

# Interference in immunoassays: avoiding erroneous results

by Dr. James Miller

Interference is the effect of a substance present in a sample that causes the measured result of an assay to be erroneous. This bias is an analytical laboratory error that is difficult to detect and that many laboratory scientists feel is beyond the ability of the laboratory to control. However, knowledge of the types of interferences in immunoassays, strategies to detect and confirm them and a good working relationship with clinicians can help avoid diagnostic dilemmas and inappropriate testing and therapy. This article reviews these aspects of interferences in immunoassays.

Immunoassays are assays that use one or more antibodies as reagents. Some types of immunoassay interference are similar to interferences in other chemistry assays and some types are unique to immunoassays. The interfering substance (interferent) in the sample may be exogenous to the patient, for example a drug, or endogenous, for example antibodies produced by the patient. The bias caused by interference may be positive or negative and, in some cases, the degree and direction of interference may vary with the concentration of the interferent or the concentration of the analyte. Types of interference in immunoassays include: 1. crossreactivity; 2. the hook effect; 3. antibody interference; 4. signal interference; and, 5. matrix effects. Some immunoassay designs are especially prone to particular types of interference. These are summarised in Table 1. Several publications review these interferences in detail [1-4].

## Crossreactivity

Crossreactivity is non-specificity, whereby a substance in the sample with structural similarity to the analyte competes for antibody binding. Crossreactivity is probably the most common type of interference in immunoassays. The crossreactant may be a metabolite or precursor of the analyte, e.g., conjugated cortisol metabolites in urinary cortisol assays; or a co-administered drug of similar structure, e.g., tricyclic antidepressants. Crossreactivity usually causes positive interference, but negative interference is possible with certain assay designs. For example, Figure 1 shows the crossreactivity of oleandrin, a cardiac glycoside similar to digoxin, in the AxSYM digoxin assay at different digoxin concentrations [5]. At low digoxin concentration, oleandrin causes the typical positive interference, but at high digoxin concentration, the interference is negative. Crossreactivity can affect any type of immunoassay, but it is most problematic in competitive assays. In two-site immunometric ("sandwich") assays, two reagent antibodies must bind the analyte at the same time, making these assays much more specific.

## The hook effect

The hook effect is a state of antigen excess, in which very high concentrations of analyte saturate all of the available binding sites of the reagent antibodies without forming complexes. This results in falsely lower measured values. This has been reported for many analytes and affects turbidimetric and nephelometric assays, as well as immunometric assays performed in one step. Most modern nephelometers and dedicated turbidimeters have built in checks for antigen excess and automatically dilute the sample when necessary. However, turbidimetric assays performed on routine chemistry analysers are usually not able to perform antigen excess checks. In immunometric assays performed in one step, i.e., the sample, and both capture and detection antibody are added at the same time, the same effect can occur. The hook effect can be eliminated from immunometric assays if a wash step is included between the incubation of the sample and the capture antibody and the addition of the detection antibody. The hook effect used to be relatively common, but antigen excess testing and design improvements have greatly reduced the incidence of this type of interference.

## Antibody interference

Sometimes antibodies in patients' samples interfere with immunoassays, usually by binding to the reagent antibodies. Endogenous antibodies against fluorescein-labelled or enzyme-labelled tracers and exogenous antibodies, such as Digibind, may also interfere with immunoassays. There are two general types of endogenous antibodies that interfere with immunometric assays, anti-animal antibodies and heterophile antibodies. While some properties of these differ [6], they interfere by the same mechanism. Both types interfere with immunometric assays by binding the capture antibody to the detection antibody in the absence of the analyte. Most reports of antibody interference in immunometric assays have been false increases, but negative interference is possible when the interfering antibody binds only one of the reagent antibodies and is present at high titre. The best known anti-animal antibodies are the human anti-mouse antibodies (HAMA). There has been an increasing use of mouse monoclonal antibodies given intravenously for therapeutic and imaging purposes and some patients generate HAMA, which may interfere with assays that use mouse monoclonal antibodies. Other persons may develop anti-animal antibodies from exposure to antigens from other species, for example, vaccines from rabbit or chicken, anti-snake venom from horse, or from casual or occupational contact with pets and other animals. Some of these, including HAMA, may interfere with assays using antibodies from other species. The reagents for immunometric assays include serum or other blocking agents to minimise antibody interference, but some samples still cause interference. When this is suspected, it is useful to have additional blocking agents available to treat individual samples. Several of these are available and were reviewed by Reinsburg [7].

