Medical Laboratory Techniques Department

Lab 4 : Preparation of different antigens

Msc. A'laa Khalaf Bediwi

Washing Cells and Making Red Cell Suspension

- Suspension of washed red cells is needed for all haemagglutination tests.
- The cells must be washed to remove plasma, cell suspensions are used in haemagglutination tests since the ratio of serum to cells affects the sensitivity of most tests – a minimum number of antibodies must bound to RBCs in order to bring about agglutination.

How washing red blood cells:

1- Place 0.2 -0.5 ml of blood into the tube (2-3 drops).

2- Fill 3/4 of the tube with normal saline (0.9% NaCl).

3- Centrifuge for 1-2 minutes until the RBCs are packed.

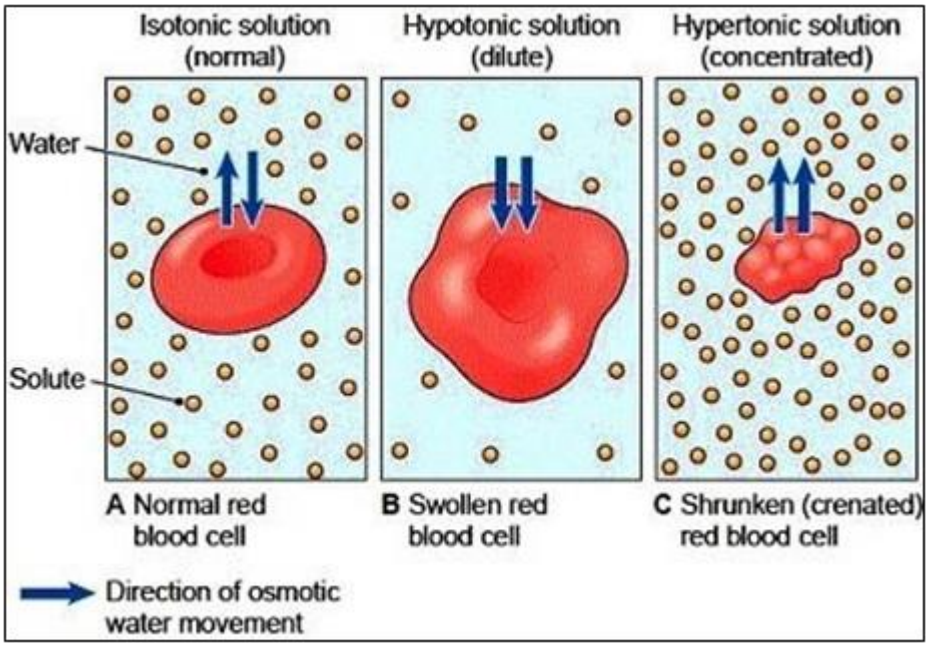
4- Decant the supernatant.

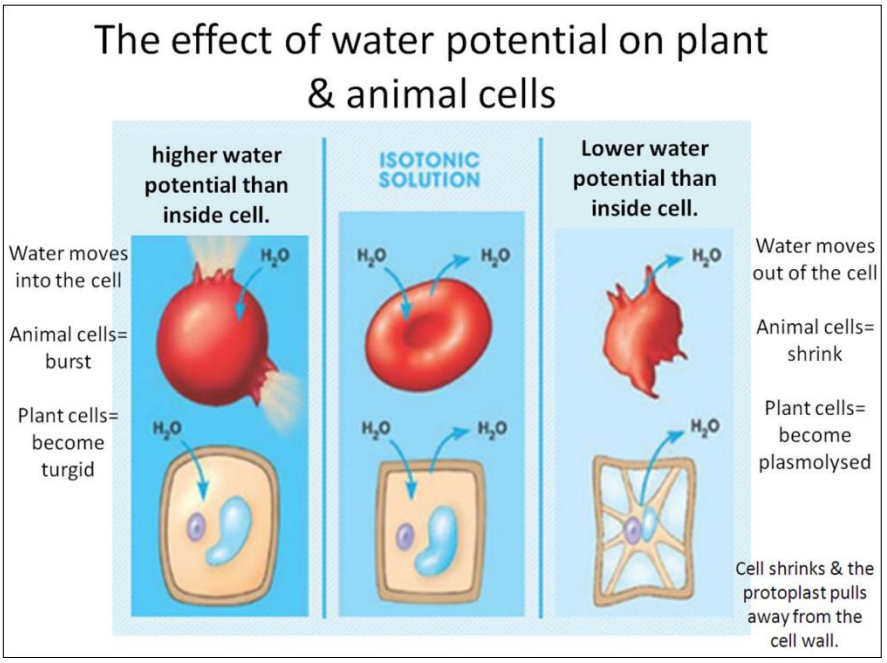
Repeat steps 2-4 at least twice. The last wash should always have a clear supernatant with no signs of hemolysis

Why normal saline (NaCl 0.9%) used in red cell suspension?

