

Industry Division Newsletter

Winter 2013



2013 Division Officers

Chair: Jennifer Huff, D.V.M., Ph.D.
Chair-Elect: Open
Secretary: Agim Beshiri, M.D.
Treasurer: Suzette Chance, Ph.D.
Past Chair: Doug Clark

Division Goals

- To provide a forum for AACC members to meet, identify common interests and concerns, and develop programs that assist them in the workplace. Although the focus of the Division is on issues relevant to industry, or issues from the industry perspective, membership in the Division is open to any member of the association.

Nominating Committee:

Karl De Vore
John Ellison
Mihaela Necula, Ph.D.
Jim Pierson-Perry

- To identify and develop synergies between industry and clinical laboratory members which contribute to the health care system and enhance patient care.

Contents

| | |
|---|--------|
| Message from Past Division Chair..... | 1 - 2 |
| Industry Division Awardees of recent years..... | 2 |
| Election Update..... | 2-3 |
| Assay Interference: A Need for Increased Understanding and Ease of Testing..... | 4 - 11 |

Message from Past Division Chair

Doug Clark

We welcome our new Chair of the Industry Division, Jennifer Huff, along with all members of the Industry Division. Please encourage your colleagues to join the Industry Division, if they have not, and to actively participate if they already are members.

Our Division's annual business meeting was held during this year's AACC National Meeting in Los Angeles, CA, on Tuesday, July 17th from 4-6 pm. We had two guest speakers at the meeting. Dr. Herbert Vesper from the CDC spoke about the progress and future plans for steroid standardization projects. Standardization programs are available for Testosterone and Estradiol and the development of a program for Vitamin D is well underway.

Dr. Michael Bennett from the Children's Hospital of Philadelphia spoke about the need for Pediatric Reference ranges for methods provided by Industry. Availability of patient samples that cover all of the age and gender groups needed to develop pediatric ranges for children is a problem for Industry.

At the AACC annual meeting in Los Angeles our Division sponsored the following seminars and workshops:

- OEM Lecture Series
- Challenges and Clinical Impacts of Standardization of Immunoassays
- JCTLM/IVD Industry meeting

- Testing for Chronic Kidney Disease (CKD): Creatinine, GFR, UA1b and More: Industry Responses to Clinical Needs

For 2013, Jennifer Huff will lead the Division's annual business meeting and technical discussions. The Industrial Division meeting in Houston will be held on Monday, July 29, 2013 from 2-4 pm. Jennifer will provide more details in the near future so save the time slot to attend the meeting. Refreshments will be provided.

Keep an eye out for two upcoming webinars sponsored by the Industry Division on our changing regulatory landscape.

See you in Huston!

Industry Division Awardees of Recent Years

Best Abstract Award

This award is made to the author(s) of an abstract for the AACC national meeting which is deemed to present a significant contribution to the IVD industry in one of the following areas: management, regulatory affairs, or improved patient care through a new or improved medical device. The selection is done through a vote of the Division's officers and Nominating Committee members.

Awardees for the past three years were:

2010 A Panel of Cervicovaginal Fluid Biomarkers Efficiently Predicts Gestational Age at Delivery by Alison Woodworth, Ph.D., DABCC, FACB, Assistant Professor of Pathology, Associate Director of Clinical Chemistry, Vanderbilt University Medical Center, Nashville, TN

2011 Development of Real-time PCR assay for the Detection of Five Major Pneumonia Pathogen Panels by Yu-Jeong Kim, Ph.D., Daejeon, Korea

2012 Direct Identification of Microbes in Urine Specimens Using Mass Spectrometry by Mari DeMarco P.h.D, Clinical Chemistry Fellow Washington University School of Medicine Laboratory and Genomic Medicine

Louis J. Dunka, Jr. Award

The Louis J. Dunka, Jr. Memorial Service Award is presented annually by the AACC Industry Division to an individual for outstanding accomplishment that is evidenced by the initiation or support of a single event or continuous effort that has served the AACC Industry Division Membership and/or the IVD industry.

Awardees for the past three years were:

2009 Ronald H. Laessig, Ph.D. (posthumously) Emeritus Director, Wisconsin State Laboratory of Hygiene, Madison, WI

2010 Jack Levine, M.B.A Siemens Healthcare Diagnostics, Tarrytown, NY

2011 James Pierson-Perry Ph.D. Siemens Healthcare Diagnostics, Glasgow, De

Election Update

After reviewing the by-laws for the Industrial Division, we found that we are behind in our elections of some positions for 2013. The terms for Karl Devore and Mihaela Necula on the Nominating Committee were up for renewal at the end of 2012. Also the term of Suzette Chance, our Treasurer was up for renewal at the end of 2012. We need a Chair Elect for 2013 who will serve as the Chair for 2014.

