Feline Blood Transfussion

Online webinar

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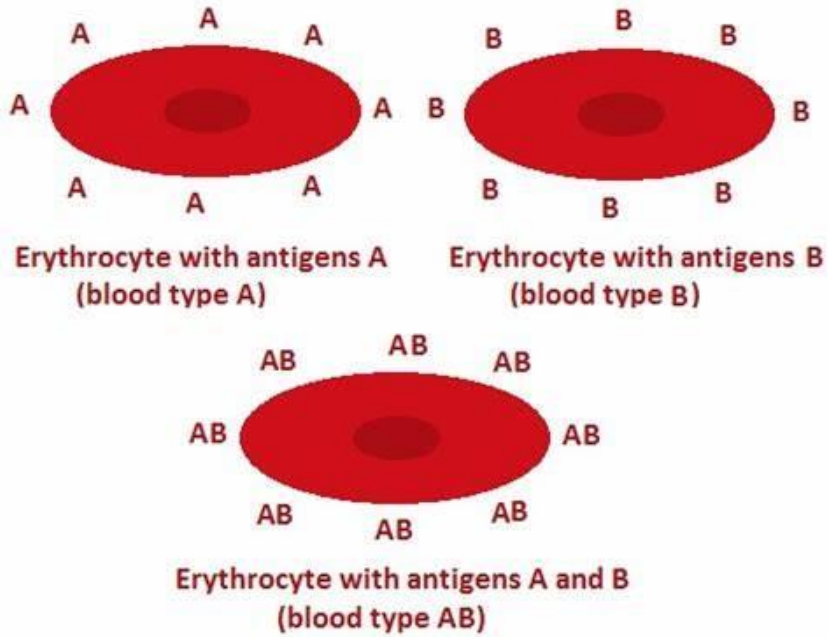
Feline blood group system

- Important in mediating blood transfusion reactions and neonatal isoerythrolysis (NI).

- AB blood group system:
  - ✓ consists of A, B & AB (C) blood group
  - ✓ With three alleles; A, b, & a^{ab}

- Mik; a new feline blood group antigen.

