

Interfering Antibodies in Immunoassay

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Immunoassay

- analytical sensitivity
- adequate specificity?

Specificity is dependent on :

- property of the antibody
- composition of the sample antigen
- Sample matrix
- reagent composition
- immunoassay format
- False positive and false negative interference are possible

interferences

- Analytical interference is defined as the effect of a substance present in the sample that alters the correct value of the result
- deviation from true value

interference

- **Analyte-independent interferences**
hemolysis, lipaemia and effects of anticoagulant and sample storage
- **Analyte-dependent interferences**
 - Interaction between constituents in the sample with one or more reagent component
 - They include compounds with chemical differences but structural similarities that cross-react with the antibody,

Analyte-dependent interferences

- Heterophile antibodies
- Human anti-animal antibodies
- Autoanalyte antibodies,
- Rheumatoid factors
- Other proteins

Interference

- Interference can lead to falsely elevated or falsely low analyte concentration
- Depending on the site of the interference in the immunoassay reaction
- Interference affects a wide range of immunoassay analytes including hormones, tumor markers, drugs, cardiac troponin, and microbial serology

Interference

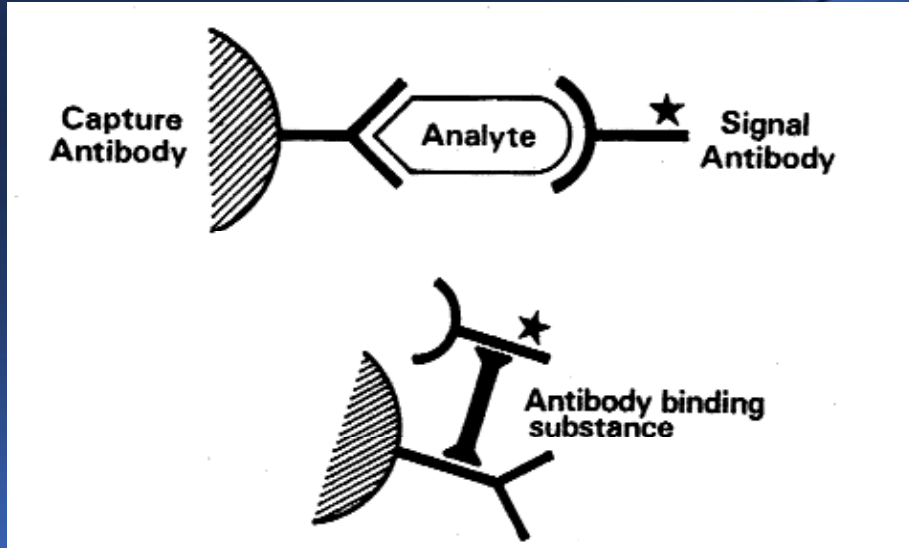
- The magnitude of the effect depends on the concentration of the interfering substance, but not necessarily in a **directly proportional way**
- It may result in the misinterpretation of a patient's results from which the wrong course of treatment is given

Interference

- occur in both **healthy** and **pathological** patient samples
- arise from properties of the specimen
- The sample properties are unique to the patient
- interference results from an interaction with one or more steps in the immunoassay procedure such that the measurable analyte concentration in the sample or antibody binding is altered

Antibody Interference

- **Exogenous**
 - Immunological drugs
 - Most common interference is Fab fragment from anti digoxin antibody
 - Ginseng (digoxin like immune reactive component)
- **Endogenous**
 - Identical mechanism
 - Saturating (sandwich)analyses



