Analytical Error in Immunoassays

In 1959, Rosemary Yalow revolutionized the field of clinical chemistry by developing a novel technique, radioimmunoassay, to measure plasma insulin. The principles of immunoassay have been refined and exploited throughout laboratory medicine to improve the sensitivity and specificity of assays for a variety of antigens including drugs, enzymes, hormones, microbes, and specific proteins. Serology employs similar methods to detect many different types of antibodies. The accuracy, availability, cost and speed of many laboratory tests have improved dramatically.

Unfortunately, the immunologic basis for these assays also introduces potential sources of analytical error, which (if not recognized) may prove detrimental to patient care. Fortunately, these occur on an infrequent basis. This brief review will illustrate several examples of such error and discuss steps that can be taken if such error is suspected. This is not intended to be an exhaustive review, but rather to serve as an alert.

Autoantibodies (Thyroid Autoantibodies)

Autoantibodies to a given antigen may interfere with test methods employing antibodies to measure the same antigen. Both the reagent antibody and the autoantibody compete for the antigen. Depending on the principle of the assay, the result may be spuriously low (single-antibody technique) or high (double antibody technique). Autoantibodies to thyroxine (anti-T4) or triiodothyronine (anti-T3) can interfere with some methods of detecting T4, T3, free T4 or T3. The prevalence of such antibodies can be as high as 10% in patients with autoimmune disease, but only a minority of samples from such patients will result in significant interference in these assays. It is difficult to predict to what extent any particular autoantibody will interfere with any particular method. Assays, which are “one step” or fail to physically separate the conjugate antibody – antigen complex from the rest of the serum prior to introducing the “detection” reagent (e.g. by a “wash” step) are more prone to interference. (At Rex, the T4 assay would thus be theoretically more prone to this interference than the free T4 assay. Another reason to stop ordering total T4s.)

Endogenous and Exogenous Interference (Digoxin-like immunoreactive factors)

Since 1965, there have been reports of false (+) digoxin assays in patients not taking the medication. It was discovered that some patients produce digoxin-like immunoreactive factors (DRIF). The prevalence of DRIF is greatest in patients with renal failure, hepatic failure, recent cardiac surgery, or newborns (particularly cord blood). Some have also reported their presence in pregnant women (particularly in the 3rd trimester.) Different digoxin assays vary (unpredictably) in their response to DRIF, and this can result in significant problems in managing digoxin therapy. In some assay systems, “digoxin levels” as high as 1.2 ng/ml may result. Assays employing polyclonal digoxin antibodies appear to be more susceptible than those using a monoclonal preparation are. Another potential problem exists when patients ingest substances which contain chemicals structurally similar to digoxin. Recent examples include Chinese medicine containing Ch’an Su (prepared from dried venom of the Chinese toad, in Asian countries over 300 tablets or powders include this compound). A similar bufadienolide-based preparation has been marketed as a purported aphrodisiac (“Stone” or “Rock Hard”) and produced fatalities due to cardiac arrhythmias. Again “digoxin levels” between 1 – 5 ng/ml have been reported due to the presence of these compounds alone.
Finally, it was recently reported that some digoxin assays (including the one currently used at Rex) are subject to interference by spironolactone, canrenone and related steroid drugs. Use of these drugs produces a falsely elevated result with some assays, and falsely low results with others. (For the assay used at Rex, the result was roughly 78% of the expected result in the presence of canrenone – an active metabolite of spironolactone available as a drug in Europe.) Thus patients taking both spironolactone and digoxin may be at risk of digoxin toxicity if dosages are adjusted on the basis of digoxin levels alone, and this phenomenon is not recognized.

