

RADIO-IMMUNE ASSAY

I. Materials

1. The BSA diluent used is a solution of 3% BSA in 0.05M Tris buffer with 0.1% Na Azide. (Used to dilute Antigen* + Inhibitors).
2. The S-dil used is 3% BSA solution as above but in addition it contains 10% of Normal Rabbit Serum. (Used to dilute antisera) NRS for C¹ source. Do not use in α G2b assays. Use
3. P-33 is a 33% saturated ammonium sulfate cut of normal rabbit serum. It is a source of Clq complement approximately 2.5 times as concentrated as normal rabbit serum. (See P-33 Procedure for details)
4. BP-33 is 3% BSA as above with 10% P-33. Complement source for Ig4_b assay only. *Bottles in freezer are labeled "5ml BP-33" to which 45ml of 30% BSA must be added.*
5. Labelled antigens are purified proteins of specific class and allotype. They are labelled with ¹²⁵I by the chloramine T method, diluted to contain $1-2 \times 10^5$ cpm/50 μ l. (See separate procedure for details.)
labelling
6. Antisera are class and allotype specific purified antisera which are diluted in S-dil for Ig-1 assays (BP-33 for Ig-4). Dilutions are determined in advance by precipitation assay.
7. Inhibitors (samples and controls) are diluted in BSA diluent to appropriate dilution.
8. Assay tubes are 6 x 50 mm culture tubes which can be centrifuged (Kimax #45048). Identify tube by white benzene ring on surface (can withstand 10,000 rpm).
9. Counting tubes are 13 x 100 flint glass tubes with lip (suitable for our gamma counter).

II. Rationale

Into each tube a constant amount of labelled antigen is added

Let Ag = μ g of labelled antigen

Ag* = cpm associated with Ag

Therefore $\frac{Ag^*}{Ag} =$ Specific radioactivity of labelled antigen (activity/unit mass)

Enough antibody is added in conditions of antigen excess, to precipitate a constant amount of antigen. This precipitate is generally 2/3 max ppt and is dependent on Clq and is inhibited by salts $> .07$ M: Use .05 M Tris, no saline.

III. B. Procedure for compupet

- a. Use labelled antigen at $1/250$ and use only 25λ ⁵⁰ (~~5 λ in, 25 λ out~~)
- b. Add 5λ of inhibitor and wash out with 20λ BSA (total 25λ).
- c. Add 50λ ²⁵ antiserum ($2/3$ max pptable point on Ab tit. curve ^{BP-33} x 2)
- d. Sample 50λ , deliver 500λ

IV. Controls

For each 50 tubes and at the end of the assay include:

1. Two tubes with only 5λ BSA instead of inhibitor and 50λ of S-dil (or BP-33) instead of antiserum. (No ppt control) Counts for these tube show the maximum radioactivity possible.
2. Two tubes with 5λ BSA as inhibitor and 50λ of antiserum. (Max ppt indication).
3. In addition, at the end do two tubes with 55λ BSA instead of antisera and 50λ labelled antigen (control for diluent).
4. Once in each assay do a series of serial dilutions of a standardized protein in order to make a calibration curve of reference protein of same class and allotype as antigen tested (or normal serum containing reference protein).

$$\text{Percent Ag* ppt} = \frac{\text{count of unknown Sample}}{\text{count of control \#1 above}}$$

Let Ab = ug of antigen bound

Then P = percent of radioactivity precipitated is

$$P = \frac{Ag^* \text{ cpm}}{Ag \text{ ugm}} \times \frac{Ab \text{ ugm}}{Ag^* \text{ cpm}} \times 100$$

$$P = \frac{Ag^* \text{ cpm}}{Ag \text{ cpm}} \times \frac{1}{Ag^* \text{ cpm}} \times Ab \text{ cpm} \times 100$$

Let I = ug of inhibitor added

Then specific activity of all antigen is

$$\frac{Ag^* \text{ cpm}}{Ag + I \text{ ugm}}$$

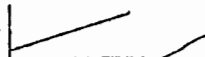
Then

$$P = \frac{\frac{Ag^*}{Ag + I} \times Ab}{Ag^*}$$

Rearranging yields

$$1/P = \frac{Ag}{Ab} + \left(I \times \frac{1}{Ab} \right)$$

Therefore, by plotting 1/P vs. I for some serial dilutions of a standard protein, a straight line is expected.