Endogenous Antibody Interference

- *Heterophile Antibodies*
- *Human Anti-animal Antibodies*
- *Auto antibodies*

Heterophile Antibodies

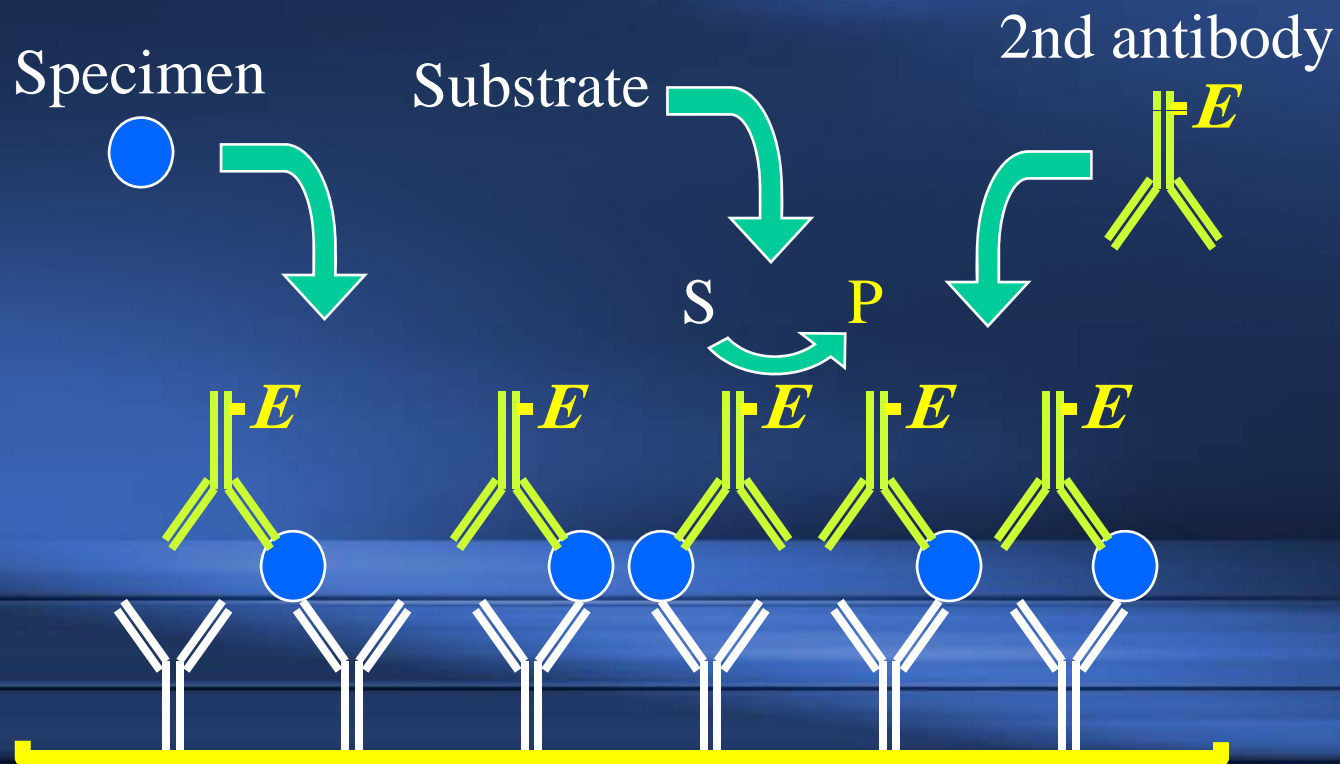
- Interfering, endogenous antibodies are called heterophile antibodies :
- no clearly defined immunogen,
- antibody reacts with immunoglobulin from two or more species
- or has RF activity
- Generally IgG class
- generally show low affinity
- natural antibodies