Heterophile Antibodies and Rheumatoid Factors

Virtually all immunoassays are subject to interference by heterophile antibodies and rheumatoid factors. Heterophile antibodies are antibodies which react against specific animal immunoglobulins of a certain species (or may cross-react with a variety of animal immunoglobulins from various species). Among the most widely recognized are human anti-mouse antibodies (HAMA). The stimulus for these antibodies is not always known, although they have been associated with the use of murine monoclonals used for diagnostic or therapeutic purposes. Heterophile antibodies have also been observed in association with vaccine use, environmental exposure (farmers, veterinarians), and autoimmune disease. Rheumatoid factors (RF) are IgM antibodies targeting the Fc fragment of human IgG. They are present not only in patients with rheumatoid arthritis, but also those with other autoimmune disease and some (otherwise healthy) elderly patients. Both HAMA and RF may interfere with immunoassays in a fashion that is not always predictable – and both artifactually high and artifactually low values may result.

This phenomenon has been recognized for a long time in the laboratory community. The fine print of most immunoassay “spec sheets” contains verbiage similar to the following. “Patient samples may contain heterophilic antibodies that could react in immunoassays to give falsely elevated or depressed results. This assay has been designed to minimize interference from heterophilic antibodies.” The latter refers to the use of “blocking reagents” (usually nonspecific polyclonal murine and human IgG) to attract and absorb any MAHA or RF present. Indeed, in most cases, this strategy is successful, but some patients may have persistent interference problems. If not recognized, problems may follow. In November 1999 a class action lawsuit was filed against Abbott Laboratories on behalf of women falsely diagnosed as having choriocarcinoma on the basis of elevated quantitative human chorionic gonadotropin levels. Part of Abbott’s defense is the assertion that the test under duress was “…designed solely for the detection of pregnancy…and even FDA documents have said for years that there are limitations and that if a doctor finds a patient who is exhibiting elevated hCG in the absence of pregnancy, and inconsistent with their total clinical profile, that the doctor should retest and do other confirmatory diagnostic techniques.” (Clinical correlation again!)

Exogenous Antibodies (Digibind®)

Digoxin immune Fab (Digibind®) is a purified preparation of the Fab fragments of digoxin antibodies obtained from sheep. It was approved by the FDA in 1986 for use in cases of serious digoxin toxicity (but has also be used in managing overdose of Ch’an Su or “Rock Hard”). This therapeutic antibody produces unpredictable and erroneous results in most digoxin immunoassays. Thus digoxin levels in patients treated with this drug are not particularly helpful, and may, in fact, be misleading. The half-life of Digibind in patients with normal renal function is 15-20 hours, but can be much longer in patients with renal failure. In one patient, Digibind interference with digoxin assays was noted > 10 days after administration.

The Role of the Laboratory

As noted above, errors due to interference are relatively rare. Vendors of immunoassays continue to improve their product to limit the effect of interference. Absent knowledge of the clinical setting, it is difficult (if not impossible) for the Laboratory to suspect interference in any given immunoassay result. (The one possible exception being a dramatic increase in a “digoxin level” in a patient with an already elevated digoxin. To the insightful laboratorian, this might raise the question of Digibind® administration, although confirmation would require conferring with the treating physician or nurse.) If alerted by the clinical staff to the fact that an
immunoassay result does not fit the clinical profile, the possibility of interference should be considered (in addition to the possibility of patient or specimen identification error, contamination with IV fluid, etc.) Practical laboratory steps to evaluate the possibility of immunoassay interference include reviewing the clinical history (underlying illness, ? recent vaccination, ? autoimmune disease, ? cancer patient subjected to diagnostic or therapeutic monoclonal antibody), performing dilutions on the specimen, and testing by an alternate method. (In some research laboratories, other steps could be employed including testing an ultrafiltrate of the specimen or testing after treatment with polyclonal mouse/human sera. These latter procedures are cumbersome, time-consuming and often do not provide practical information in a realistic time frame.) Furthermore, none of these measures will definitely establish either the presence or type of interfering substance. Often the clinical scenario is the most helpful information in evaluating apparent laboratory discrepancies. Accordingly one of the most important things the laboratory can do is educate those ordering and interpreting immunoassay tests.