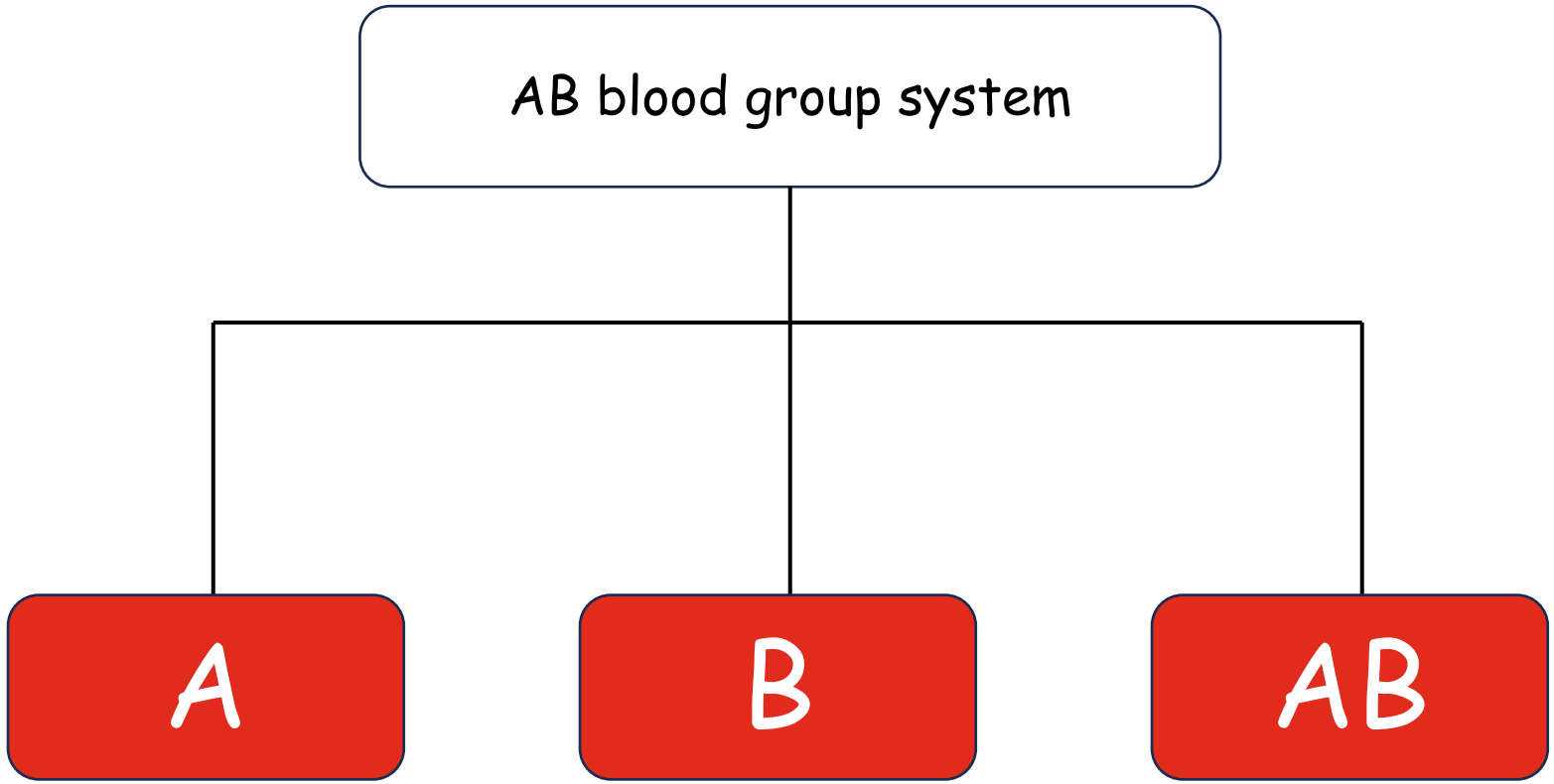


Feline blood group Prevalence

- Different prevalence in different feline breeds and geographical populations.

  - ✓ Blood group A is the most common in cats in Iran and the United States, especially among DSH & DLH.
  - ✓ Blood group B is common in European breeds (Devon rex, British shorthair, Abyssinian).
  - ✓ Blood group AB is rare.

Breed	Group A (%)	Group B (%)	Group AB (%)	Number of cats	Country	Reference
Abyssinian	100	0	0	36	Australia	Barrs *et al.*, 2009
	89	11	0	30	Australia	Malik *et al.*, 2005
	100	0	0		Hungary	Bagdi *et al.*, 2001
Bengal	100	0	0	100	UK	Gunn-Moore *et al.*, 2009
	86	14	0	7	UK	Forcada *et al.*, 2007
Burmese	100	0	0	5	UK	Forcada *et al.*, 2007
	93	3	3	30	Australia	Malik *et al.*, 2005
Carthusian/Chartreux	77.8	18.5	3.7	27	Germany	Weingart *et al.*, 2006
Persian	80	20	0	5	UK	Forcada *et al.*, 2007
	67	22	11	9	Australia	Malik *et al.*, 2005
	100	0	0	7	Portugal	Silvestre-Ferreira *et al.*, 2004
	66.6	33.3	0		Hungary	Bagdi *et al.*, 2001
Ragdoll	80	20	0	5	Australia	Malik *et al.*, 2005
Russian Blue	80	20	0	5	Australia	Malik *et al.*, 2005
Siamese	100	0	0	13	UK	Forcada *et al.*, 2007
	100	0	0	12	Australia	Malik *et al.*, 2005
	100	0	0	19	Portugal	Silvestre-Ferreira *et al.*, 2004
	100	0	0		Hungary	Bagdi *et al.*, 2001
Somali	100	0	0	24	Australia	Barrs *et al.*, 2009
	71.4	23.8	4.8	21	Germany	Weingart *et al.*, 2006
Turkish Angora	53.6	46.4	0	28	Turkey	Arikan *et al.*, 2003
Turkish Van	42.3	57.7	0	78	Turkey	Arikan and Akkan, 2004
	40	60	0	85	Turkey	Arikan *et al.*, 2003



