Farr Assay for determination of total Hib antibody (RIA)

Reagents:

Stock 3H-PRP antigen (usually 100-400 ng/ml. Good for 3-4 months at 4^{o}C.)

Antigen diluent (0.1 M NaPhophate pH= 7.4 + 0.001 % thimerosal)

Sample diluent (heat inactivated FBS+ 0.01% thimerosal + 0.02% azide)

SAS (saturated ammonium sulfate) (keep at 4 ^{o} C)

Farr pellet (Ammonium sulfate cut of FBS)

Stock $NaCl^{36}$ solution (This was pre-diluted to 25 uCi/ml.)

Ultima Gold Scintillation Fluid (Packard 6013329)

Standard serum (OBRR standard serum) from FDA. - Assigned value is 70 ug/ML.

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Materials/equipment:

Microcentrifuge assay tubes (Sarstedt 72.690)

Scintillation vials (20 ml size)

Scintillation Counter (Beckman 3800)

Microcentrifuge (Eppendorf 5414)

Procedure:

1. Determine the total number of assay tubes to be used (tube number=24 +8*samples)

2. Prepare the working solution of 3H-PRP, which has 10 ng/ml of 3H-PRP and 0.05 uCi/ml of $NaCl^{36}$. Prepare this by diluting stock 3H-PRP (10-40X) and stock $NaCl^{36}$ [1/500 v/v] with the antigen diluent. Needed volume is 50 ul/tube + 300 ul (extra).

3. Set up the following assay tubes.

One spill tube (25 ul Farr pellet + 25 ul of 200X diluted stock solution of $NaCl^{36}$. Note: The tube

has no H^3 .) (This tube checks the scintillation counter "windows" set for each isotopes.)

Three total count tubes (25 ul Farr pellet/tube)

Fourteen standard tubes (25 ul/tube) for 2 sets of serially diluted standard samples. They are prepared by diluting the standard serum 1/50, 1/100, 1/200, 1/400, 1/800, 1/1600, 1/3200 with the Sample Diluent.

Six QC tubes (25 ul/tube) for 3 quality control sera. Two tubes for each control serum. The QC sample needs to be diluted 10-50X with the Sample Diluent.

Eight sample tubes (25 ul/tube) for each sample. Four dilutions (neat, 1/5, 1/25, 1/125) in duplicate. Dilute the samples with the Sample Diluent.

4. Add 50 ul of working solution of 3H-PRP to all the tubes except the spill tube.

5. Mix well and incubate overnight at 4 ^{o} C.

6. Add 75 ul of SAS to each tube except the spill and the total tubes. Mix and incubate at 4^{o}C. for 1 hour.

7. Centrifuge for 5 minutes in the Eppendorf centrifuge at 4 ^{o} C.

8. Aspirate the supernatant with a fine tip.

9. Dissolve the pellet in 400 ul water and mix well.

10. Open the tubes and dump the tube content into scintillation vials containing 10 ml each Ultima Gold and shake well.

11. Wipe the scintillation vial with a damp cloth to reduce static. Count the tubes for 3-5 minutes for both H^3 and Cl^{36}.

12. Calculate the % of PRP bound to the antibody using the formula below.

(3H count - (spill * 36Cl count) - (36Cl count / total 36Cl) * total 3H) /

(1 - 36Cl count / total 36Cl) / 3H total.

13. Calculate the antibody concentration of each sample by comparing the % binding of the test sera to the % binding of the standard curve. We use the linear interpolation method. Correct for the dilution of the sample.