Please let Doug Clark or Jennifer Huff know if you are interested in any of these positions or if you currently hold one of the positions it you would like to serve another term. We can hold a special election to catch up with our by-laws if we can get nominees. It is not a huge time commitment so become involved in the Division. With more members involved we can work on becoming more active Division and provide useful articles, webinars, and information to all members of the Industrial Division.

The follow is an informative article from John H. Contois, PhD at Sun Diagnostics about Interference Testing for Industry.

Assay Interference: A Need for Increased Understanding and Ease of Testing

John H. Contois and Rae-Anne Nguyen
Sun Diagnostics, LLC
New Gloucester, Maine

Laboratory Directors are responsible for the quality of data that is reported from their laboratories, but they rely on the manufacturer for a great deal of information. The quality of interference testing data provided by the manufacturer is seldom questioned, and, in-house interference testing is not normally performed. Only 8% of laboratories performed in-house interference studies (2). Therefore, the burden is on the manufacturer to provide the highest quality interference data possible. While relying on the manufacturer's interference claims is considered sufficient to meet regulatory requirements, this information may not translate to performance in a specific clinical laboratory, and individual laboratories should be encouraged to verify manufacturer's data by performing in-house interference testing.

In addition, the laboratory must implement an effective detection system to identify specimens with clinically important interferences and a specific policy to prevent reporting of inaccurate results. One study reported that 9.7% of all specimens contained at least one visible interference; 76% were lipemic, 16% were hemolyzed, and 6% were icteric (3), yet visual inspection of samples alone is not effective. The use of automated, spectrophotometric measurement of bilirubin, hemoglobin, and lipemia (serum indices), along with clearly defined decision rules, is effective (4), but must include an understanding of the relationship between index scale and the impact on each test. Interestingly, recent abstract reports that only 38% of laboratories were using automated serum indices for assessment of interference (2). Manufacturers should encourage their customers to use serum indices and provide training to help end users better understand how indices can better detect problem specimens.

It is also important for the laboratory to understand how the indices were determined by the manufacturer, and for the manufacturer to describe their interference testing protocols in detail. It is important to correlate icterus and hemolysis indices with bilirubin and hemoglobin concentrations. Lipemia index is typically based on the spectrophotometric response of Intralipid®, a synthetic lipid emulsion, and cannot be correlated with the response of human triglyceride-rich lipoproteins. The manufacturer should assess lipemia using intact human lipoproteins, primarily chylomicrons, instead of Intralipid.

Information provided by the manufacturer may not be adequate for the laboratory to gauge the impact that interference will have on their results. Often, the data is reported without defining a "significant" change or what analyte concentration was tested along with the interference, and interpretation is often based on an arbitrary 10% rule (5). The experimental

design may be vague, such that the user does not have adequate information to make an informed decision about the adequacy of interference testing (5).

There are several reasons for encouraging a laboratory to perform in-house interference testing experiments, such as: verifying the commutability of interference data from the manufacturer to their specific instrument/reagent system; to provide additional information that may not be provided by the manufacturer, such as assessing interference with at least two levels of analyte, as recommended by CLSI due to potential interdependence of interferent and analyte concentrations; and determining the applicability of data generated from testing that used artificial materials, such as Intralipid, instead of human lipoproteins to test lipemia interference.

Common Interferents

Interference from **hemolysis** (hemoglobin and /or red blood cell contents), **lipemia** (triglyceride-rich lipoproteins or turbidity), **icterus** (unconjugated or conjugated bilirubin), and **proteins** (albumin and gamma-globulins, paraproteins) are so common with routine chemistry assays, that validation by manufacturers always includes interference testing for these substances. Newer instruments and reagent formulations are better able to limit the effect of common interferents; sample blanking, bichromatic measurements, and additives to inhibit interferents have all been effective to a certain degree, but problems have not been eliminated. Unfortunately, we cannot develop universal rules regarding interference, as instruments and reagent systems can vary widely in the response to specific interferents (6, 7). Some investigators have developed a mathematic formula for the correction of interference, but this is generally not a good idea. The best approach is for laboratorians to understand the limitations of the assay in their laboratory, and not report results that are inaccurate to a degree that they will impact clinical interpretation.