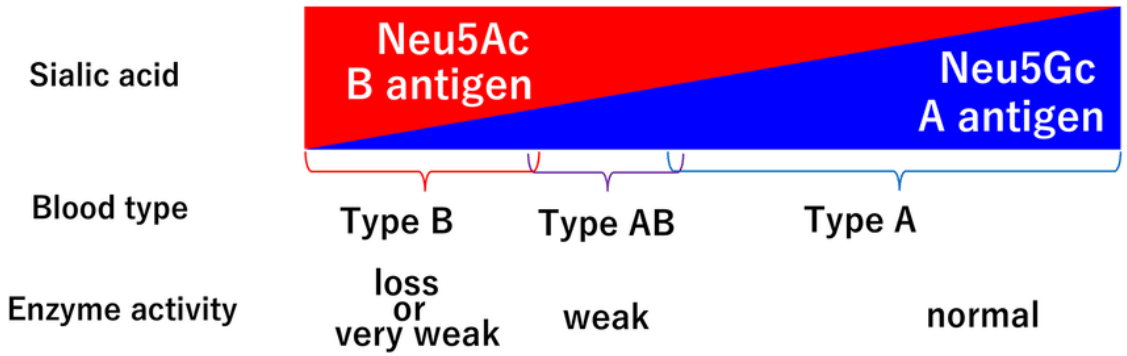
AA
Ab
Aa^{ab}

bb

$a^{ab}a^{ab}$
$a^{ab}b$

Feline blood groups & CMAH enzyme

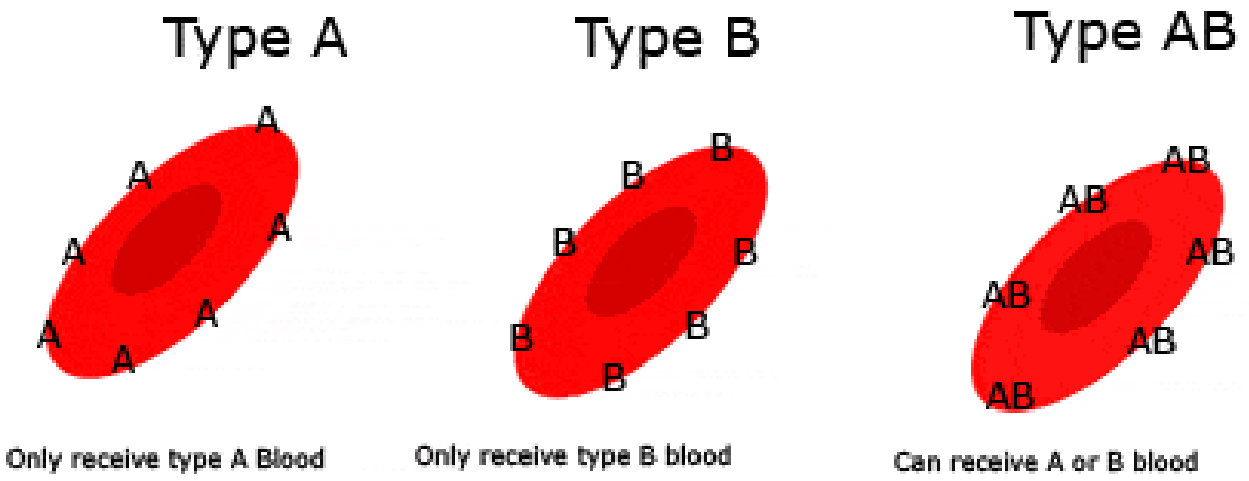
- The type A antigen is N-glycolyl-neuraminic acid (NeuGc) and the B antigen is N-acetyl-neuraminic acid (NeuAc).
- Type A cats have a dominance of NeuGc with small quantities of NeuAc, whereas type B cats have only NeuAc expression.
- Type AB cats have an equal amount of both molecules on the surfaces of their red blood cells (RBCs).
- Type B cats appear to lack the enzyme (cytidine monophosphoN-acetylneuraminic acid hydroxylase (CMAH)) that converts NeuAc to NeuGc.



