
- 1ml syringe
- 37°C water bath (can use water in bowl, just check temp or incubator)
- Access to refrigerator at 4°C
- Microscope slides
- Oil immersion lens (x 100)
- Stopwatch

Preparation

Step 1

Collect 1ml EDTA plasma from the recipient (spin blood and place supernatant plasma in a plain tube, discard pellet of RBC's) and 1ml of donor RBC's (this can be an aliquot from a Pet Blood Bank product or EDTA blood from an in-house donor).

Step 2

Place donor red cells in a plain tube.

Centrifuge at 3400rpm for 2 minutes.

Step 3

Decant supernatant leaving the red cells. The red cells should then be washed by filling the tube with 0.9% saline to resuspend the cells before centrifuging at 3400rpm for 1 minute.

After centrifuging remove and discard the supernatant.

Repeat the washing procedure two further times.

Preparation

Step 4

Prepare a 4% red blood cell suspension by adding 0.2ml packed red blood cells to 4.8ml saline and label with the donor ID.

Step 5

Now prepare six tubes (3 control and 3 donor) by labelling them as indicated in the table below. The numbers indicate the incubation temperature of the tube during cross match.

1. Donor 37	2. Control 37
3. Donor 25	4. Control 25
5. Donor 4	6. Control 4

Mixture preparation and incubation

Step 6

The cross match is performed by placing 2 drops of recipient plasma and 1 drop of the prepared 4% red blood cell suspension into each of the 3 tubes labelled as donor.

Step 7

The control tubes are prepared by putting 2 drops of 0.9% saline and 1 drop of the prepared 4% red blood cell suspension into the 3 tubes labelled as control.

Step 8

Incubate each pair of tubes for 15 mins at the marked temperature:

37°C – incubator or temperature controlled water bath

25°C – room temp

4°C – refrigerator

Centrifugation and initial assessment

Step 9

Following incubation, centrifuge all 6 tubes for 1 minute.

Step 10

Examine the supernatant for haemolysis by comparing it to the control sample.

Step 11

Gently invert each tube and assess for macro-agglutination of cells.

Step 12

Prepare the slide by placing a drop of the solution on a slide and assess for microscopic agglutination (Fig.1) (take care to differentiate from rouleaux formation).

If haemolysis, macro-agglutination, or micro-agglutination are observed the donor is **NOT COMPATIBLE**, and the result should be interpreted accordingly.