Heterophile Antibodies

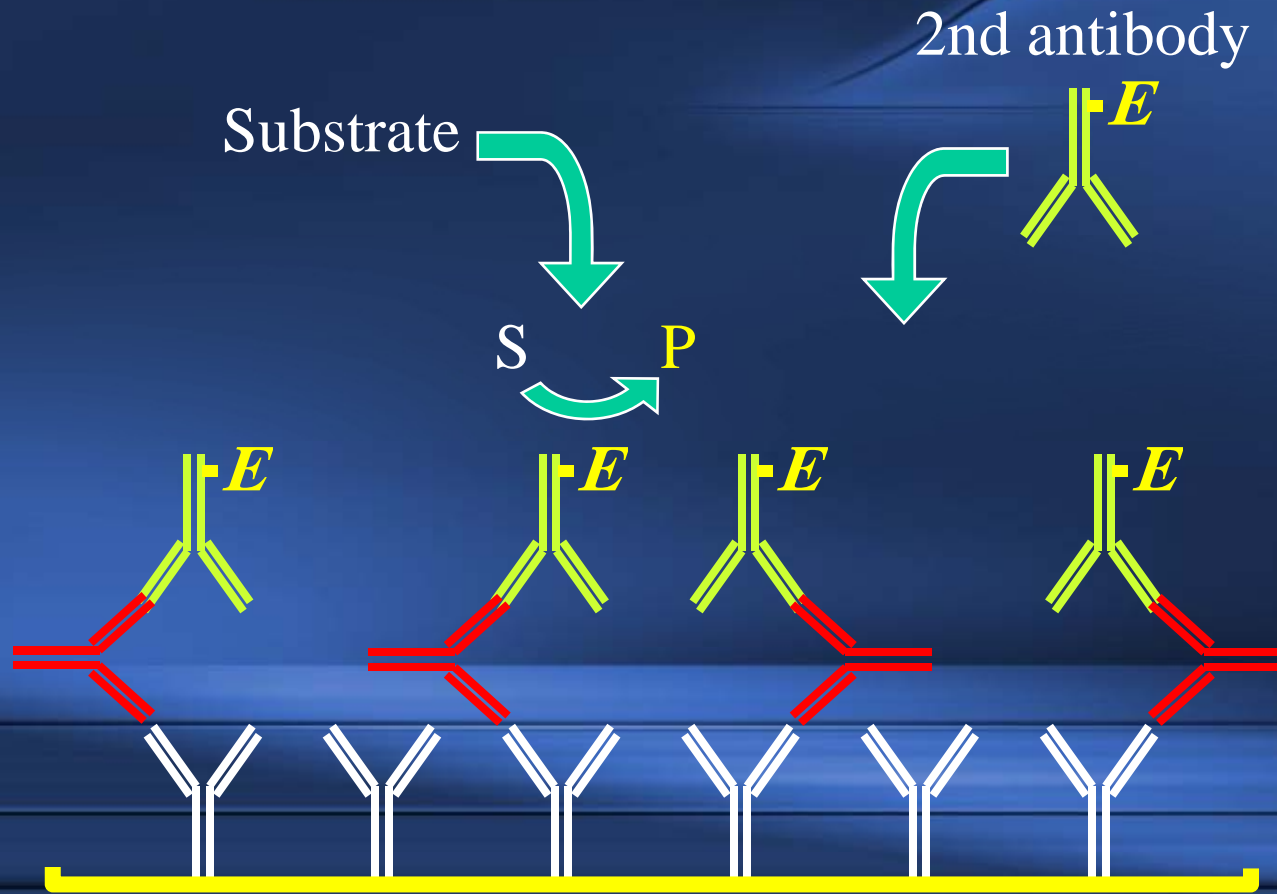
- The same heterophile may react differently for different antibody combinations hence giving rise to a falsely elevated result in one assay but a lower result in another assay
- steroid hormones, thyroid function tests, and digoxin
- Interfere during determination of cytokine by ELISA
- Patients with monoclonal gammopathies

- Despite the occurrence of immunoassay interference having been initially reported in the 1970s the problem remains today with heterophile antibody
- Preissner et al. reported a high heterophile antibody interference rate of >1.5% for a new commercial Tg assay

