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PEARLS OF LABORATORY MEDICINE

Heterophile Antibodies

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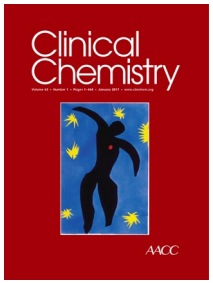

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Outline

Overview of antibodies

- Basic structure
- Definition of heterophile antibody

Heterophile antibodies and immunoassays

- Immunoassay overview
- Examples of interference mechanisms/scenarios

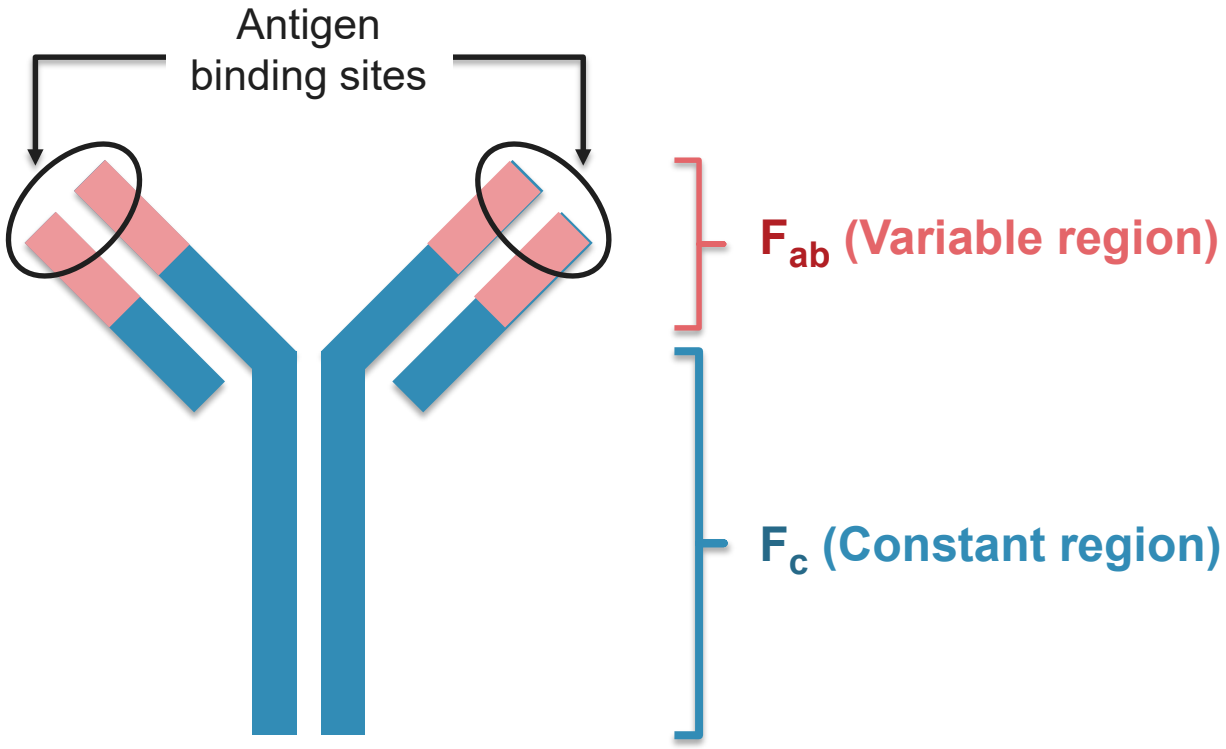
"The real world"

- How to identify erroneous results
- Workup strategies & caveats

Summary

Antibody (Ab) – Definition and Structure

"a blood protein produced in response to and counteracting a specific antigen" (Google Dictionary)



WHAT is a heterophile antibody?

"Hetero-"

het·ero \ het-ə-rō\

- "different"
- "other than usual"

"-philic"

- "having an affinity for"
- "loving"

WHAT is a heterophile antibody?

"antibodies produced against poorly defined antigens […] generally weak antibodies with multispecific activities"

Kaplan and Levinson. *Clinical Chemistry.* 1999

"these nonspecific antibodies interact poorly with immunoassay antibodies"

Datta. *Clinical Laboratory News*. 2015

"…are a group of poorly defined endogenous antibodies that react with many different molecules"

Jones and Honour. *Clinical Endocrinology*. 2006

WHERE do “heterophilic antibodies” come from?

Endogenous antibodies

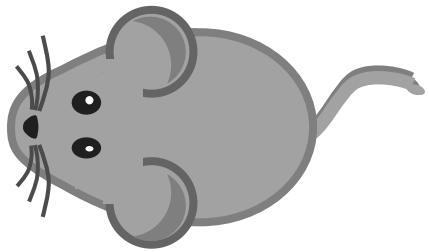
present in more than 10% of patients
(Klee GG. *Arch Pathol Lab Med*. 2000)

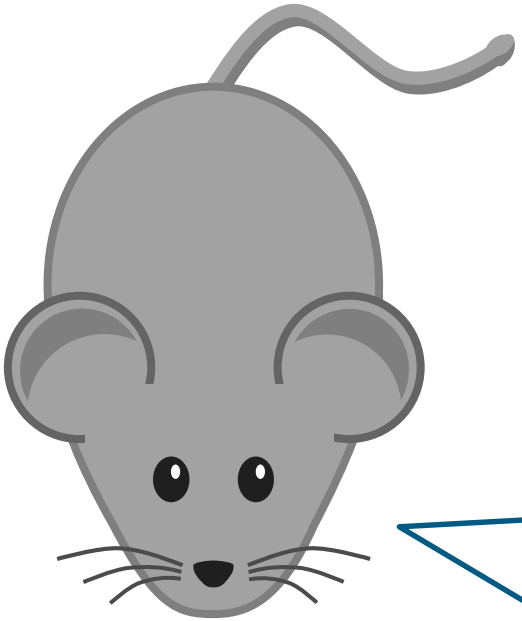
- Naturally occurring from antigen diversity
- Present in patients with autoimmune or inflammatory conditions
- Present in healthy individuals

“Heterophile antibodies” is often used to refer to **human anti-animal antibodies (HAAA)**
e.g., human anti-mouse antibodies (HAMA)

up to 40% of people have Ab with affinity to animal Abs
(García-Gonzále E. *Pract. Lab. Med.* 2016)

- **Exposure to antigens from animals**





For the purpose of this discussion, we will use the term "*heterophile Ab*" to describe Abs that can interfere with clinical immunoassays

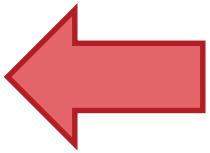
Main concern for the laboratory:

Assay interference leading to incorrect results!

HOW do these heterophile Ab interfere with immunoassays?

WHAT can heterophile antibodies (Ab) bind to in an immunoassay?

