PHARM.D • YEAR 1

Medicinal Biochemistry

CHAPTER 12

Immunochemical Techniques

PPT CHAPTER COVER



IMMUNOCHEMICAL TECHNIQUES: OVERVIEW

Immunochemical tests use antigen–antibody binding to identify or measure a target in a biological sample.

TARGETS

Hormones, proteins, microbial antigens or patient antibodies.

SAMPLES

Serum, plasma or urine, depending on the assay.

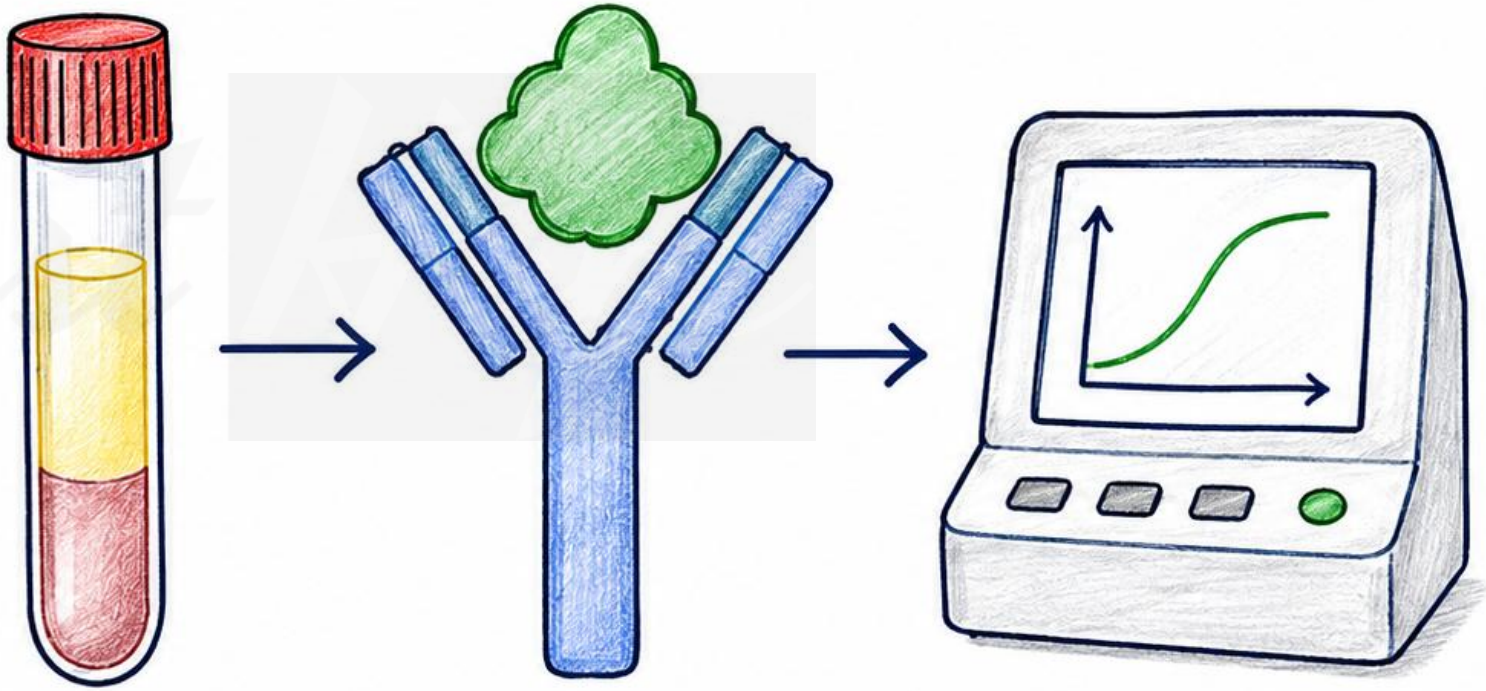
READOUT

A radioactive, enzyme or fluorescent label produces a measurable signal.

USES

Supports endocrine testing and infectious-disease diagnosis.

The result depends on assay design, calibration and the clinical setting.