(Congratulations on reading this far…and thank you!) In addition, as we become aware of potential problems with specific assays, we will try to inform you through articles such as these, and brief comments appended to report results. (We recently added comments to our quantitative hCG and digoxin results warning of potential interferences.) If you suspect a problem with interference in a particular laboratory result, please call the Core Laboratory (784-3020) and ask to speak to the “Charge Tech” or the pathologist on call. We will do our best to evaluate this possibility, although it may require referral of specimens to a reference laboratory and take several days or longer to resolve.

John D. Benson, MD

References


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Anatomic Pathology will convert to a new information system (Co-Path®) on May 1, 2002. If all goes well, this should be a relatively seamless (and hopefully transparent) conversion. If all does not go well, contact Dr. John Sorge with your comments or concerns.

Pap Smear Reports to Incorporate Bethesda 2001 Revisions

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The Bethesda System for reporting of cervical/vaginal cytologic diagnoses was introduced in 1988 as a means to standardize Pap Smear reporting and to encourage the use of specific, descriptive diagnoses and to standardize specimen adequacy criteria. In late 2001 recommendations from the third modification of the system were issued. These latest modifications were made in conjunction with representatives from the obstetrics and gynecologic community. The Rex Healthcare Cytology Department plans to incorporate these new recommendations into our Pap Smear reports beginning May 1, 2002.

SPECIMEN ADEQUACY: Bethesda 2001 recommends that the category “Satisfactory but limited by…” be eliminated. Specimens will now be categorized as either Satisfactory for Evaluation or Unsatisfactory. The presence or absence of an endocervical component as well as any other factors that could hinder evaluation of the specimen including obscuring blood, lack of menstrual data, etc. will still be mentioned in the comment section of the report.

INTERPRETATION: Bethesda 2001 recommends that all cases be classified either as “Negative for Intraepithelial Lesion or Malignancy” or, if there is an Epithelial Cell Abnormality, then the abnormality (Low Grade Squamous Intraepithelial Lesion, etc.) will then be the diagnosis of record. In other words, all Pap smears will be classified as either Negative or Positive. We will no longer use diagnostic terms such as Within Normal Limits, Reactive Changes, Benign Cellular Changes, or Infection. If a significant infectious organism such as Trichomonas or Candida is present then its presence will be documented in the comment section.

ASCUS: This term will be broken down into two categories. ASCUS (Atypical Squamous Cells of Undetermined Significance) will be used when there are atypical cells present which raise the question of low-grade dysplasia but insufficient criteria for a definitive diagnosis are present. ASC-H (Atypical Squamous Cells Cannot Exclude High Grade Squamous Intraepithelial Lesion) will be used when findings suspicious for high-grade dysplasia are present but are insufficient for a definitive diagnosis. There will no longer be a category of ASCUS favor reactive changes.

GLANDULAR LESIONS: Bethesda 2001 recommends the reporting of endometrial cells out of menstrual phase only in women greater than 40 years of age. This recommendation was made in light of the fact that endometrial cells reported in women under the age of 40 are rarely associated with endometrial pathology and almost never associated with endometrial pathology that is not already apparent to the clinician. In addition, the diagnostic category of AGUS (atypical glandular cells of undetermined significance) has been eliminated. It was felt that this diagnosis was to easily confused with the diagnosis of ASCUS. It is now recommended that if atypical glandular cells are identified they simply be reported as “Atypical Glandular Cells” with a comment as to whether or not they appear to be endocervical or endometrial and a comment as to whether a neoplastic process is favored.

These changes will be reflected in our Pap Smear reports beginning on May 1, 2002. If there are any questions or concerns please contact Keith V. Nance, MD at 784-3286.

Keith V. Nance, MD

For further information, call the Laboratory (784-3040). Telephone extensions are: Pathologists’ Direct Line (3201), Sharon Logue (Lab Director 2400), Robin Ivusic (Microbiology Lab Manager 3053), Elaine Patterson (Core Lab Manager 3054), Jackie Okoth (Core Lab PM Manager 4248), Diane Young (Anatomic Pathology Manager 3888), Nga Moore (Customer Service Manager 3396)