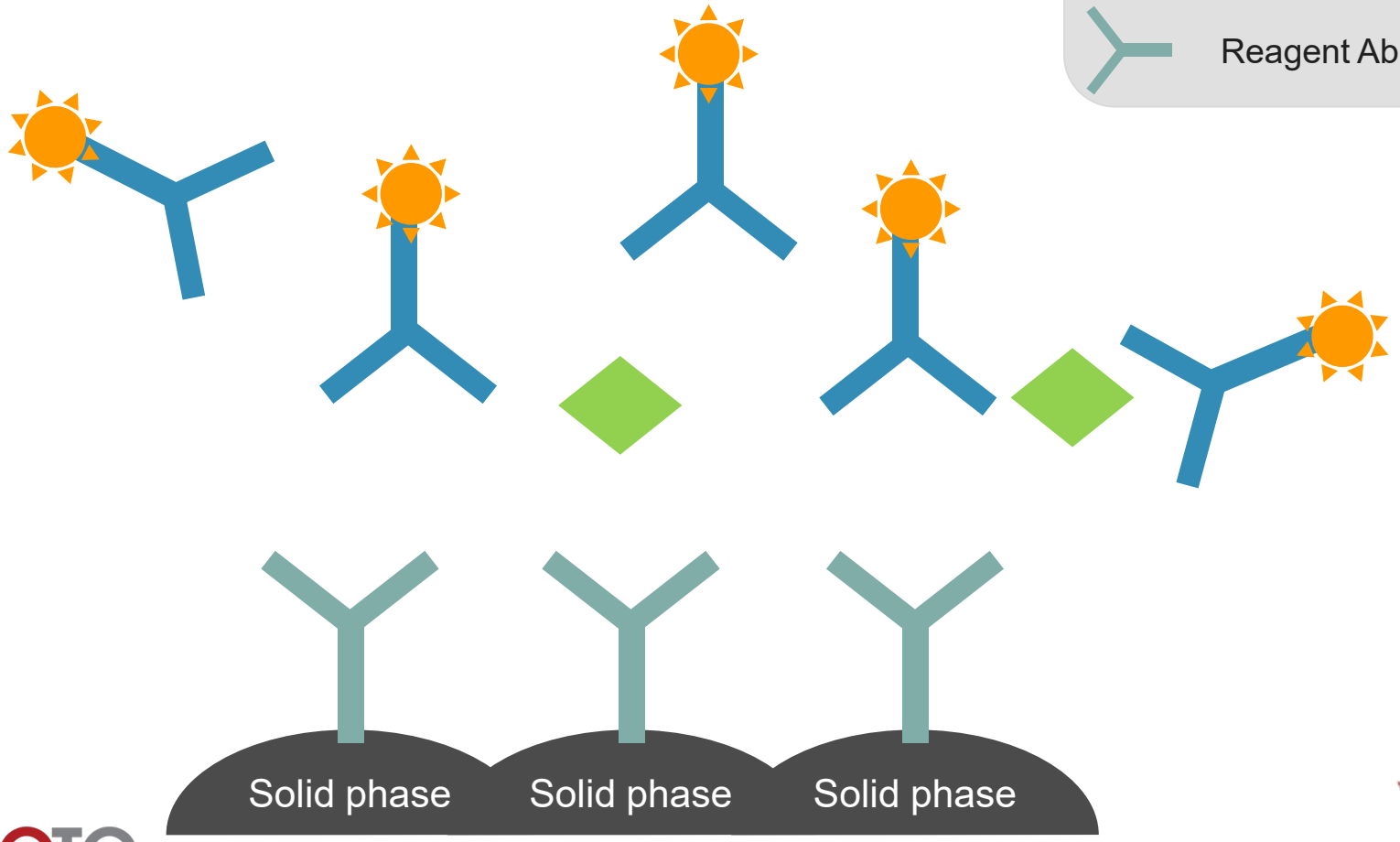
- Antigens / analytes of interest
- Other antibodies
  - E.g., capture and signal antibodies
- Other components of an immunoassay
  - E.g., Conjugate part of the detection system



Tate and Ward. Clin Biochem Rev. 2004

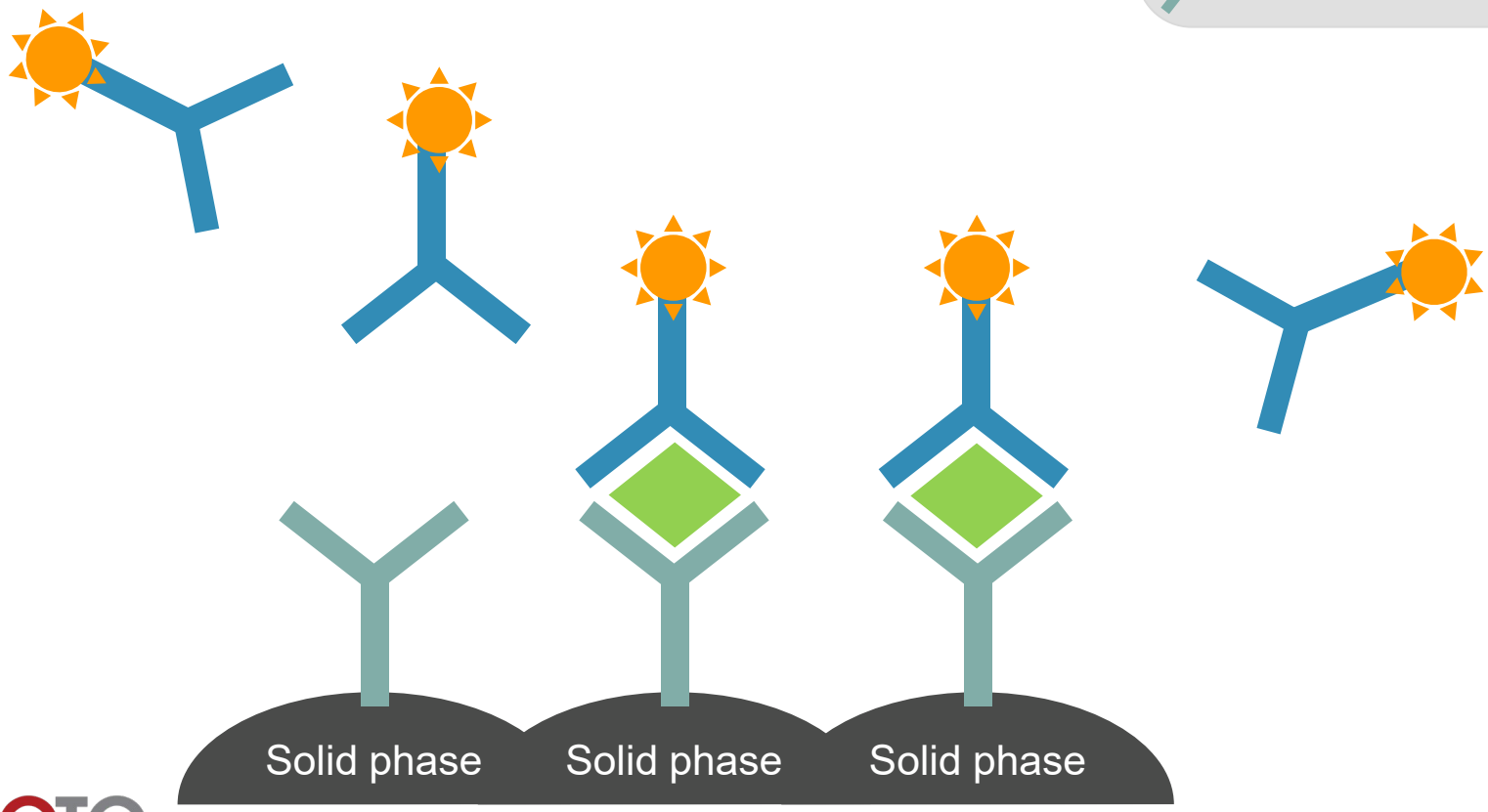
Scenario 1: Sandwich Immunoassay
Heterophile Ab **absent**