Hemolysis

Hemolysis occurs in about 3% of all specimens received in the laboratory and accounts for 40% to 70% of all unsuitable specimens (8). Dimeski describes four mechanisms for interference from hemolysis: additive, spectral, chemical, and dilutional (9). Interference may be due to hemoglobin or to other red cell constituents that are released into plasma or serum. These substances can directly or indirectly interfere with a number of assays, including ALT, AST, creatinine, CK, iron, LDH, lipase, magnesium, phosphorus, potassium, urea, albumin, ALP, chloride, GGT and sodium (10-12).

Differentiating between *in vivo* and *in vitro* hemolysis is vitally important to patient management. It is sometimes possible to differentiate between the two biochemically. Free hemoglobin in the circulation binds to haptoglobin and the complex is rapidly cleared. Therefore, a decrease in serum haptoglobin is consistent with *in vivo* hemolysis. Increased

bilirubin and reticulocyte counts are also characteristic of *in vivo* hemolysis. The bilirubin is derived from the breakdown of heme, while the presence of reticulocytes indicates the physiological response to anemia. Another clue differentiating *in vitro* and *in vivo* hemolysis is the concentration of intracellular contents: *in vitro* hemolysis is typically characterized by an increase in cellular components such as K, LDH, and AST, which would be quickly normalized during *in vivo* hemolysis. Given the clinical significance of hemolysis, the cause of hemolysis should be investigated and documented. Procedures for handling and reporting hemolyzed specimens should be available, including appropriate rejection criteria. As a rule of thumb, when most or all specimens from a patient are hemolyzed, *in vivo* hemolysis should be suspected and reported to the physician. When intermittent specimens are hemolyzed, it is likely to be preanalytical, *in vitro* hemolysis. *In vivo* hemolysis is relatively rare, accounting for only about 3% of all hemolyzed specimens (10), which is all the more reason to be vigilant.

Hemoglobin, which absorbs light strongly at around 550 nm, can cause an apparent increased analyte concentration when monitored near this wavelength. Bichromatic readings and sample blanking will minimize interference; however, the high background absorbance may still be problematic (13). Hemoglobin may also have “pseudo” peroxidase activity which may interfere with bilirubin measurement by diazonium methods (3). Artfactual release of red cell constituents will have obvious effects on the accuracy of serum concentrations, especially K, Mg, P, LDH, AST and ALT (10); AST activity is approximately 40-fold higher in erythrocytes than in plasma, so that even slight hemolysis can alter results (10). Potassium is typically 25 times more concentrated in red blood cells than in plasma. Adenylate kinase released from red cells can increase creatine kinase (CK) activity (10), although the addition of AMP or other analogs can inhibit adenylate kinase (11). Although most phosphate in red blood cells is organic, it can stimulate the release of inorganic phosphate in serum (6). It is inappropriate to use hemoglobin to assess interference from hemolysis because only the direct effect of hemoglobin is evaluated. The effect of dilution and other red cell components should be considered, as well. CLSI therefore recommends interference testing by spiking hemolysate into a non-hemolyzed serum pool (14).

Various techniques have been used to reduce or correct for hemolysis interference. Deproteinization by ultrafiltration or precipitation can remove hemoglobin but cannot correct for the release of intracellular contents. Sample blanking and bichromatic measurement will decrease the absorbance effect of hemoglobin but, again, will not correct for the release of intracellular contents. It is critically important that the laboratory have a clear understanding of the effect of hemolysis on each laboratory test and clearly know when a test result cannot be accurately reported.

Lipemia

Lipemia is best defined as turbidity in a sample caused by elevated triglycerides, mostly as chylomicrons and very low density lipoproteins (VLDL), visible to the naked eye and caused by recent dietary fat intake, abnormal lipoprotein metabolism, the infusion of lipids (parenteral or enteral feeding), and heparin therapy. Interference from lipemia is due to light scattering or absorbance or, with severe lipemia, volume displacement (9). Turbidity is visually evident with triglycerides above about 300 mg/dL. Chylomicrons, because they are larger and more triglyceride-rich, are the more common source of turbidity, while VLDL contributes much less (15). Other causes of turbidity include elevated proteins (monoclonal gammopathies) and cold agglutinins.

Lipemia interferes with almost all spectrophotometric measurements by absorbing and scattering light. Sample blanking and bichromatic measurements help but do not eliminate this interference. Triglyceride-rich lipoproteins in large concentrations also have a volume depletion effect, whereby the apparent concentration of analyte is decreased because lipoproteins replace the volume of available water. In other words, the volume taken up by lipoproteins is included in the analyte concentration. With immunoassays, the analyte may not be accessible to antibody, resulting in falsely low results.

