

Hemagglutination Procedure

Testing a Red Cell Agglutination Serum

A. Reagents and Equipment

1. ACD (Acid Citrate Dextrose)

27.2 gm. $\text{Na}_3\text{Citrate} \cdot 2\text{H}_2\text{O}$

1.7 gm. $\text{H}_3\text{Citrate} \cdot \text{H}_2\text{O}$

2.2 gm. $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$

25.0 gm. Dextrose

Add distilled water to bring volume to 1000 ml. Filter and store at 4°C in sterile bottles. + Merthiolate

2. PBS (Phosphate Buffered Saline) pH 6.8

 adjust pH

3. PVP (Polyvinylpyrrolidone), 1.0% in PBS with 0.1% BSA (Bovine Serum Albumin)

The PVP is supplied as a 45% aqueous solution. It is made up in PBS to 1% solution, aliquoted out, and autoclaved. It can be stored at room temperature. Just before using, BSA is added to make the 1% PVP contain 0.1% BSA. After the BSA is added, the PVP will keep in the cold for a few days but I prefer to add BSA fresh daily. The PVP is available from General Aniline and Film Corporation, 140 West 51st Street, New York, N Y 10020. Specify Polyvinylpyrrolidone (PVP), Type K-60, 45% aqueous solution Lot #29. The PVP varies from lot to lot, and lots other than 29 may not work at 1%.

4. Disposable test tubes, 10 X 75 ← smaller size doesn't accommodate Oxford pipet.

5. Hamilton syringe with automatic dispenser, pipets, and serial diluter.

6. Alloantiserum (anti-H₂ serum)

B. Collection of Cells

1. For titering antiserum - the red blood cells are collected in a graduated centrifuge tube containing a large excess of ACD, washed twice in PBS, and resuspended in PBS to a 2% suspension. *her.*

2. For typing the red blood cells are more conveniently collected by bleeding 75 lambdas from the retro-orbital plexus into 2.0ml of ACD. The cells are then washed twice with 2 ml aliquots of PBS and resuspended in 2 ml of PBS. This gives a 2% suspension.

C. Titering Antiserum

The test is set up in duplicate with a NMS control. For example, let's assume serial dilutions from 1/10 to 1/5120 (10 tubes across). Dispense 0.18 ml PVP solution (A,3 above) into the first tube and 0.1 ml in the remaining tubes. Add 20 lambdas alloantiserum to the first tube and serially dilute across the row by transferring 0.1 ml, discarding the final 0.1 ml. Repeat for each row. For each set (2 rows antiserum dilution and 1 row NMS dilution) add 0.05 ml 2% RBC suspension to each tube. Shake rack, incubate at R.T. for 2 hours, and read.

D. Typing Red Cells

Again the test is set up in duplicate with a NMS control, but this time only the dilution of antiserum which gives optimal agglutination is used. For each tube, 0.1 ml of serum dilution is added (either antiserum or NMS). Then, 0.05 ml of 2% suspension of test cells is added to each set, the rack is shaken, incubated at R.T. for 2 hours, and read.

E/. Controls

In addition to NMS for a serum control, it is advisable in titering and typing to use known positive and known negative cell controls.

F. Reading

To read, tubes are "stroked" smoothly across the length of an Rh typing box 8 or 10 times and scored for red cell agglutination on a scale of 1+ to 4+. I consider clarity of the suspending medium as well as clump size in scoring. Generally, a good positive will be in clear suspension. Always use a NMS control.

Make enough negative control tubes so that one can be read with each tube of test sera.

B. Deak 10/7/71