Step 1: Incubation



Scenario 1: Sandwich Immunoassay Heterophile Ab **absent**

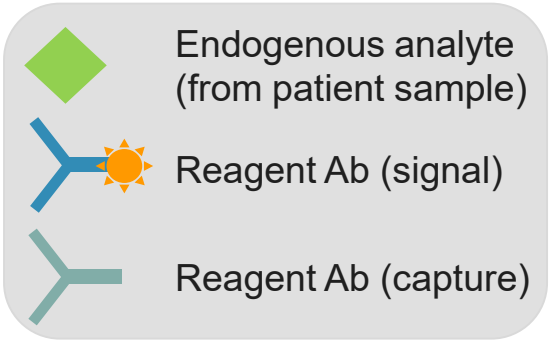
Step 2: Ab-analyte binding followed by washing

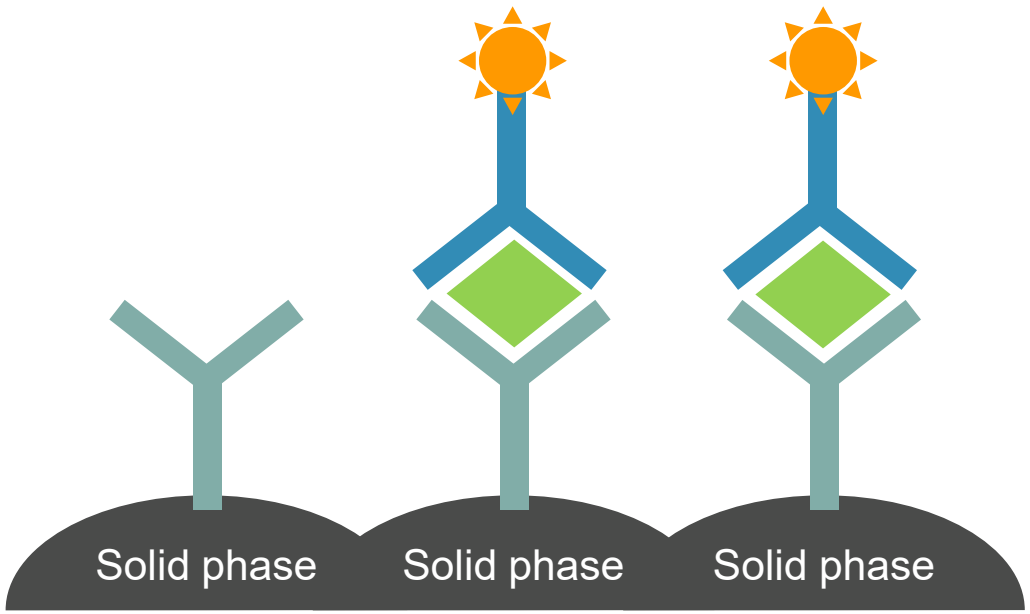


Scenario 1: Sandwich Immunoassay Heterophile Ab **absent**

Step 3: Signal detection

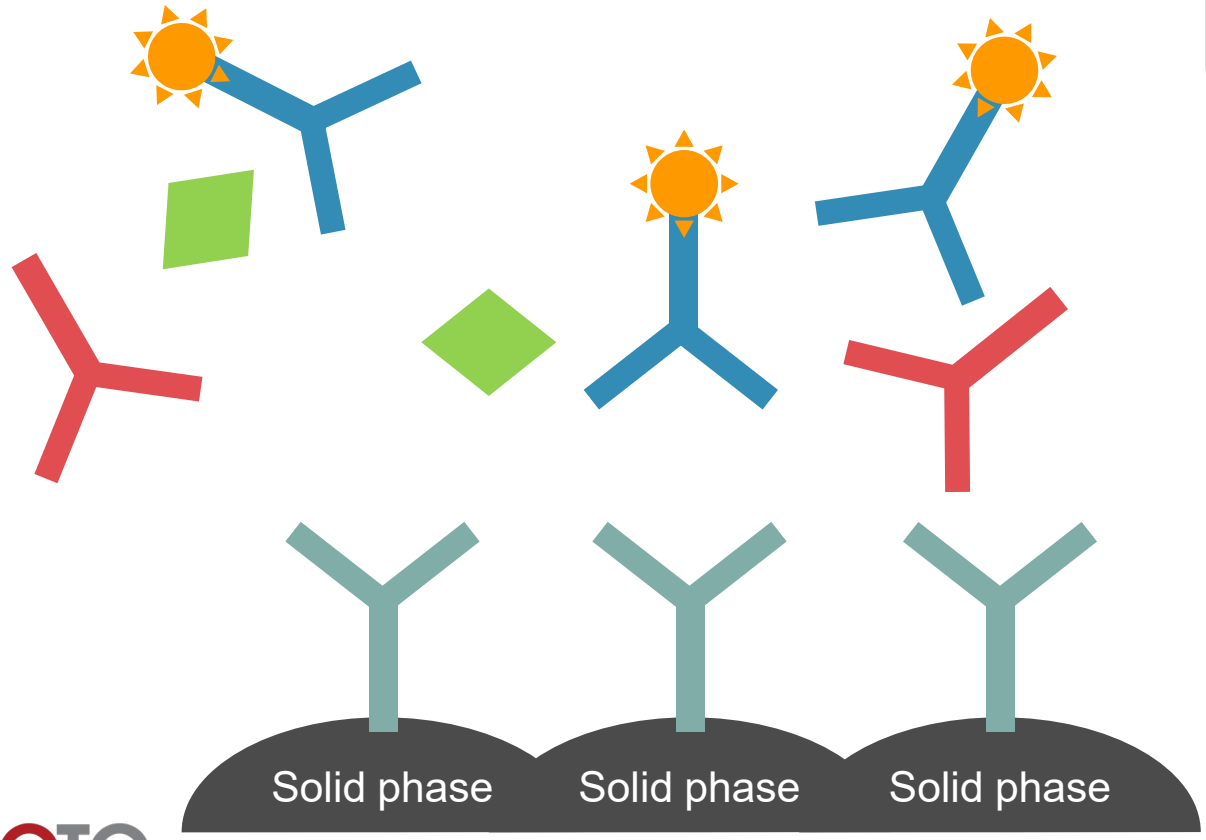
- Signal proportional to amount of analyte present





Scenario 2: Sandwich Immunoassay
Heterophile Ab **present**
(binding to the *capture Ab* in the reagent)

Step 1: Incubation



Endogenous analyte (from patient sample)

Reagent Ab (signal)

