Radioimmunoassay

RIA

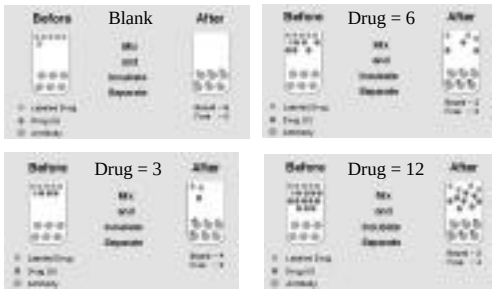
Radioimmunoassay

- Separation using the specificity of antibody - antigen binding
- Quantitation using radioactivity

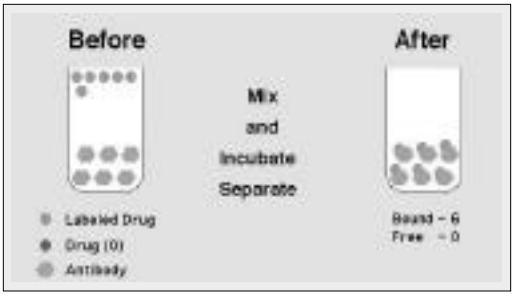
RIA Components of the Assay

- Drug
- Antibody
- Labelled Drug
- Mix, incubate, separate free labelled drug
- Quantitate by measuring radioactivity
- Determine standard curve

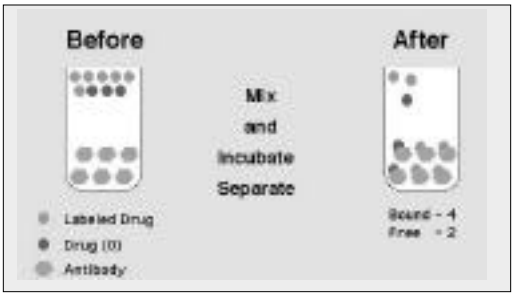
Principle of RIA - Overview



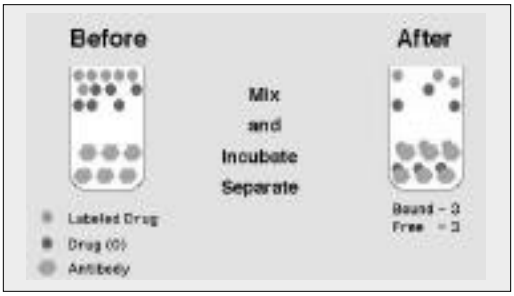
Principle of RIA



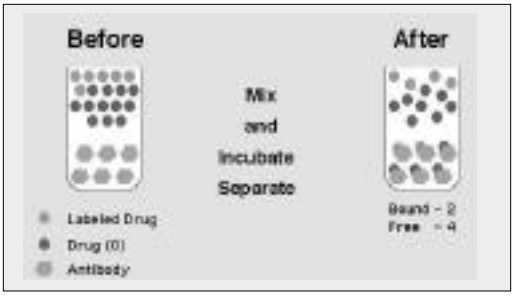
Principle of RIA



Principle of RIA



Principle of RIA



Principles of RIA

[Drug]	Bound*	Free*
0	6	0
3	4	2
6	3	3
12	2	4

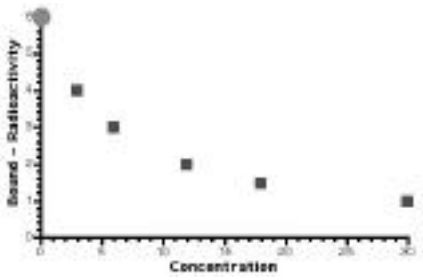
Procedure

- Mix sample containing drug with fixed quantity of labelled drug and antibody
- Allow to equilibrate - incubate
- Separate drug bound to antibody from unbound drug
  - Charcoal adsorption of antibody (and bound drug)
  - Antibody - antibody binding precipitates bound drug
  - Antibody bonded to container

Procedure contd.

- Measure radioactivity associated with bound labelled drug
  - low drug concentration means more bound radioactivity and higher measurement
  - high drug concentration means less bound radioactivity and lower measurement
- Non-linear plot of radioactivity versus concentration
- Logit-log concentration plot is linear

Bound versus Concentration



Logit-Log C Plot