- Since cats have naturally occurring alloantibodies (body gains immunity against antigens of another individual of the same species, which are perceived as foreign) against the blood-type antigen that they lack, <u>there is no universal feline blood donor</u>.

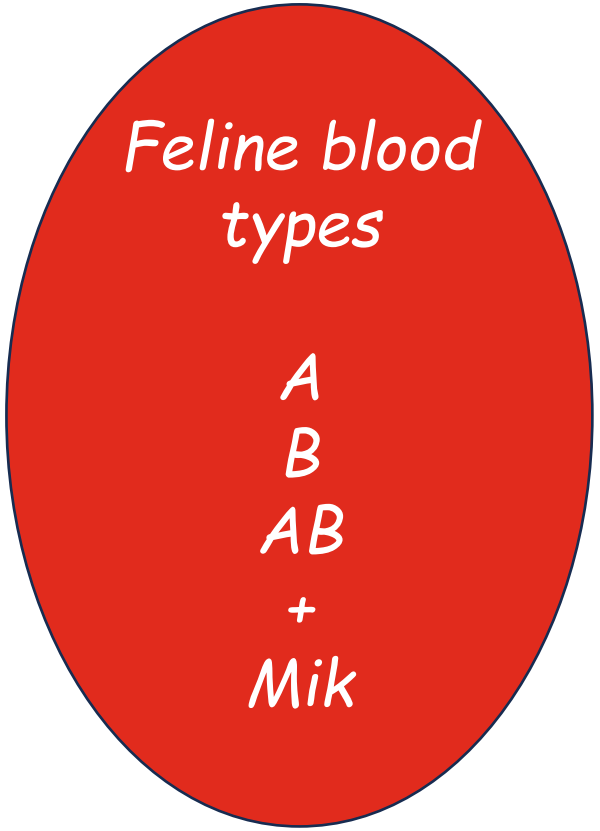
- Blood typing and cross-matching should <u>ALWAYS</u> perform on cats. Each day and every time, even in the first blood transfusion!

- Type A cats should receive type A blood.
- Type B cats should receive type B blood.
- Type AB cats can receive either type A or type B blood with no clinical reactions.



Mik antigen

- A new feline blood group antigen, Mik, has been identified recently.
- Some type A cats lack expression of Mik and therefore have serum alloantibody specific for this antigen.
- In these cats, even an AB matched blood transfusion may potentially lead to a reaction, so crossmatching would be required to detect this incompatibility

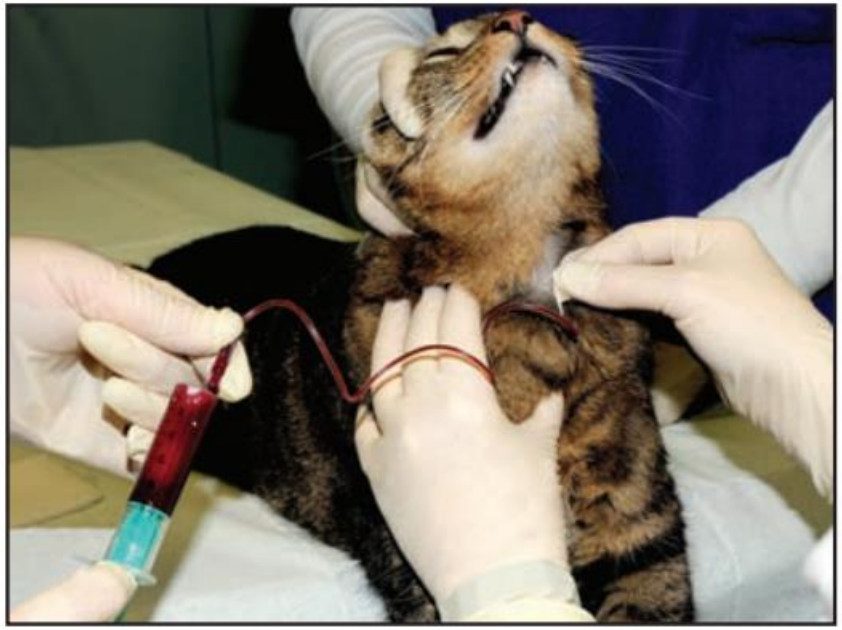


Feline blood donors

Donor cats should be:

- Healthy large adults
- With no history of previous blood transfusion
- Vaccinated and dewormed
- Indoor
- Not pregnant
- Normal CBC, biochemistry, & physical situation
- have a HCT of > 30, and preferably of > 35.
- Negative for infectious agents such as:

  - ✓ FeLV
  - ✓ FIV
  - ✓ haemotropic Mycoplasma spp.
  - ✓ Bartonella spp
  - ✓ Cytauxzoon felis
  - ✓ Babesia spp
  - ✓ Anaplasma phagocytophilum
  - ✓ Dirofilaria immitis
  - ✓ Neorickettsia risticii

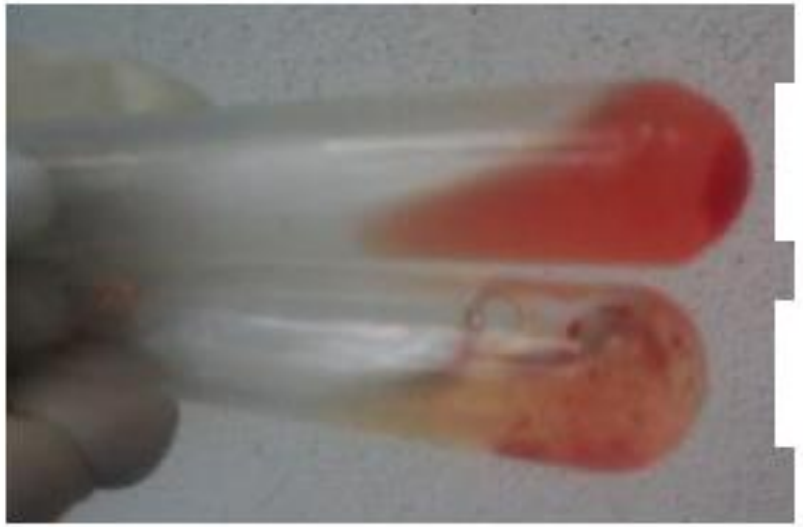


Feline blood donors

Other pre-transfusion tests

- To ensure efficient and safe transfusions, blood from both donor and recipient should be typed.
- Contrary to the situation in dogs, blood typing or cross-matching should be performed on all cats at the time of the first blood transfusion, owing to the frequency of naturally occurring alloantibodies.
- Owing to the existence of blood groups outside the AB system (e.g. the Mik antigen) the cross-match has become an important pre-transfusion test, because blood cross-matching demonstrates the serological compatibility between donor and recipient.