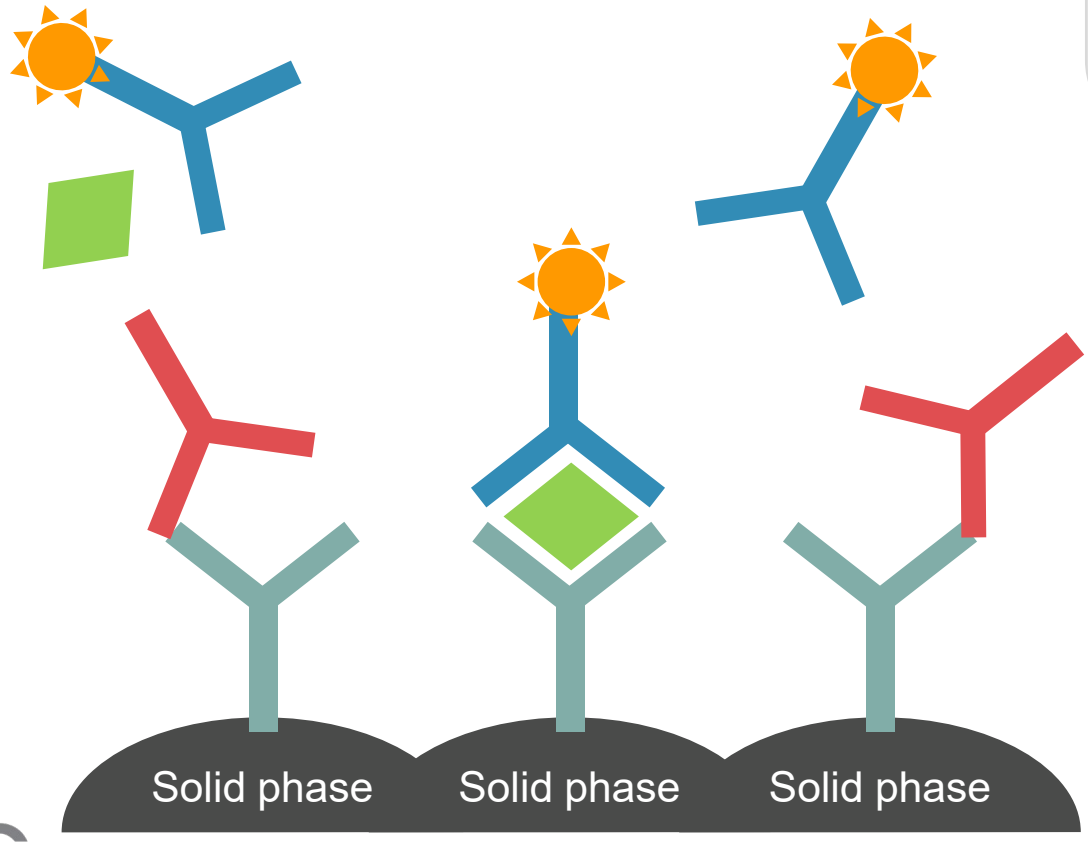
Reagent Ab (capture)

Heterophile Ab (from patient sample)

Scenario 2: Sandwich Immunoassay Heterophile Ab **present**

(binding to the *capture Ab* in the reagent)

Step 2: Ab-analyte binding followed by washing



Endogenous analyte (from patient sample)

Reagent Ab (signal)

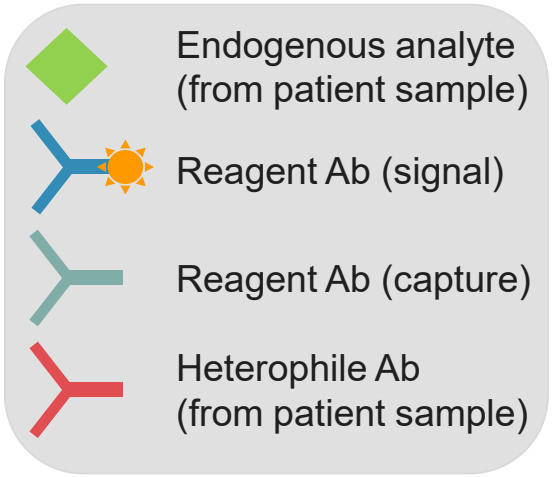
Reagent Ab (capture)

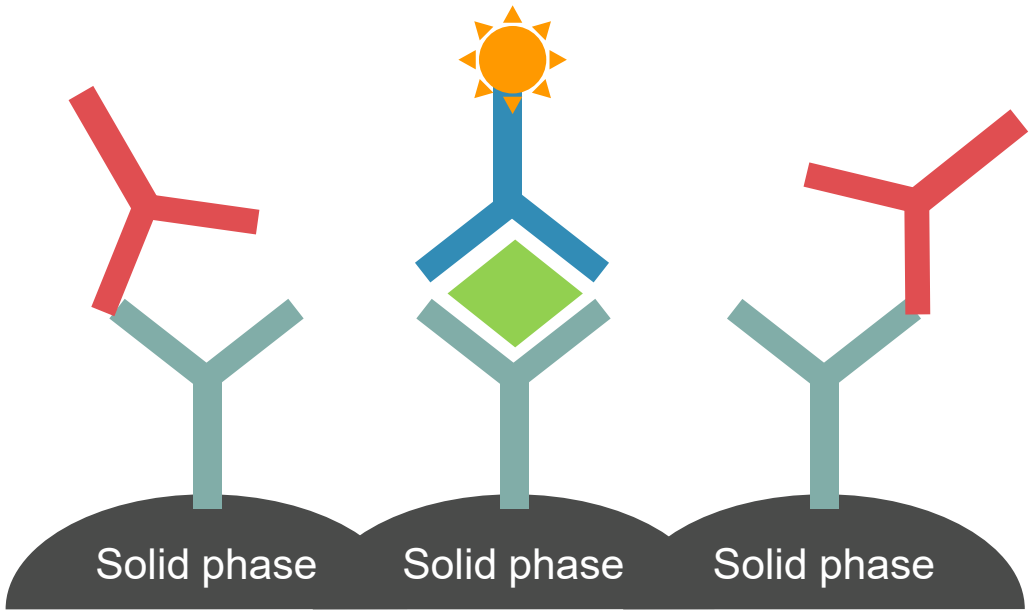
Heterophile Ab (from patient sample)

Scenario 2: Sandwich Immunoassay
Heterophile Ab **present**
(binding to the *capture Ab* in the reagent)