Interfering antibodies can affect all types of immunoassays, but this is most commonly seen in immunometric assays. There are two reasons for this. Immunometric assays are run under reagent excess conditions, that is, the

Type of Interference	Assay Types Affected
Crossreactivity	All, but primarily competitive assays
Hook Effect	Nephelometric, turbidimetric and immunometric assays
Antibody Interference	All, but primarily immunometric assays
Signal Interference	All
Matrix Effects	All

Table 1. Types of interferences in immunoassays.

concentration of both reagent antibodies is much greater than the usual concentration of analyte. This drives the reaction to completion, making this type of assay very rapid and analytically sensitive. However, any antibodies in the sample that have even weak affinity for both reagent antibodies may cause measurable complexes to form in the absence of analyte. In addition, a large number of these assays are performed. Almost all analytes large enough to bind two antibodies simultaneously are assayed by immunometric assays and a few of these assays, e.g., chorionic gonadotropin, thyrotropin, and cardiac markers, are performed on large numbers of individuals. The reported frequency of heterophile and anti-animal antibodies varies greatly depending on the method of detection. Heterophile antibodies are present in more than 10% of patients [8] and 41% of patients who received injections of murine monoclonal antibodies developed HAMA [9]. However, with adequate blocking agent in the assay the frequency of clinically significant interference may be less than 0.05% [6, 10].

### Other types of interference

The two other types of interference in immunoassays are signal interference and matrix effects. Some samples may contain compounds that artifactually increase or decrease the magnitude of the detection mechanism, e.g., fluorescence. This is called signal interference. Because there are many types of indicator mechanisms used in immunoassays, there are many subtypes of signal interference. The matrix of a sample is the environment of the analyte and includes properties like pH, ionic strength, and the protein and lipid concentrations of the sample. Matrix effects are caused by variations in the reactivity of the analyte due to variations in its environment in the sample. Immunoassays are often quite sensitive to the matrix due to effects on antigen antibody binding, efficiency of separation of bound and unbound fractions, and the extent of nonspecific binding. Further details about signal interference and matrix effects can be found in references 1-4.

### Detecting and checking interference

Detecting inaccurate results due to interference is difficult because the true result is not known. However, there are several mechanisms that might be included in an interference screening program. Results from patients with clinical conditions commonly associated with more frequent occurrences of interference, such as liver disease, renal failure, or pregnancy, may be scrutinised for possible interference. If certain drugs are known to interfere with a particular assay, collection of samples from patients taking those drugs might be timed to coincide with trough concentrations of the drugs or delayed until the drugs are discontinued. Samples should be inspected visually for haemolysis, icterus, and lipaemia, or better, evaluated objectively using serum indices. Any results with instrument error codes should be investigated for possible interference. In addition, unexpected changes over time in a patient's results (delta checks), inconsistencies among physiologically related results (e.g., thyroxine and thyroid stimulating hormone), and exceptionally extreme results may be caused by interferences. Finally, inquiries from clinicians regarding test results that do not fit the patient's clinical picture may provide clues to possible interferences.

When suspect samples are identified by these mechanisms, three checks are especially helpful for deciding whether the results are accurate: 1. test for linear dilution; 2. assay the sample by another method; and, 3. treat the sample to remove, destroy, or inhibit potentially interfering substances. Interferents frequently dilute non-linearly and respond differently among assays, but these characteristics are not always present. In addition, one can treat the sample to remove, destroy, or inhibit the interfering substance. Possible treatments include extraction, chromatography, ultrafiltration and addition of a heterophile blocking agent. Addition of a heterophile blocking preparation is quite easy and quick, but the other techniques may be time consuming and their effect on the analyte needs to be known or checked with control samples.