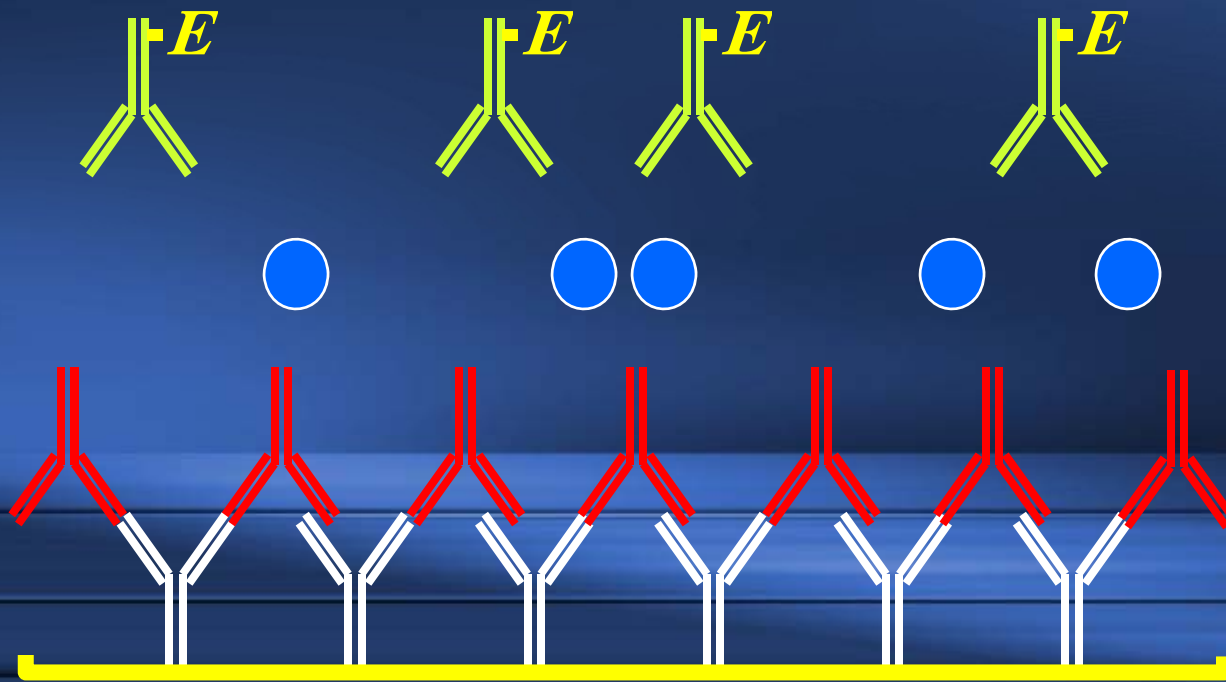
Two-site “sandwich” enzyme immunoassay



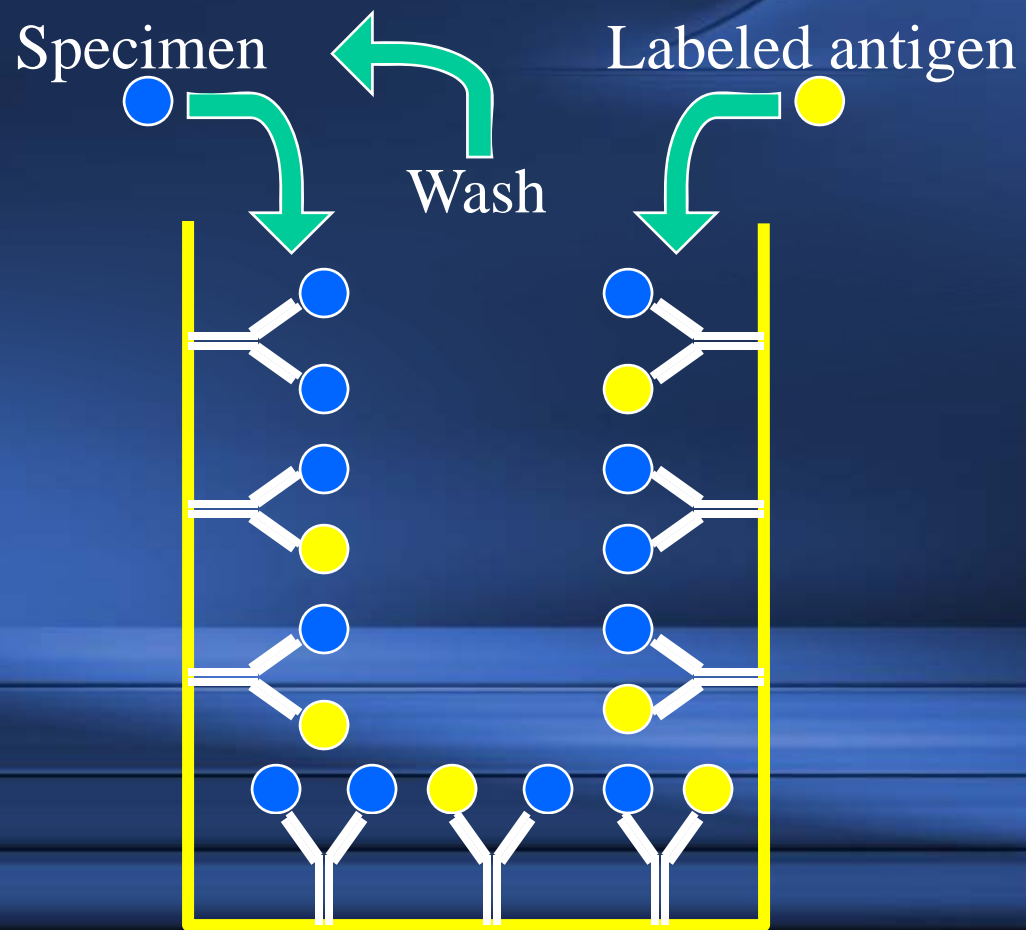
HAMA “bridging” interference



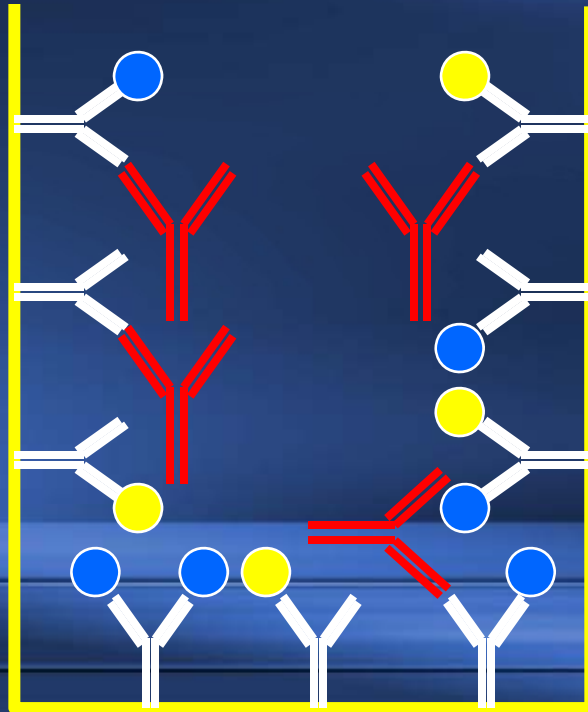
HAMA blocking interference



Competitive immunoassay



HAMA blocking interference



- Natural idiotypic antibodies are antibodies produced by an idiotype (anti-id) that can bind other antibodies
- They may affect antigen binding to antibody in immunoassays by binding to the antigen and affecting analyte concentration, or by mimicking the binding of antigen due to its mirror-image structure.
- Anti-ids together with polyspecific and natural or autoimmune rheumatoid factor (RF), account for most heterophile interference in immunoassays

- routinely add blocking agent to their assay formulations
- pooled globulin from several species