Microscopic agglutination

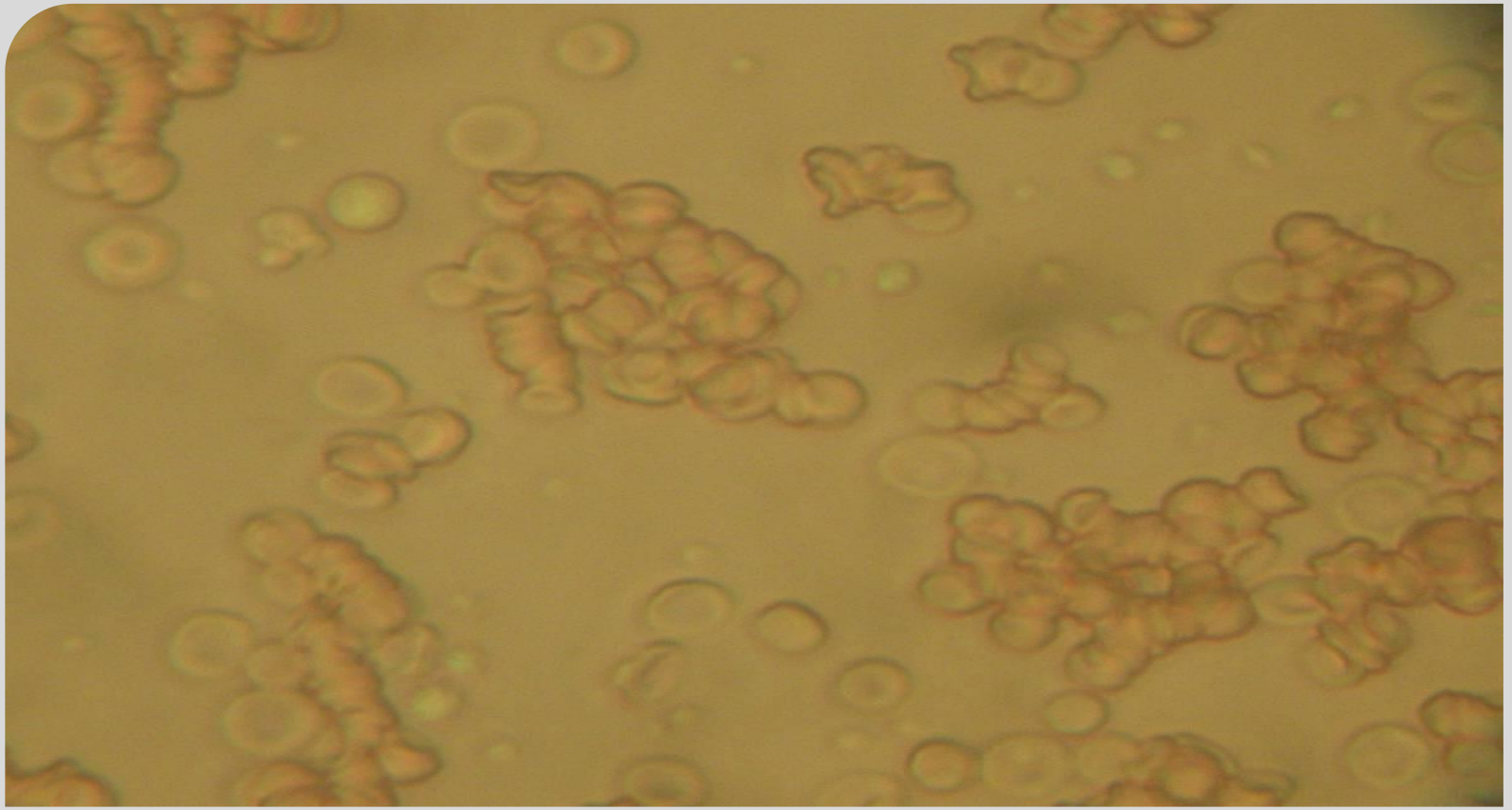


Fig.1 Microscopic agglutination

Haemolysis chart

Haemolysis is assessed by viewing the plasma component immediately after centrifuging the samples.