ANTIGEN-ANTIBODY RECOGNITION

ANTIGEN

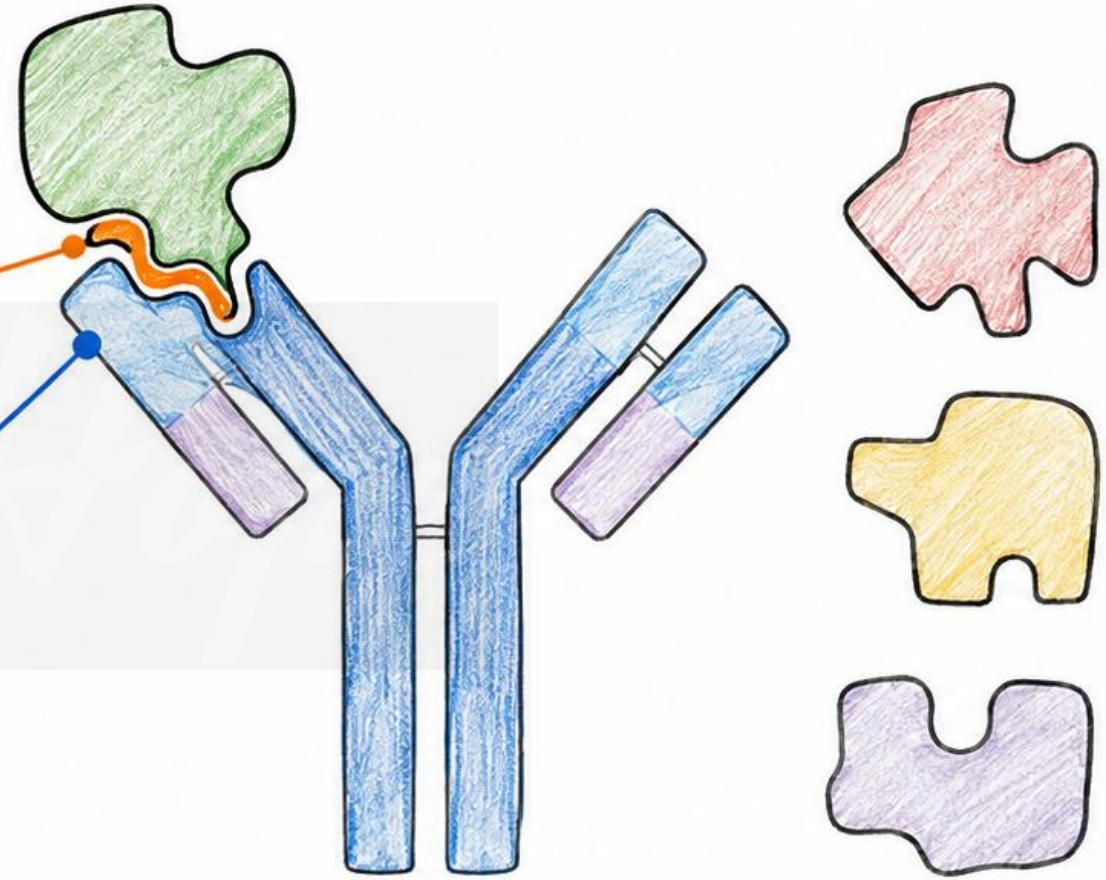
The molecule recognized by an antibody.

EPITOPE

The particular region of the antigen that is bound.

ANTIBODY

Its binding site recognizes a compatible epitope.



Specific binding allows a target to be detected among many sample components.

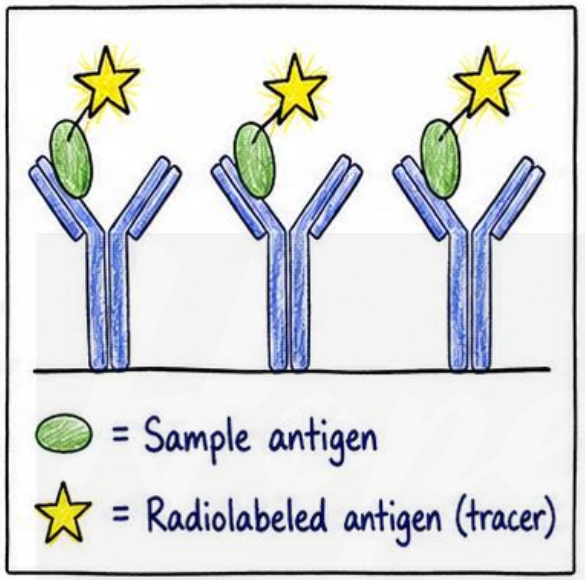
Cross-reactivity occurs when another molecule also binds and may distort the result.

Validated reagents and suitable controls help distinguish true signal from interference.