- Heterophile antibodies may show reactivity to idiotypes that are not present in the blocking agent

Human anti-animal antibodies(HAAA)

- First described in 1971 by Ammann and Hong
- Potential to cause positive or negative interferences

Human Anti-animal Antibodies (HAAA)

- High affinity,
- Polyclonal antibodies
- Produced against a specific animal immunogen strong binding with antigen of a single chemical composition
- They compete with the test antigen by cross-reacting with reagent antibodies of the same species to produce a false signal
- most commonly human anti-mouse antibodies (HAMA)
- **HAMA APPLIED INTERA VENOUSLY**
- rats,rabbits, goats, sheep, cattle, etc

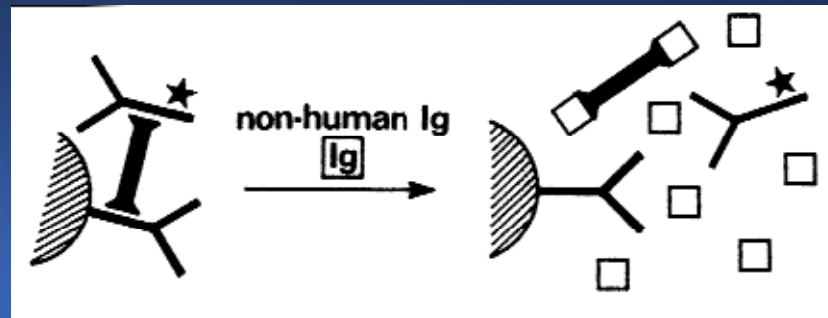
Human Anti-animal Antibodies

- Higher in patients with IgA deficiency
- Vaccine
- Anti –snake venom
- Professional exposure to pet and animals