Feline blood typing



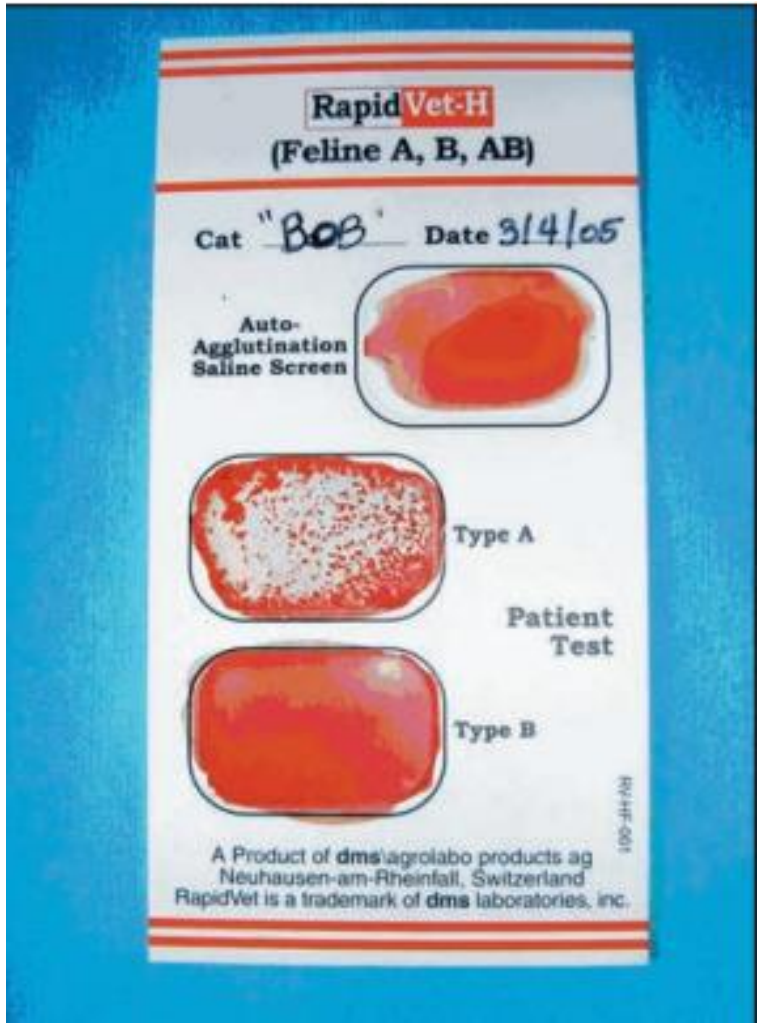
Agglutination tests

- The 'gold standard' for feline blood typing remains the tube or microplate agglutination test.
- A washed suspension of patient RBCs is incubated with reagents for the detection of blood group antigens A and B, and in a negative control well containing phosphate-buffered saline only.
- The standard reagent for detection of the A antigen is serum from a type B cat known to contain anti-A alloantibodies.
- The standard reagent for detection of the B antigen is the lectin from Triticum vulgaris, which at low concentration will only agglutinate RBCs that express the type B antigen.

Feline blood typing

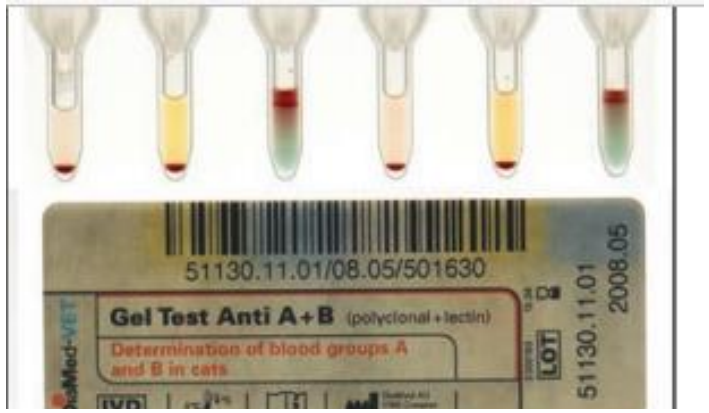
Card tests

- The cards have three 'wells' containing reagents for detection of A and B, and a negative control well to check for autoagglutination of the patient RBCs.
- The reagent for detection of the A antigen was initially serum from type B cats, but this has been replaced more recently with a monoclonal antibody specific for A.
- The anti-B reagent is T. vulgaris lectin. A diluent is added to each well, followed by the test blood.
- After mixing and a brief incubation with gentle rocking, the test is read by determining the presence of agglutination



Feline blood typing

- Gel-based test



- Immunochromatography

- Genetic testing

Cross-match test

- Cross-matching determines the serological compatibility between the patient and donor blood, based on an agglutination reaction, and allows the detection of naturally occurring alloantibodies or alloantibodies produced as a consequence of sensitization.

  There are two types of cross-match tests:

The major cross-match is an assessment of the compatibility between donor RBCs and patient plasma/serum.