Step 3: Signal detection

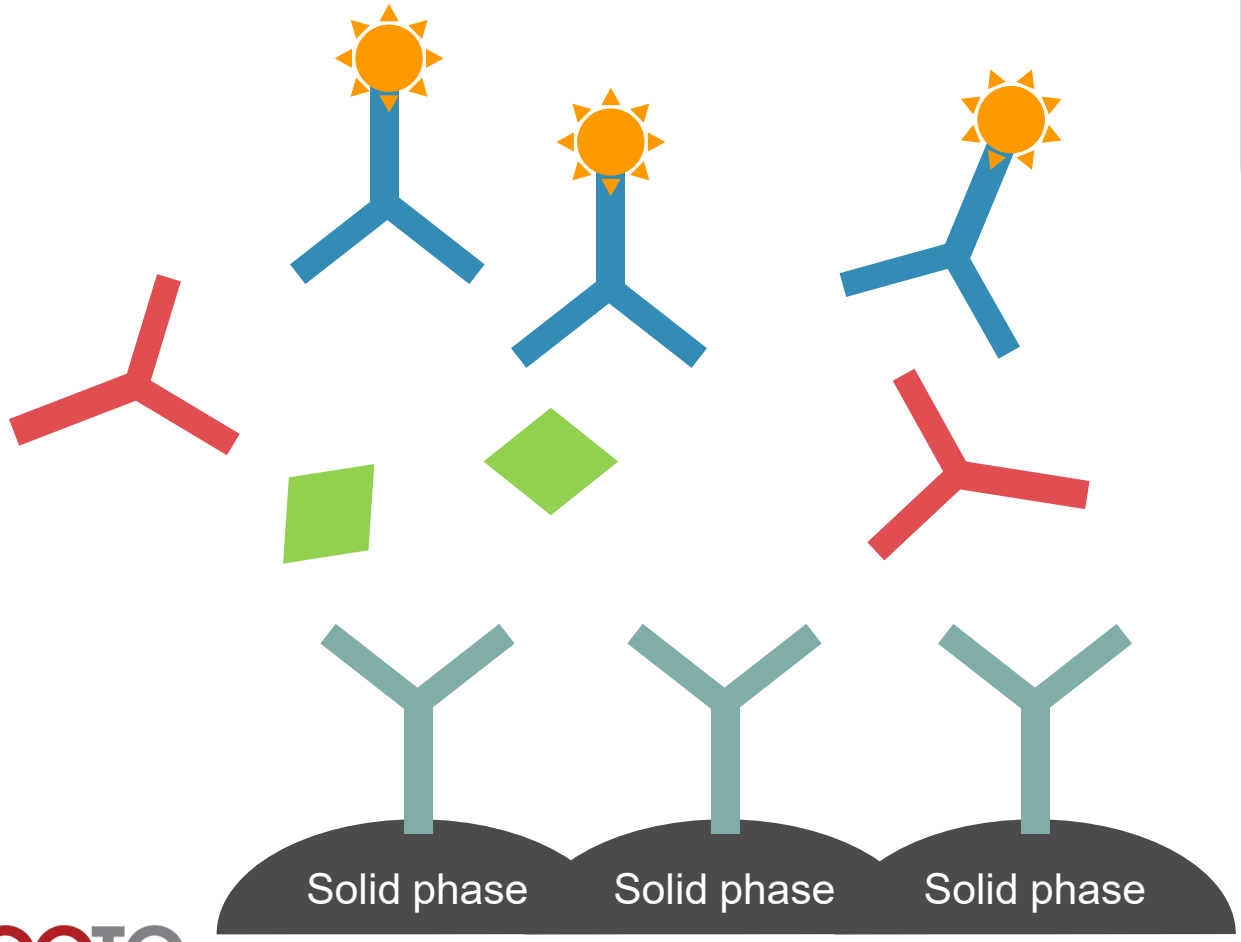
- Decreased signal detected
- **Falsely low result**





Scenario 3: Sandwich Immunoassay
Heterophile Ab **present**
(binding to the *signal* Ab in the reagent)

Step 1: Incubation



Endogenous analyte (from patient sample)

Reagent Ab (signal)

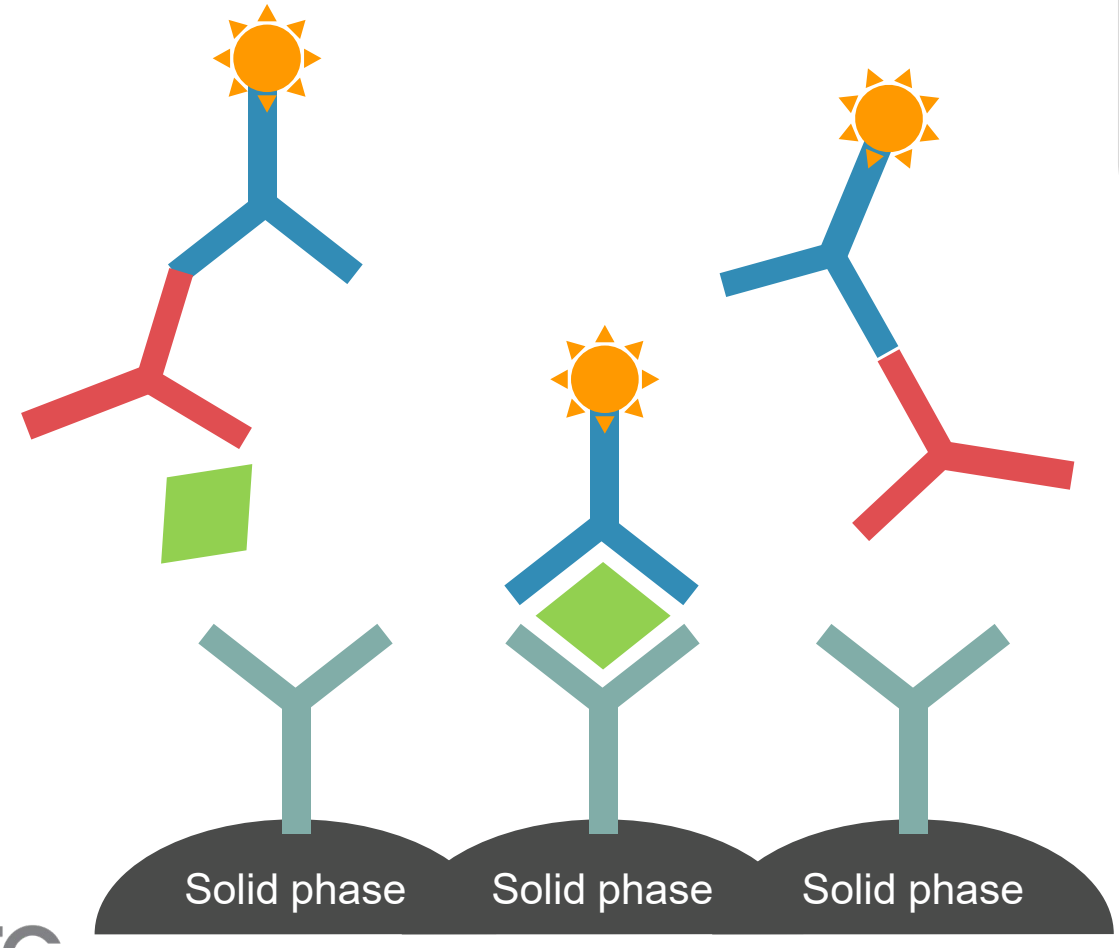
Reagent Ab (capture)

Heterophile Ab (from patient sample)

Scenario 3: Sandwich Immunoassay Heterophile Ab **present**

(binding to the *signal* Ab in the reagent)

Step 2: Ab-analyte binding followed by washing



Endogenous analyte (from patient sample)

Reagent Ab (signal)

Reagent Ab (capture)

Heterophile Ab (from patient sample)