HAMA

- Mouse antibody is especially prevalent in the serum of animal workers, patients on monoclonal antibody for therapy or imaging, and others exposed to mice
- HAMA interference has been reported for numerous analytes
 - cardiac marker assays
 - thyroid function
 - drugs
 - tumor markers
- Two-site IMA methods are more prone to Interference from antibodies to animal IgG in human

HAAA



Incidence of Immunoassay Interference

- Dependent on the type of antibody interference
- It may vary from 0.05% for interference from heterophile antibody and HAMA, to $\geq 6\%$
- The addition of blockers does not guarantee the elimination of interference

- The blood was from donors with RF-positive illnesses, multiple sclerosis, or lupus, and had detectable RF (31 to >1000 IU/L) and/or HAMA (3-589 $\mu\text{g/L}$).

Properties of interfering substances

- Unique to an individual
- Interfering antibody concentration can change over time within the same individual
- Low affinity polyspecific antibodies can be present in high concentrations or high affinity in low
- Can produce falsely high (false-positive) or falsely low (false-negative) results
- May interfere within one or more manufacturers' immunoassay systems but not necessarily in all assays
- The inclusion of one or more interference blocking agents in manufacturers' immunoassay reagents may be insufficient to overcome the interference

Techniques to Minimise Antibody Interferences in Immunoassay

- Prior extraction of analyte from sample:
 - by chromatography
 - addition of murine or other animal species serum immobilised onto Sepharose beads
 - addition of immobilised Protein A suspension.
 - precipitation with PEG 6000 can remove an anti-animal interference
 - Heating to 70-90°C (for heat-stable analytes only)

Techniques to Minimise Antibody Interferences in Immunoassay

- Addition of low concentrations of serum or immunoglobulin from the same species as the antibody reagents in the reaction mixture can prevent interference in some samples by neutralising or inhibiting the interference

- Non-immune serum, species-specific polyclonal IgG, anti-human IgG or polymerised mouse IgG, non-immune mouse monoclonals, or species-specific fragments of IgG [Fc, Fab, F(ab')₂] from the same species used to produce the reagent antibodies, are commonly used as blocking agents by the manufacturers of kit assays

- Several heterophile blocking reagents (HBR), immunoglobulin inhibiting reagent (IIR), and antibody blocking tubes are commercially available
- Determination of the exact amount of blocker sufficient to eliminate interference in all patient samples is difficult to determine in practice as the immune response to interfering antibodies is so variable between individuals

When should someone suspect interference?

- **Unacceptable result**
- **Non linearity during dilution**
- **No agreement with other test result**
- **No agreement with other clinical data**

Detection and Testing for Interference in Suspected Samples

- both laboratories and physicians aware of the potential for immunoassay interference, which can lead to clinical misinterpretation.
- on-going education
- review of patient results
- protocols for the testing of suspected interference
- notification of interferences both to the physician and to the diagnostic manufacturer

Cont...

- To minimise the reporting of false-positive or false-negative results, a constant dialogue is required between physician and laboratory about unexpected immunoassay results

Cont...

- Physicians should be encouraged to communicate specifically with the laboratory about discordance between results and clinical findings.
- At the same time senior staff should be proactive in improving the laboratory-clinical communication link by presentation and discussion of laboratory data at local journal club meetings

Testing for interference in suspected samples

- Use of an alternate immunoassay that preferably uses antibody raised to a different species
- Measurement before and after addition of a blocking reagent, especially bovine, or a series of concentrations of the blocker, or a combination of blockers from different species
- Measurement of dilutions of the sample using the manufacturer's diluent containing non-immune globulin
- Sample pre-treatment
- Radioimmunoprecipitation of labelled thyroid hormones to detect anti-T3 and anti-T4 autoantibodies

*“Something is better than nothing, unless
‘something’ is wrong, in which case nothing is
better than something.”*

- John Savory