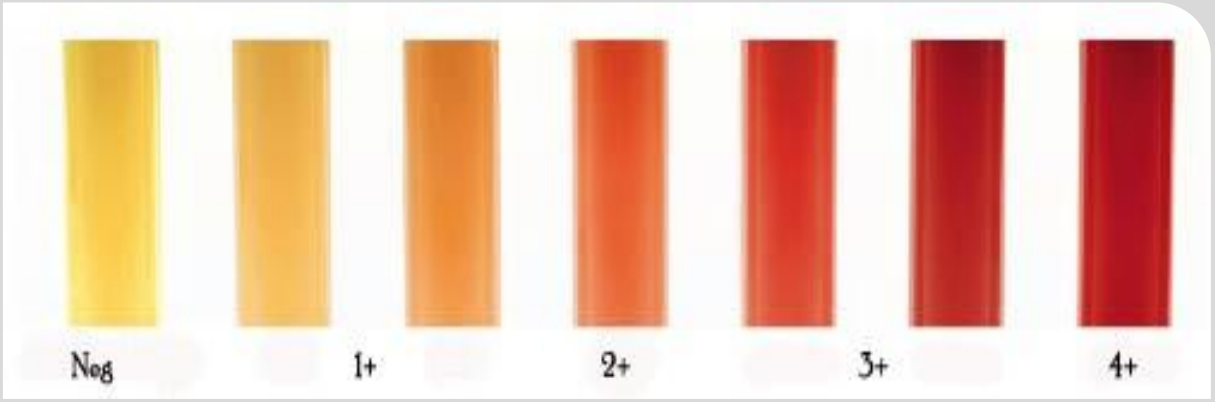


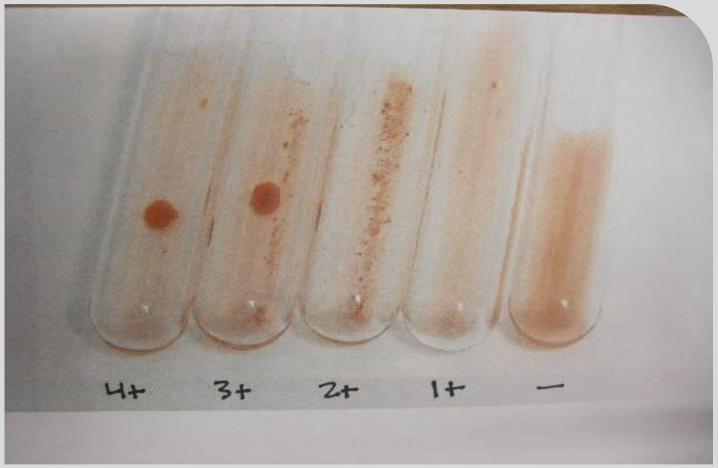
Fig.2 Haemolysis chart for serum or plasma

Interpretation of haemolysis

4+ HEMOLYSIS	all red blood cells have been lysed, liquid phase is red, no red blood cell clump	**POSITIVE**: do NOT use this donor for transfusion with this recipient
3+ HEMOLYSIS	a small red blood cell pellet is left after centrifugation, liquid phase is red, often seen with 2+ agglutination	**POSITIVE**: do NOT use this donor for transfusion with this recipient
2+ HEMOLYSIS	a moderate red blood cell pellet is left after centrifugation, liquid phase is red, often seen with 2+ agglutination	**POSITIVE**: it is recommended that a different donor be utilised
1+ HEMOLYSIS/ NEGATIVE	a large red blood cell pellet is left after centrifugation, liquid phase is pink or light pink, red blood cells re-suspend normally	**NEGATIVE**: this donor may be utilised for transfusion of this recipient

Agglutination guide

Macroscopic agglutination is assessed by gently agitating tubes and assigning a score based on the red cell movement/separation.



Agglutination Guide

An illuminated mirror can be used to assist in assessment of macroscopic agglutination as shown in Fig.3.

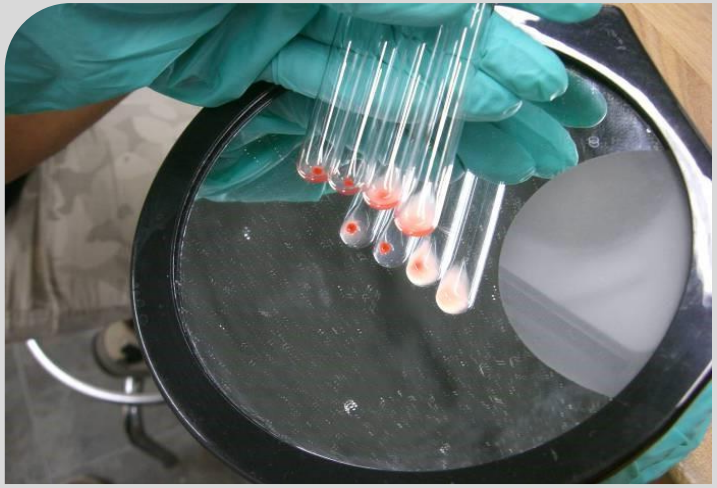


Fig.3

Interpretation of macroscopic agglutination

4+ AGGLUTINATION	one large clump of red blood cells, no free cells	**POSITIVE**: do NOT use this donor for transfusion with this recipient
3+ AGGLUTINATION	one large clump of red blood cells, some tiny clumps of red blood cells	**POSITIVE**: do NOT use this donor for transfusion with this recipient
2+ AGGLUTINATION	many medium size clumps of red blood cells, some free cells	**POSITIVE**: it is recommended that a different donor be utilised
1+ AGGLUTINATION	many tiny clumps of red blood cells, many free cells	**NEGATIVE**: this donor may be utilised for transfusion of this recipient
NEGATIVE	all free red blood cells	**NEGATIVE**: this donor may be utilised for transfusion of this recipient

Thank you for using this guide.
We hope you found it useful.

To make transfusion medicine as easy for you as possible, we also provide:

- Blood deliveries around the clock
- Quality tested products that reduce the risk of complications
- Advice on cross matching and selecting blood products
- Administrative equipment

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