For interference testing, lipemia is often simulated using Intralipid[®], an emulsion containing 20% soybean oil, 1.2% egg yolk phospholipids, 2.25% glycerin, and water. Unfortunately, the photometric response to this synthetic “fat” differs from physiological lipemia, making it inappropriate to use Intralipid[®] for interference studies (16). It is far more accurate to assess lipemia using intact human triglyceride-rich lipoproteins (15) as lipids are far more complex than Intralipid[®]. CLSI recommends use of a high-triglyceride serum sample to evaluate lipemia interference (14).

To prevent lipemia it is advisable to request fasting specimens (8-12 hour fast) and to halt parenteral feedings for 8 hours prior to specimen collection. If a turbid sample is received in the laboratory and the requested test(s) are affected, it is possible to remove lipoproteins by centrifugation. Other approaches such as extraction of lipids with organic solvents, precipitation of lipoproteins with polyanions, cyclodextrin, or polyethylene glycol, and delipidation with detergents, lipase, and deoxycholate, have all been suggested. It is important to document the process used to remove the turbidity from the sample and to remember to measure cholesterol, triglycerides, and apolipoproteins before centrifugation or other means of lipid removal. Laboratories will also want to ensure that their process to eliminate lipid interference does not introduce additional errors. Although equations can be used to correct for volume depletion, the equations do not consider other interfering effects, such as light scattering, that may also introduce error.

Icterus

Icterus is another name for jaundice, but it also specifically refers to serum or plasma with elevated levels of conjugated or unconjugated bilirubin. Bilirubin occurs in serum as relatively insoluble free bilirubin, as water soluble conjugate (mono- and di-glucuronides), and covalently bound to albumin. Conjugated bilirubin is often seen in urine when present in high concentrations in serum. Unlike hemolysis and lipemia, icterus is not easy to detect visually. Icterus has been reported to interfere with many assays, including creatinine (Jaffe reaction), phosphate (molybdate), albumin (dye-binding), and assays based on oxidase or peroxidase reactions (glucose, cholesterol, triglycerides, and uric acid). Hyperbilirubinemia is relatively common in hospitalized patients (ICU, gastroenterology, surgical, pediatrics), making assessment of bilirubin interference, and choice of methods, especially important.

Bilirubin causes spectrophotometric interference because of its strong absorbance between 340 and 500 nm (3). Spectrophotometric interference is due to the fact that the absorption of bilirubin and the chromophores typically used for reaction with hydrogen peroxide overlap (17). It is important to evaluate interference from both conjugated and unconjugated bilirubin.

Possible solutions to the problem of icterus interference have been proposed, including use of different chromogens, longer wavelengths, and additives such as ferrocyanide (17, 18) or bilirubin oxidase (18). Unfortunately, none of the methods fully resolve the problem. The best guidance is for laboratorians to evaluate or understand the effect of icterus on their test systems and ensure that inaccurate results are not reported.

Proteins

Most protein interference is caused by paraproteins (monoclonal immunoglobulins) that may affect many different assays, including bilirubin, phosphate, HDL cholesterol, GGT, CRP, and glucose (19). The interference may be due to physical or chemical alteration in the sample or it may be due to a volume displacement effect. A very high protein concentration will, like very high concentrations of chylomicrons and VLDL, reduce the available water (9). The practical effect of these "solids" is volume depletion; the aspirated sample is diluted with protein so that the analyte concentration is artifactually low. As with lipemia interference, the use of equations to calculate for volume depletion is discouraged. The equations do not consider other interfering effects, such as light scattering, that may also introduce error and the mean specific volumes are estimates that can vary widely between individuals.

Viscosity is a common problem with specimens containing monoclonal immunoglobulins, especially IgM, and this may affect sample delivery.

Serum Indices

Visual inspection alone is not an effective method for screening for interferences that may be present in a sample. Serum indices are semi-quantitative estimates of hemoglobin, bilirubin, and lipemia (turbidity) in a sample using spectrophotometry. These indices are typically expressed in arbitrary units (+1, +2, etc) but can also be correlated to actual concentrations of hemoglobin, bilirubin, and triglycerides. Absorbances are recorded at several wavelengths to allow the calculation of the indices, and although they are similar, each manufacturer has a unique procedure. In general, we can assume that absorbance for turbidity is first monitored at wavelengths >600 nm. Then absorbance at ~450-575 nm is measured to assess bilirubin (icterus), and absorbance at 400-600 nm is used to assess hemoglobin interference. Algorithms are used to resolve overlapping areas and calculate indices. Jay reported clinically meaningful changes in AST, chloride, LDH, potassium, and sodium in specimens without visually apparent hemolysis (11). Although carotenoids and other pigments may interfere with this assessment, automated measurement of serum indices is necessary for an effective program (3). Manufacturers should encourage laboratories to use serum indices as part of their program to detect abnormal specimens.