- A 0.9% NaCl solution is **isotonic**: when blood cells stay in such a medium, the intracellular and extracellular fluids are in osmotic equilibrium across the cell membrane, and there is no influx or efflux of water.
- In **hypotonic** (e.g. 0.4% NaCl or distilled water), the influx of water occurs that leads to cells swell, membranes disrupted, and hemolysis.

- In **hypertonic** (e.g. 1.8% NaCl), the cells lose their normal shape and shrinks due to the rapid osmotic efflux of water.





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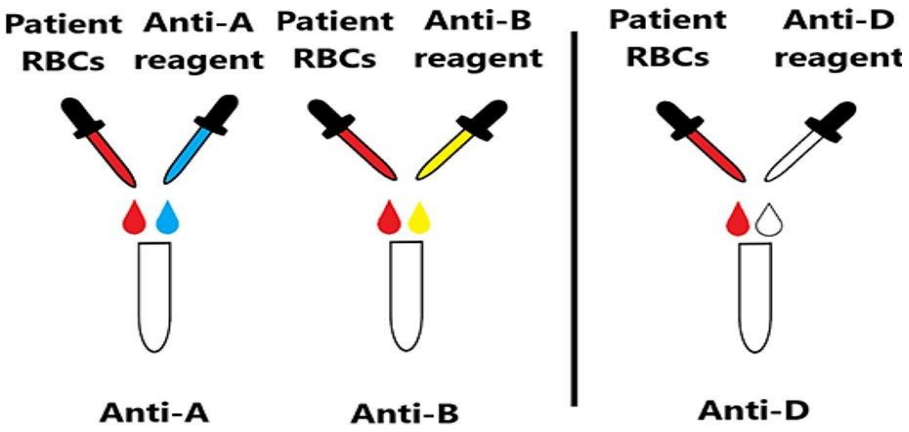
Name of the test: Test tube method of blood grouping.

Procedure:

1) Collect the blood by vein puncture method.
2) Added the blood into the anticoagulant tube (EDTA-tube) and then mixed.
3) Added an isotonic solution such as normal saline (N.S) to the mixture.
4) Wash the RBCs suspension by centrifugation for 5min. at 3000 Rpm.
5) Discard the supernatant and again add normal saline (N.S) to precipitate.
6) Mixed the precipitate and repeat steps 4 and 5.
7) Re-suspended the precipitate again with saline and standardized it.
8) Label three khan tubes with A, B, and Rh (D) and add 2-3 drops of Anti-A, 2-3 drops of Anti-B, and 2-3 drops of Anti-D to tubes A, B, and D. Then, in each of the three tubes, add 2-3 drops of RBC suspension.
9) Mix and centrifuge for 1 minute before checking the result.



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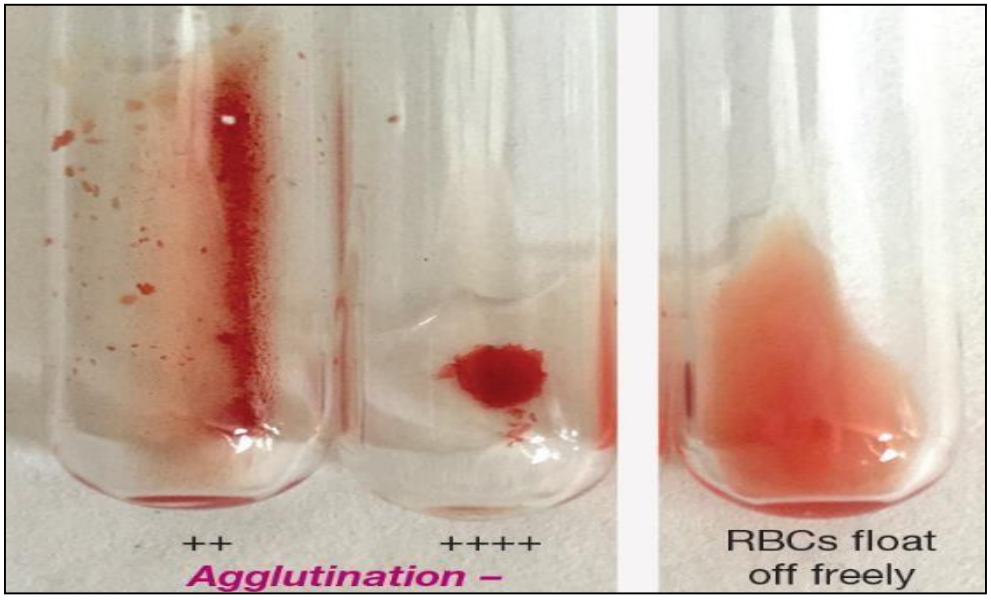
Result:



Agglutination (positive)

Non-agglutination (negative)