How To - Saline Agglutination Test

WHY?

The saline agglutination test is used to determine whether red cell agglutinates are present in a blood sample. When red cell agglutinates are present this indicates that anaemia is due immune-mediated haemolysis.

WHEN?

You may decide to perform this test because the animal is anaemic or because you have seen red cells sticking together on a blood film in what appears to be agglutinates (like bunches of grapes) or rouleaux formation (stacks of coins). Rouleaux formation is due to increased amounts of inflammatory proteins in the blood and the red cells will disperse when saline is added to the blood. Agglutinates are caused by antibodies targeting the red cells and binding the cells together – this will not disperse when saline is added.

Consider performing a saline agglutination test every time you have an anaemic dog. That way you will be comfortable with recognizing what a normal or negative result looks like before you have a positive sample.

HOW?

1. Place a few drops of saline onto a glass slide.
2. Next touch a very small amount of EDTA blood either using a capillary tube or a needle and syringe on to the surface of the saline, enough to make it very slightly pink in colour when mixed (make sure your EDTA tube is well-mixed before removing any blood).
3. Gently mix the sample by rocking the slide backwards and forwards lengthways.
4. Place a coverslip on top of the saline.
5. Examine the slide on 10x objective of your microscope or higher if needed.
6. With a negative result all the red cells can be seen freely floating apart from each other. With a positive result, clumps of red cells like bunches of grapes will be floating around interspersed by a few free cells.
7. If the sample looks positive, leave it on the microscope stage with the light remaining on for a few minutes then come back and reconfirm that it is positive (sometimes strong rouleaux formation takes a little time to disperse).

Figure 1. A positive agglutination test - sample on gross exam (left) and under the microscope (right).