



# Principle of immunoassay

# *labeled material*

- All immunoassays require the use of labeled material in order to measure the amount of antigen or antibody present.
- A label is a molecule that will react as part of the assay, so a change in signal can be measured in the blood:reagent solution.

# Labeled immunoassays

- Labeled immunoassays are designed for antigens and antibodies that may be
- small in size
- or present in very low concentrations.
- The presence of such antigens or antibody is determined indirectly by using a labeled reactant to detect whether or not specific binding has taken place.

# 1- Labeled analyte

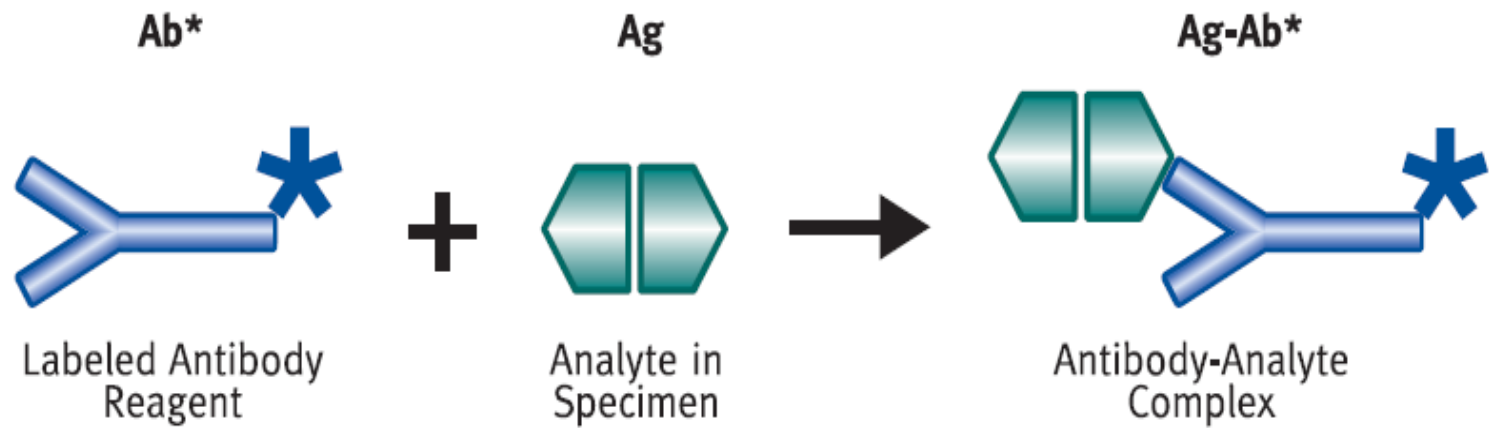
- A labeled reactant is used to detect whether or not specific binding has taken place.
- The label used in immunoassay
  - must not alter the reactivity of the molecule,
  - and it should remain stable for the shelf life of the reagent.
- Labels attached to analytes and antibodies may be
  - radioactive, usually iodine-125 (radioimmunoassay and immunoradiometric assays),
  - enzymes such as alkaline phosphatase and horseradish peroxidase, (enzyme immunoassay or immunometric assay, or enzyme-linked immunosorbent assay ELISA),
  - chemiluminescent (e.g., acridinium ester),
  - or fluorescent (e.g., fluorescein).

# The biotin-Streptavidin/ Avidin indicator label system

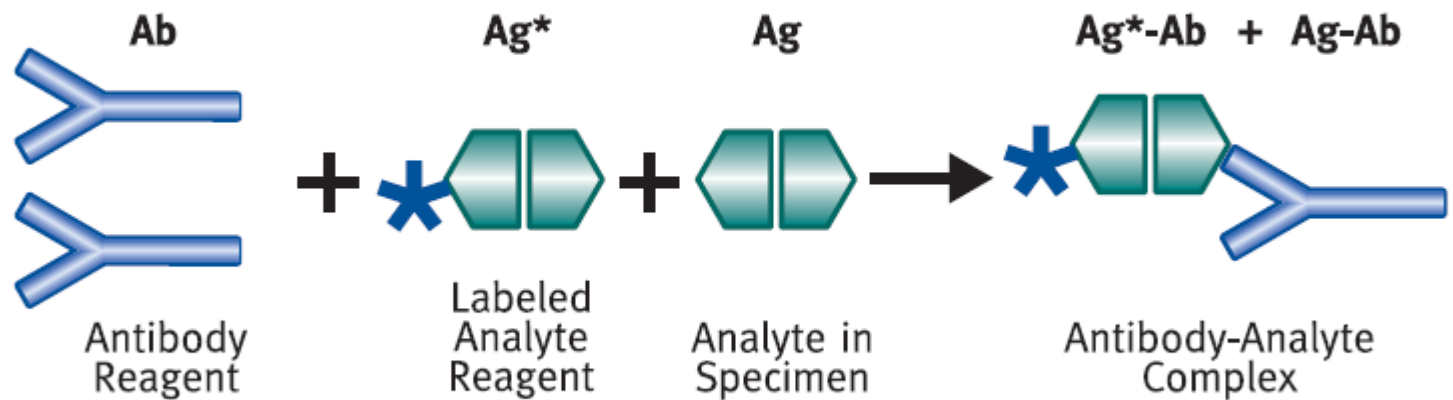
- Biotin is a vitamin that can bind tightly to either avidin or streptavidin.
- Avidin streptavidin are proteins.
- The natural attraction of these two proteins for one another is a property that has been exploited to facilitate coupling of indicator molecules to antigens or antibodies.

# Examples of a label

- Examples of a label include a **radioactive compound**, an **enzyme** that causes a change of color in a solution, or a **substance** that produces light.
- The label can be applied during the manufacture of the reagent to either the **antibody** (Ab\*, see Figure 1-5) or **antigen** (Ag\*, see Figure 1-6).
- **Immunoassay technologies** utilize different formats to distinguish the bound antigen-antibody complex from the free unbound label.



**FIGURE 1-5** Labeled antibodies allow detection of antigen/antibody complexes in immunoassays



**FIGURE 1-6** Labeled antigen also allows detection of antigen/antibody complexes in immunoassays