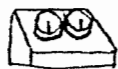
% Ag-ppt →  ugm inhibitor added

Thereafter knowing the 1/P value of a sample, one can calculate its concentration of inhibitor.

III A. Procedure - Hand Technique

1. Put 50 λ of labelled antigen in BSA diluent in each tube.
Counts \approx 10,000.
2. Add 5 λ of inhibitor in BSA. Do duplicate tubes per inhibitor.
3. Mix on vortex with small angle of tube to rotor.
4. Add 50 λ of antisera or conc to ppt \sim 60-70% Ag and mix thoroughly.
5. Incubate at 37°C for 3 hours--incubator in plaquing room.
6. Chill in refrigerator usually overnight. (*at least hrs*)
7. Centrifuge for 15 minutes at 10,000 rpm

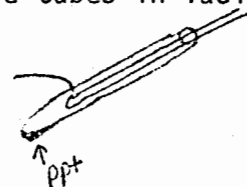
⑦ black line to outside of carrier



black line DOWN for sampling.

8. Sample 50 λ of supernatant from each tube and wash with 200 λ of saline into a counting tube containing \sim .4 ml saline. (Count on our counter) usually 1 minute. Put reaction mixture tubes in lucite racks as they are sampled.

sample probe opening



PROCEDURAL DETAILS

Reagents: S-dil, 3% BSA and BP33 are stored frozen on the upper left-hand side of the silver freezer,

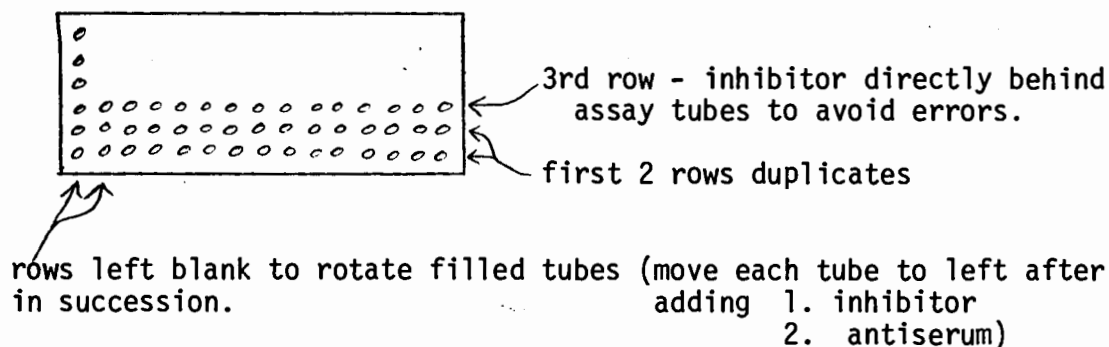
a x b is kept on the shelf labelled "Isoantisera."

I - inhibitor mouse sera is in freezer VI where all bleeds are kept.

Mix all sera and Ag* well before diluting.

When diluting labelled Ag*, open Beckman tube with a kimwipe and discard in dry waste. Radioactive pipet can be put in ^{radioactive} pipet washing solution in the hood room.

Set up rows of tubes in lucite rack with 2 rows to the left as rows to be filled as each reagent is added.



Standards are diluted to yield strong to no inhibition.

Radioactivity Precautions:

Lab coat - badge on radiation level

Diapers (dry waste)

Don't leave area without radioactive hazard sign

Wash hands after Ag addition

Lead racks for scintillation tubes @ sampling time

Compupet

1. Watch for bubble from back vacuum. Change BSA silastic vs. labelled protein tubing.
2. Ag* "pickup and deliver" to get uneven # λ - not available on "deliver" volumes only. Right foot - deliver for accessory.

3. Lubricant must be cleaned off silastic when finished with tubing, as it solidifies.
4. "Input" and "Deliver" on side of rollers indicates direction of flow in silastic tubing.