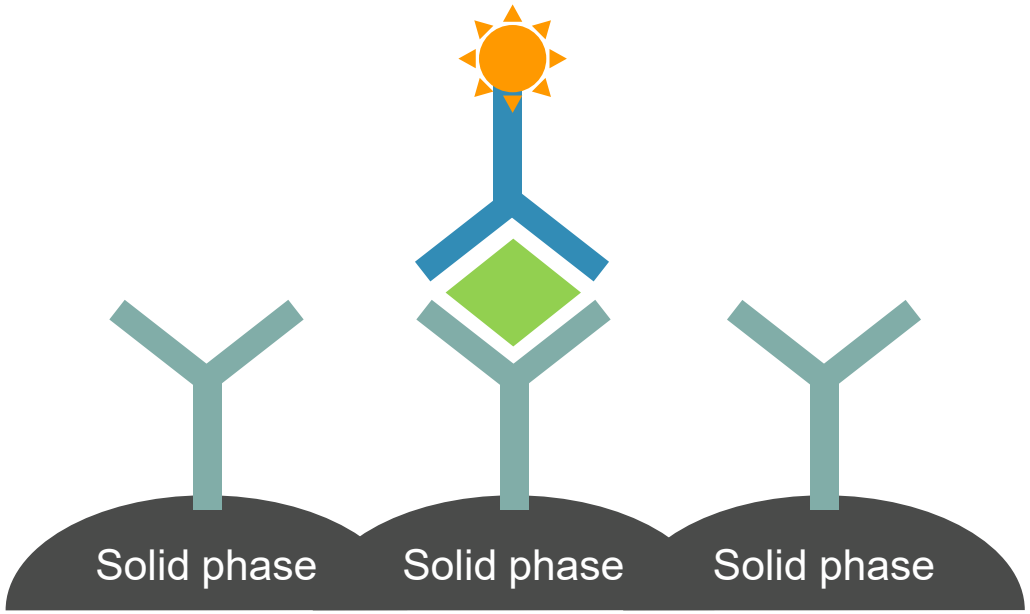
Scenario 3: Sandwich Immunoassay

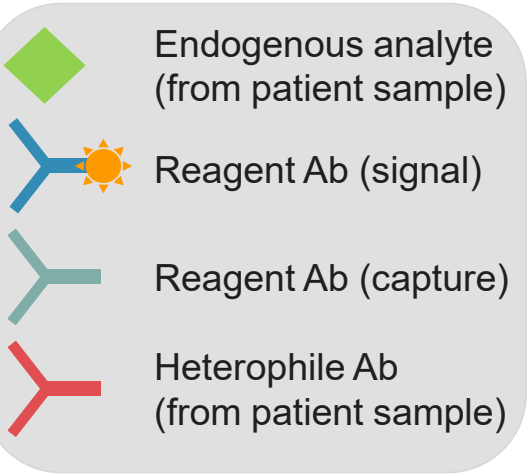
Heterophile Ab **present**

(binding to the *signal* Ab in the reagent)

Step 3: Signal detection

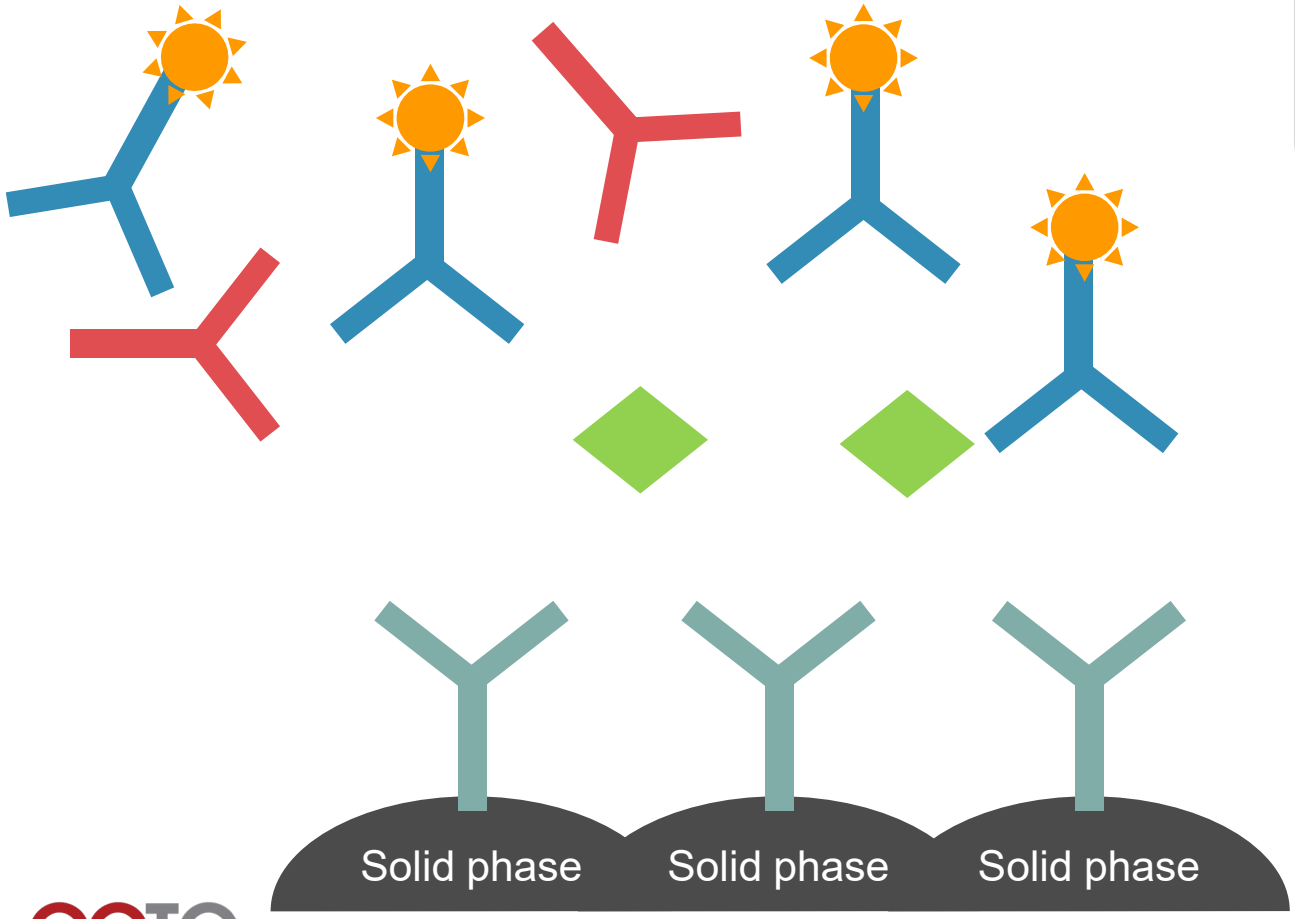
- Decreased signal detected
- **Falsely low result**





Scenario 4: Sandwich Immunoassay
Heterophile Ab **present**
(binding to *both* capture and signal Abs)

Step 1: Incubation



Endogenous analyte (from patient sample)

Reagent Ab (signal)

Reagent Ab (capture)