The minor cross-match is an assessment of the compatibility between donor plasma/serum and patient RBCs

Cross-match test

❖ Rapid slide method

1. Collect blood into an EDTA tube from the recipient and donor. Alternatively, for the donor sample, a segment of anticoagulated blood from the donor blood tubing may be used.

2. Centrifuge tubes to settle the red blood cells (RBCs), remove the supernatant and transfer to a clean, labelled glass or plastic tube.
3. Label four glass slides as:

- Donor control = Donor RBCs and donor plasma
- Major cross-match = Donor RBCs AND recipient plasma
- Minor cross-match = recipient RBCs and donor plasma
- Recipient control = Recipient RBCs and recipient plasma

4. On to each slide place 1 drop of undiluted RBCs b and 2 drops of plasma a. Rapidly mix together with an applicator stick.

5. Gently rock the slides back and forth and observe for macroscopic agglutination within 2 minutes. Place a coverslip and observe for microscopic agglutination (with a ×40 objective or ×100 oil immersion lens) within 5 minutes.

Cross-match test

- Tube method

1. Collect blood into an EDTA tube from the recipient and donor. Alternatively, for the donor sample, a segment of anticoagulated blood from the donor blood tubing may be used.
2. Centrifuge tubes to settle the red blood cells (RBCs), remove the supernatant and transfer to a clean, labelled glass or plastic tube.
3. Wash the RBCs three times with normal saline solution, discarding the supernatant after each wash. To wash RBCs: add approximately 4 ml saline, mix well and centrifuge for 1–2 minutes. The saline is removed from the supernatant, leaving a packed RBC pellet at the bottom of the tube.
4. Resuspend the washed RBCs to create a 3–5% solution by adding 0.2 ml of RBCs to 4.8 ml normal saline (or 1 drop RBC to 20 drops saline).
5. For each donor prepare three tubes labelled 'major', 'minor' and 'recipient control'.
6. Add to each tube 1 drop of the appropriate 3–5% RBCs and 2 drops of plasma according to the following:
7. Incubate the tubes for 15 minutes at room temperature b .
8. Centrifuge the tubes for approximately 15 seconds to allow the cells to settle.
9. Examine the samples for hemolysis (reddening of the solution).
10. Gently tap the tubes to resuspend the cells, and examine and score the tubes for agglutination.

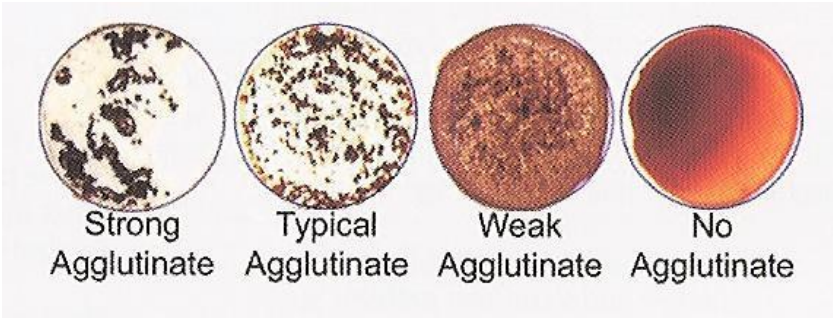
Collect blood into an EDTA tube from the recipient and donor. Alternatively, for the donor sample, a segment of anticoagulated blood from the donor blood tubing may be used.