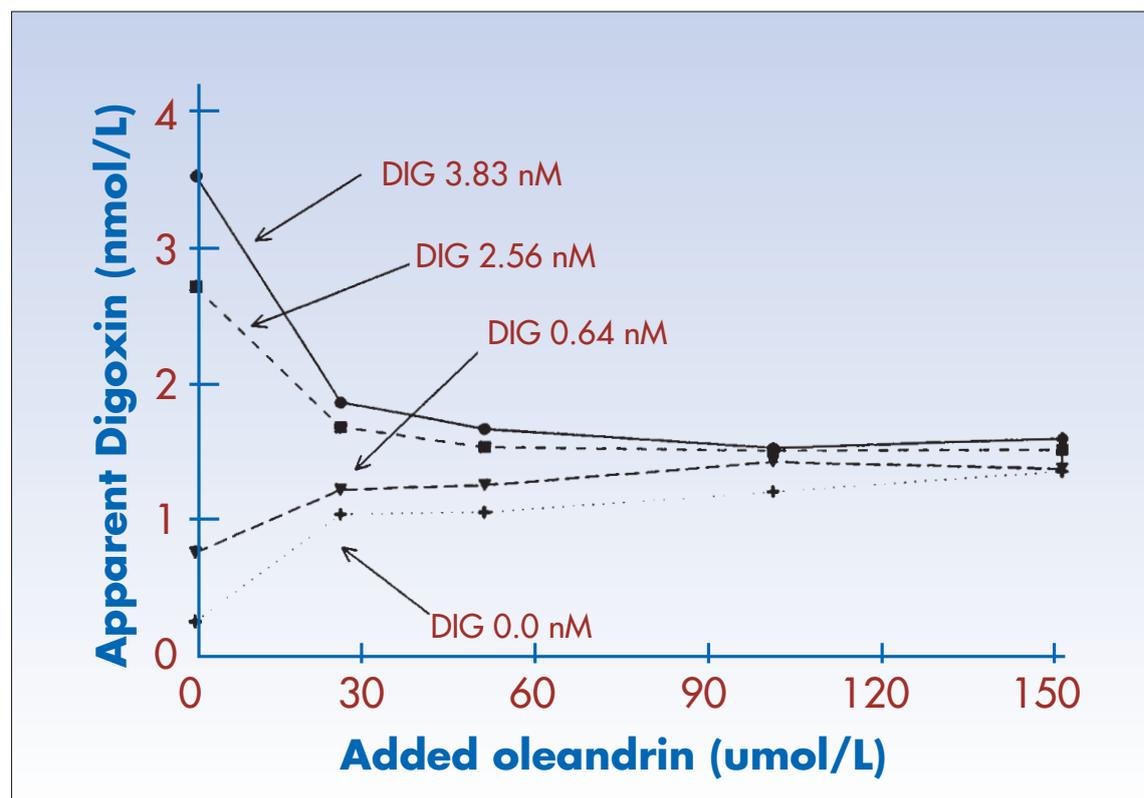


Figure 1. Negative crossreactive interference caused by the presence of oleandrin in the MEIA (AxSYM) digoxin immunoassay. Various mixtures of oleandrin and digoxin were prepared and analysed. Notice that lower than expected values of digoxin were measured at digoxin concentrations of 2.56 and 3.83 nM. At lower digoxin concentrations, oleandrin exhibited typical positive interference in this assay. Reproduced from [5] with permission.

### Consequences of interference

Falsely abnormal immunoassay results, whether falsely increased or falsely decreased, suggest the presence of disease and may lead to the ordering of additional diagnostic tests and unnecessary therapy, including surgery. The most striking and most publicised examples have been persistently false positive chorionic gonadotropin results due to interfering antibodies suggesting post-gestational choriocarcinoma or trophoblast disease and leading to unnecessary chemotherapy and surgery [11]. On the other hand, falsely normal results may contribute to an unrecognised diagnosis and failure to treat. For example, Steimer, *et al.* [12] reported on a case of digoxin toxicity that was masked by negative crossreactive interference by canrenoate administered simultaneously. This occurred by the same mechanism as that shown in Figure 1.

Manufacturers continually try to improve the specificity of their assays and laboratories have begun to be more vigilant in screening for spurious results. These efforts have reduced the risk of diagnostic errors due to interferences. However, this type of laboratory error cannot be abolished. Because of the increasing number of analytes measured by immunoassay and the increasing demand for rapid turn around times and analytically sensitive assays, erroneous results due to interferences will continue to be a problem.

Consequently, it is only through better communication between clinicians and laboratory scientists that diagnostic misadventures and patient catastrophes are going to be avoided. Laboratory scientists and manufacturers need to educate clinicians about the limitations of assay performance and the potential for inaccurate results. Clinicians should notify the laboratory when ordering tests on patients known or at risk for having interfering antibodies or other potential causes of interference. Clinicians should also consult with a laboratory scientist whenever test results do not fit the clinical picture. In such cases, further testing can be arranged to investigate the accuracy of the results. Finally, laboratorians should confer with the clinician whenever the possibility of interference is identified by the screening mechanisms described above. Hopefully, through this improved collaborative dialogue between laboratory scientists and clinicians, diagnostic dilemmas and inappropriate testing and therapy can be avoided.

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