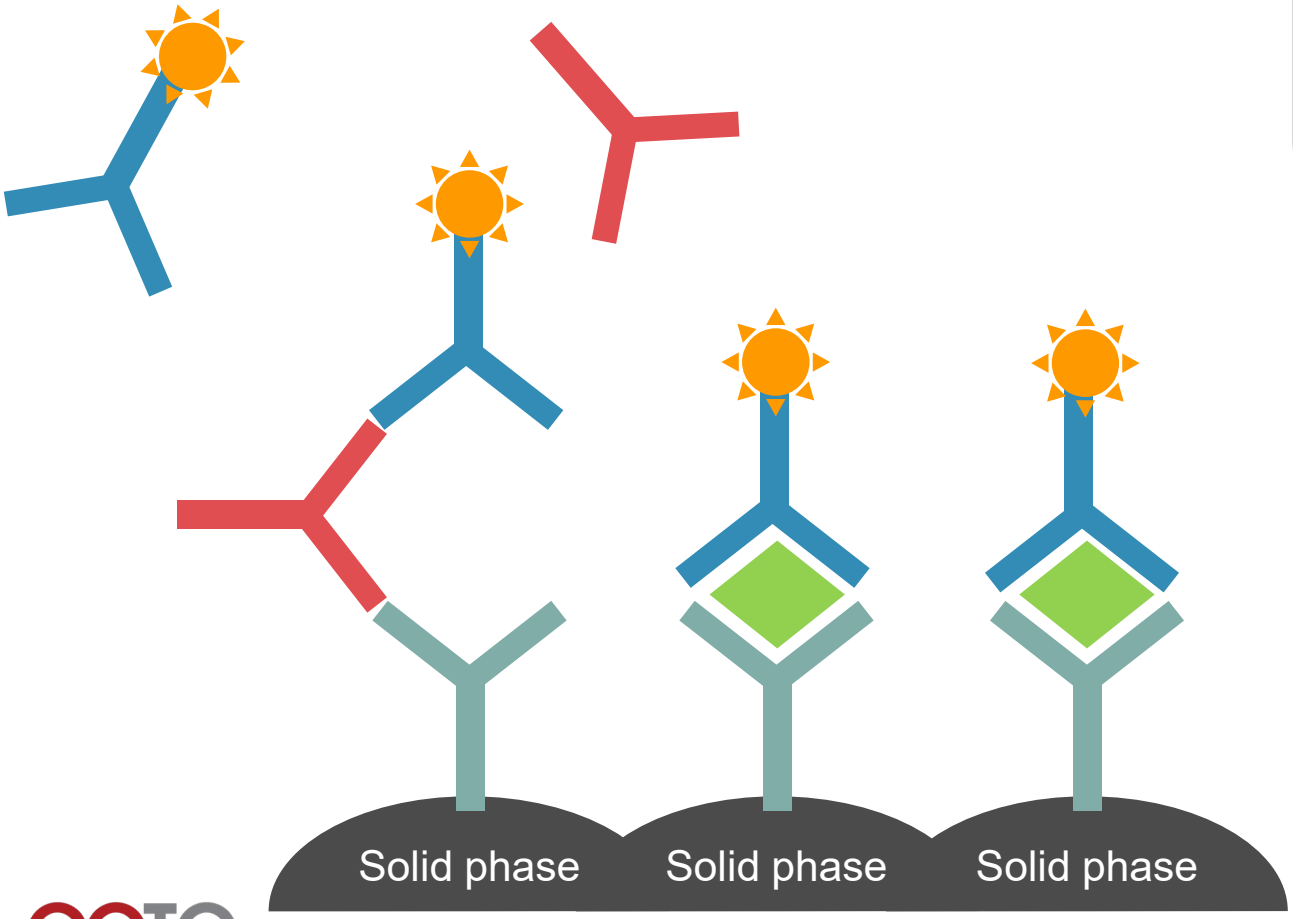
Heterophile Ab (from patient sample)

Scenario 4: Sandwich Immunoassay

Heterophile Ab **present**

(binding to *both* capture and signal Abs)

Step 2: Ab-analyte binding followed by washing



Endogenous analyte (from patient sample)

Reagent Ab (signal)

Reagent Ab (capture)

Heterophile Ab (from patient sample)

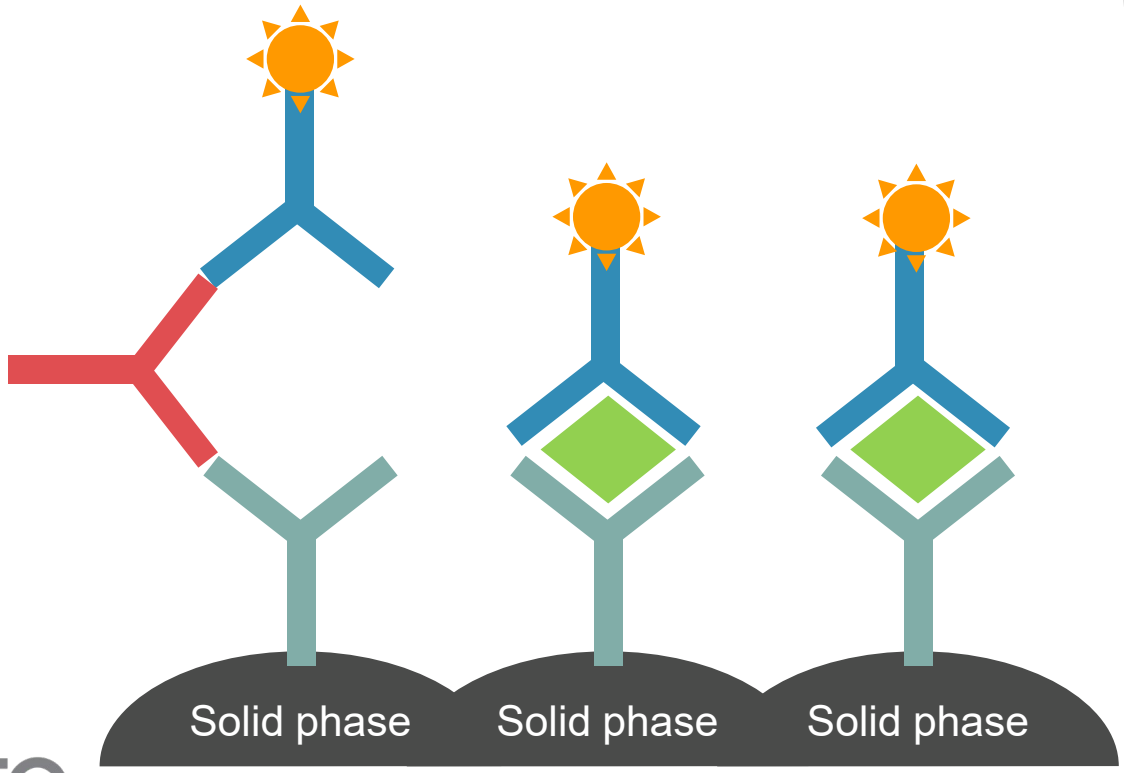
Scenario 4: Sandwich Immunoassay

Heterophile Ab **present**

(binding to *both* capture and signal Abs)

Step 3: Signal detection

- Increased signal detected
- **Falsely high result**



Endogenous analyte (from patient sample)

Reagent Ab (signal)

Reagent Ab (capture)

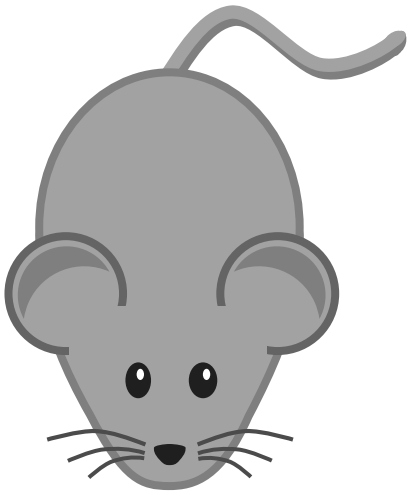
Heterophile Ab (from patient sample)

Quick recap

- Both **false negative** and **false positive** results can occur

Heterophile Ab **typically** cause **false positive** results in sandwich immunoassays

So how do we catch these "false" results?