Cross-match test

❖ Tube method

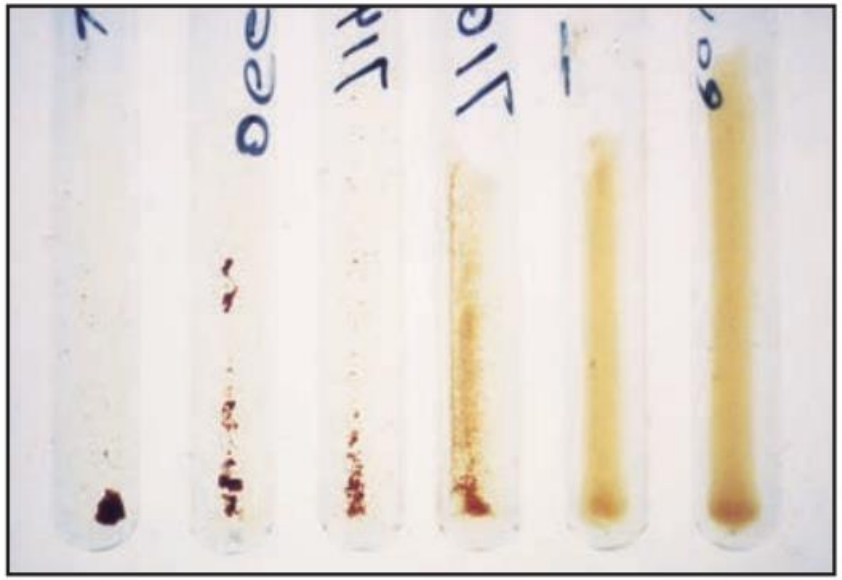
Evaluation of agglutination

- 4+ One solid aggregate of cells
- 3+ Several large clumps/aggregates of cells
- 2+ Medium-sized clumps/aggregates of cells, clear background
- 1+ Small/microscopic aggregate of cells, turbid reddish background
- +/- Microscopic aggregates



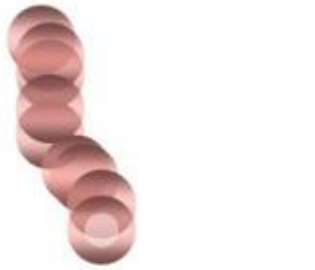
Recipient control

- If there is no hemolysis or agglutination noted in the recipient control tube, the results are valid and incompatibilities can be interpreted.
- If there is hemolysis or agglutination present in the equal scoring to the donor test samples, the compatibility and suitability of the donor can not be accurately assessed.



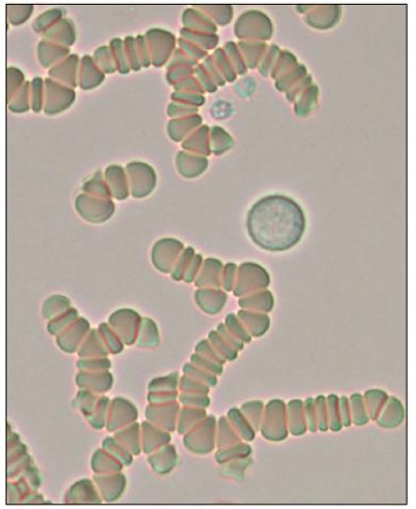
Microscopic examination of agglutination

- If macroscopic agglutination is suspected or not observed, transfer a small amount of the tube contents to a labeled glass slide and examine for microscopic agglutination.
- Take care not to confuse it with rouleaux formation
- With rouleaux, RBCs align to resemble a standing or fallen stack of coins. This type of formation is often normal and is not indicative of the presence of alloantibody.
- With agglutination, RBCs aggregate in 'grape-like' clusters.
- In some cases it may be difficult to distinguish between agglutination and rouleaux, in which case further RBC dilution may assist in clarification.

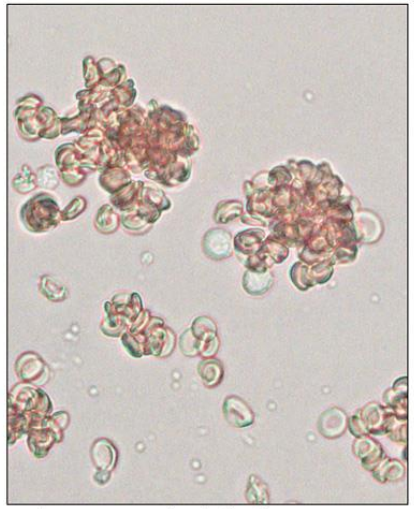


Rouleaux: Cells are arranged 'like stacks of coins'

Agglutination: Cells are randomly clumped together



Rouleaux



wet mount

Agglutination

Thanks for your attention

Any question?