RADIOIMMUNOASSAY: PRINCIPLE

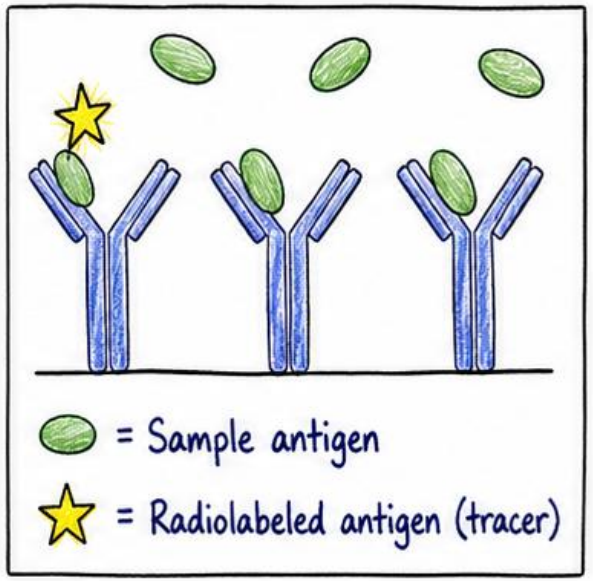
- Classical RIA is a competitive immunoassay.
- Sample antigen and a fixed amount of radiolabeled antigen compete for limited antibody binding sites.
- More sample antigen means less labeled antigen becomes bound.
- After separating bound from free tracer, measure the bound radioactivity.
- Compare the signal with a standard curve to estimate concentration.
- Iodine-125 is a commonly used label.

LOW SAMPLE ANTIGEN



More tracer bound
→ Higher radioactivity signal

HIGH SAMPLE ANTIGEN



Less tracer bound
→ Lower radioactivity signal

KEY RELATIONSHIP

Sample antigen ↑ → bound tracer signal ↓

COMPETITIVE RIA: STEP BY STEP

1. MIX

Sample or standard + radiolabeled antigen + specific antibody.

2. INCUBATE

Allow competition for the limited binding sites.

3. SEPARATE

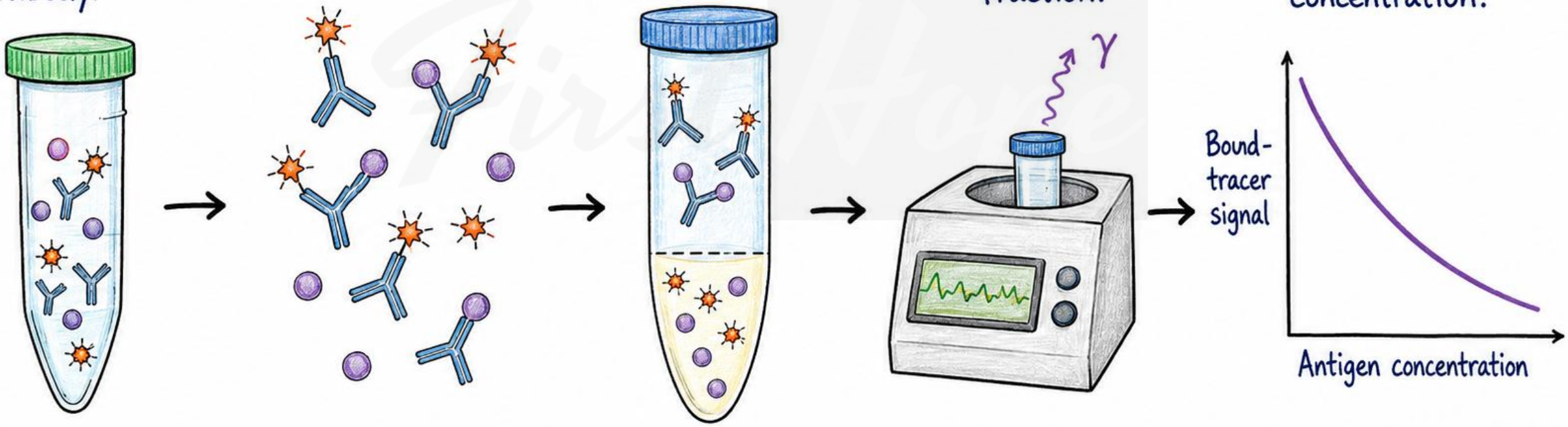
Separate antibody-bound tracer from free tracer.

4. COUNT

Measure radioactivity in the bound fraction.

5. CALCULATE

Use the standard curve to find sample antigen concentration.



The calibration curve slopes downward: greater antigen concentration produces a lower bound-tracer signal.

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