Catching misleading results

Is there something going on with your assay?

What's wrong with your lab test?

This lab result doesn't make sense.

Are you sure this is my patient's result?

Clinically discordant results!

i.e., unexpectedly high or low results

What can be done in the workup?

LABORATORIAN ⟷ CLINICAL TEAM

Communication, Communication, Communication!

- **Obtain additional clinical information**

"Does the patient have any underlying autoimmune or chronic inflammatory condition?"
"Has the patient been exposed to animal antigens or Abs?"
"Does the patient have other unexplainable lab results?"

- **Inform the physician**

e.g., follow-up and findings

What can be done in the workup?

Sample check

Rationale

- **Sample mix-up** could have caused the unexpectedly high or low results
  - Check for patient name
  - Check for specimen type

What can be done in the workup?
Dilution study

Rationale

- Check for **high-dose hook effect** and/or **interfering substances**
  - Middleware rules may be implemented to flag extremely high or low results for review
  - Nonlinear relationship is often observed if interfering substance is present (García-González et al. *Pract Lab Med.* 2016)

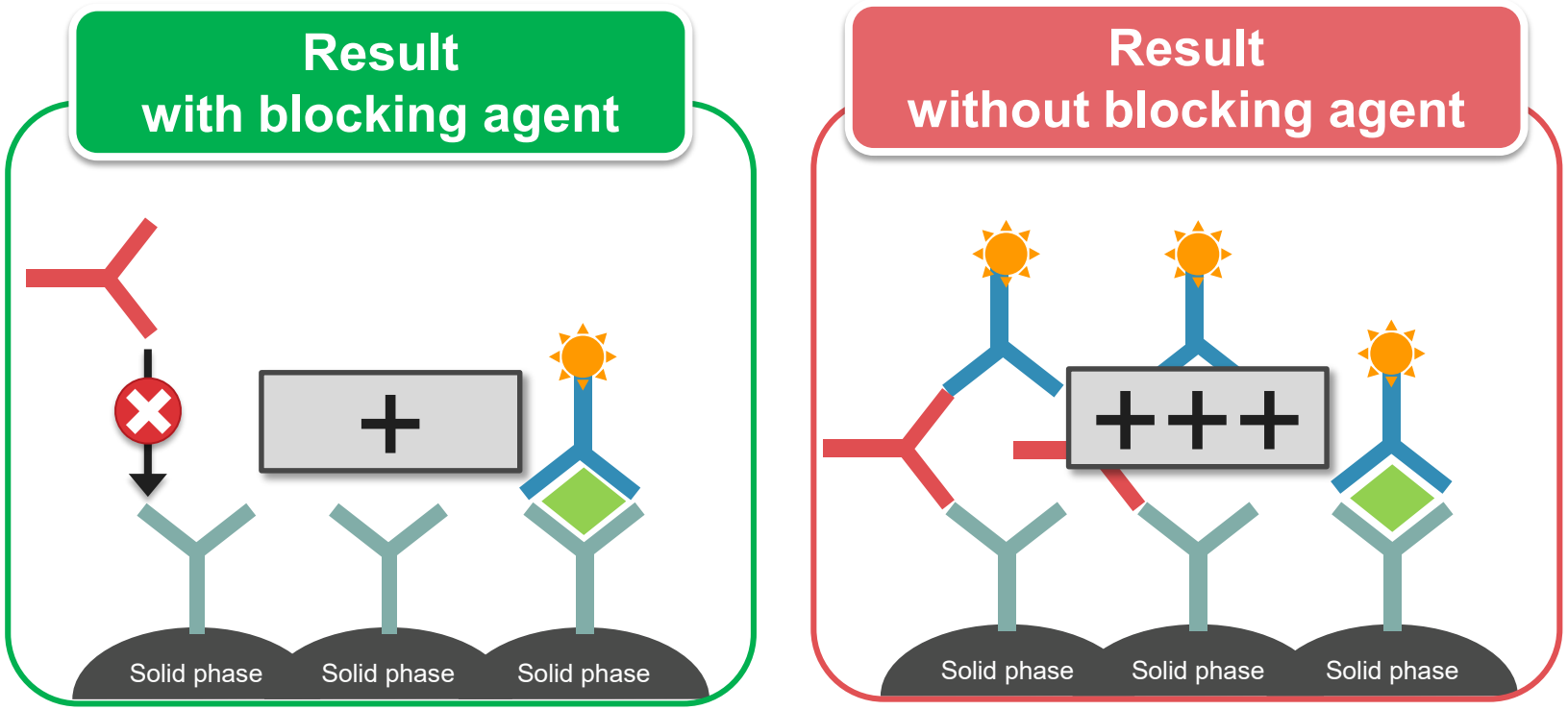
What can be done in the workup?

Heterophile Ab blocking reagent study

Rationale

- **Blocking reagent** (e.g., pooled proteins from different species) may **help neutralize or inhibit the heterophile Ab interference**

Blocking agents can help neutralize or inhibit interference from heterophile Abs (which tend to have weak, non-specific binding)



What can be done in the workup?

Heterophile Ab blocking reagent study

Rationale

- **Blocking reagent** (e.g., pooled proteins from different species) may **help neutralize or inhibit the heterophile Ab interference**
  - Assay should be validated to ensure **compatibility** with the blocking reagent (Preissner. *J Clin Endocrinol Metab.* 2003)
  - May be ineffective in demonstrating interfering Abs in approximately **20–30%** of cases (Ismail. *J Clin Pathol.* 2009)

What can be done in the workup?
Re-test with different assay

Rationale

- **Alternative** assay may have **different susceptibility or resistance to patient's heterophile Ab**
  - Re-analyze using immunoassay using Ab from different species
  - Re-analyze using mass spectrometry-based method

Summary

- Heterophile Abs are non-specific Abs that can bind to multiple molecules
- **Falsely high** or **falsely low** results may occur depending on the immunoassay type and the heterophile Abs present
- Suspicion of heterophile Abs often triggered by clinicians due to unexpected results
- Blocking reagents may be used during the workup
  - **Blocking reagent** (e.g., pooled proteins from different species) may **help neutralize or inhibit the heterophile Ab interference**
  - Assay should be validated to ensure **compatibility** with the blocking reagent
  - Not always effective
  - Re-testing sample with alternative platform may be useful

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Disclosures/Potential Conflicts of Interest

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- **Patents:** None Declared

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