

CLIENT NOTICE:

FALSE IMMUNOASSAY RESULTS DUE TO INTERFERING ANTIBODIES

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The interpretation of test results of some analytes such as TSH, HCG, and PSA may be complicated by presence of various types of antibodies in the patient samples.

What are Heterophilic Antibodies and Anti-animal Antibodies (HAAA)?

Heterophilic antibodies are “weak” antibodies and may be generated in patients who suffer from autoimmune diseases, and other inflammatory diseases, allergies, viral infections such as Epstein-Barr or influenza or after a vaccination. They are produced against diverse antigens and have multi-specific activities and include proteins such as rheumatoid factor.

In contrast, **HAAA antibodies** have strong avidity for well-defined antigens and may be present as a result of receiving therapeutic treatments containing animal derived monoclonal antibodies. One of the most documented HAAA is human anti-mouse antibodies (HAMA), which has seen increased prevalence following the introduction of *in vivo* treatments using mouse monoclonal antibodies. Interestingly, animal handlers may produce antibodies through coincidental immunization that exhibit the characteristics of either HAAA or heterophilic antibodies.

Macro-molecules

Although more rare, the presence of “**macro**” (IgG or IgM) bound forms of some molecules including prolactin and TSH have been reported in the literature. These immune complexes are formed in the circulation and have prolonged half-life compared to the “native” molecule. Evidence suggests that these molecules have minimal bioactivity and are not of pathological significance. However, they may be immunoreactive to a variable extent in immunoassays and contribute significantly to the measured amount of the molecule, leading to possible misinterpretation of the result.

Macroprolactin is a known diagnostic issue, but the literature also reports observation of “macro TSH” in some instances.

What is the effect on laboratory results?

During analysis, presence of these antibody interferents may result in either false positive or false negative results. The mechanism of action is illustrated in Figure 1. The impact of the antibody on measured values may be variable depending on the analyte measured and the technology used by the laboratory.

Fortunately, most manufacturers of analytical systems successfully neutralize interference from these antibodies with little or no impact on the accuracy of the assay with the addition of blocking agents. Large



concentrations of the interferent or antibodies with high binding avidity can, however, overwhelm the analytical system leading to false results leading to conflicting and confusing data.

Some analytes that may be affected by this type of interference are listed in Table I.

Table I: Tests that may be affected by HAAA or Heterophilic Antibodies

ACTH	CEA	LH	Renin
AFP	CK-MB	Prolactin	Somatomedin-C
β_2 - Microglobulin	Cortisol	PSA Total	Thyroglobulin
Calcitonin	C-Peptide	PSA Free	TSH
CA 15-3	FSH	PTH	TG
CA 19 -9	Gastrin		
CA 125	HCG		

What can the clinician do?

Although immunoassays are generally quite robust, erroneous results can occur with any specimen and there is no practical means of identifying samples likely to cause interference problems in immunoassay.

The ordering client knows the medical history and data for their patient(s) and is in the best position to alert the laboratory of a potential issue. If a patient result inconsistent with clinical presentation and other analytical markers is observed, the clinical evidence should not be discarded. If there is any doubt about a reported value, physicians are urged to contact the Clinical Biochemist for discussion. It may be decided that a repeat analysis using an alternative technology or specimen type would be helpful.

For example, discrepant TSH and Free T4 values are one indicator of a possible interference issue. If interference is suspected, TSH results may be confirmed by the laboratory using:

- Alternative immunoassay that uses different antibody pairs or
- Dilution analysis or
- Repeat analysis after treatment with reagents that remove the interfering antibodies from the serum.

Similar mechanisms can be used to confirm the presence of macro-prolactin. To resolve discrepant HCG data, analysis of parallel serum and urine HCG levels may be requested.

As the interfering antibody activity may be non-specific in nature, documentation of known analyte interference(s) should be included in the patient chart and all future immunoassay analyses reviewed and interpreted with caution.

Note: *The interfering antibody may not affect all analytes measured for an individual patient.*

For further assistance please contact a LifeLabs Clinical Biochemist at **1 -877-849-3637**.

References:

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Points to Remember

- Heterophilic and HAAA antibodies may be present in up to 20% of the population.
- Interference to analytical measurement due to these antibodies varies by technology and analyte and may cause false increase or decrease in the analyte of interest.
- Always consider the clinical presentation and other measured variables when interpreting immunoassay results.

Figure 1: Immunoassay Reaction Mechanisms

A. Normal Reaction

The solid-phase capture antibody and tracer antibody bind with the antigen (analyte of interest) to form an antibody-antigen “sandwich” complex. After a wash step to remove unbound tracer antibody and the addition of select reagents, a chemical change in the tracer occurs to produce a measurable signal proportional to the concentration of analyte in the sample.

B. False Positive Reaction Product Measured

A false positive result occurs in the absence of the analyte of interest, when the interfering antibody (Red) binds with both the capture and tracer antibodies to produce a measurable complex.

C. False Negative Reaction Product Measured

A false negative result occurs when the interfering antibody (Red) binds to the analyte forming a non-detectable complex which blocks the binding of the capture antibody and analyte. The apparent concentration of the analyte of interest will, therefore, be decreased or negative depending on the relative concentration and binding affinity of the interfering antibody to the analyte, compared to the tracer antibody.

Figure 1: Immunoassay Reaction Mechanisms