Increase Understanding and Evaluation

The responsibilities of ensuring assay results are accurate falls on both the instrument/reagent manufacturer *and* the laboratory.

Manufacturers should provide specific details of their experiments including the materials used, the protocols followed and the levels of interferent and analyte tested. Manufacturers must provide a full description of the experimental design and statistical methods used to assess interference with each assay (5). When serum indices are listed, the procedure used to determine the indices should be clearly outlined.

Laboratories should verify how their instruments and assays perform in their environment, with their patient population, and compare that to the claims of their manufacturer. They must assess the quality and completeness of the data supplied by the manufacturer. There should be clear methods for identifying samples with interferences and procedures for dealing with affected samples. Labs must understand assay limitations and interference thresholds so that inaccurate results are not reported.

CLSI guidance document EP-7A outlines procedures for evaluating assays for interference and is intended for both manufacturers and laboratories. The recommendation includes an initial screening for interferences and then, if necessary, a dose response experiment to determine effect at different interferent concentrations.

Using the guidance outlined in the CLSI document, EP-7A (14), Sun Diagnostics has developed an interference test kit to aid manufacturers and laboratories in evaluating interference from

the substances described in this paper. ASSURANCE™ Interference Test Kit contains human triglyceride-rich lipoproteins for evaluation of **lipemia** interference; human hemolysate for evaluation of **hemolysis** interference; a mixture of human serum albumin and gamma-globulins to evaluate **protein** interference; and conjugated and unconjugated bilirubin to evaluate interference from **icterus**. The samples are highly concentrated (up to 20x; the CLSI recommendation) and contain no added stabilizers or preservatives so that the sample matrix is largely intact. Additionally, Sun Diagnostics has developed two very useful tools to accompany the interference test kit: a recommended testing protocol that clearly outlines the steps for both screening and dose response interference experiments and a data analysis spreadsheet that performs the calculations necessary to help determine if a substance is interfering with an assay.

With the availability of a kit, interference testing becomes more practical. Manufacturers and laboratories no longer need to find patient samples that fit their needs, store untested patient samples, or obtain artificial interferent substitutes, such as IntraLipid®. ASSURANCE™ contains stable, highly concentrated, purified and/or human-sourced materials that are representative of actual interferents encountered during testing. The human-sourced material is tested and found negative/non-reactive for infectious disease. The recommended testing protocols and data analysis tools can be worked into the routine procedure for interference testing. ASSURANCE™ Interference Test Kit from Sun Diagnostics will simplify interference testing.

References

1. Ji JZ, Meng QH. Evaluation of the interference of hemoglobin, bilirubin, and lipids on Roche Cobas 6000 assays. *Clin Chem Acta* 2011; 412:1550-1553.
2. Aslan B et al. Abstract A71: Clinical chemistry sample interferences reporting patterns in Ontario laboratories. *Clin Chem Supplement* 2012; 58(10):A23-A24.
3. Roche Diagnostics. Cobas analyzer (c501) handbook: Serum Indices: Reduction of clinical errors in laboratory medicine. http://www.roche-diagnostics.cz/download/program/Serum%20Indices_maly.pdf
4. Vermeer HJ, Thomassen E, de Jonge N. Automated processing of serum indices used for interference detection by the laboratory information system. *Clin Chem* 2005; 51:244-247.
5. Kazmierczak SC, Catrou PG. Analytical interference: more than just a laboratory problem. *Am J Clin Pathol* 2000; 113:9-11.
6. Glick MR, Ryder KW, Jackson SA. Graphical comparisons of interferences in clinical chemistry instrumentation. *Clin Chem* 1986; 32(3):470-475.
7. Glick MR, Ryder KW. Analytical systems ranked by freedom from interferences. *Clin Chem* 187; 33(8):1453-1458.
8. Lippi G e. al. Abstract: Haemolysis: An overview of the leading cause of unsuitable specimens in clinical laboratories. *Clin Chem & Lab Med.* 2008 46(6):764-772.
9. Dimeski G. Interference testing. *Clin Biochem Rev* 2008; 29 Suppl (i): S43-S48.
10. Thomas L. Haemolysis as influence and interference factor. *eJIFCC vol 13 no 4*: <http://www.ifcc.org/ejifcc/vol13no4/130401002.htm>.

11. Thomas L. Haemolysis as influence and interference factor. eJIFCC vol 13 no 4:
<http://www.ifcc.org/ejifcc/vol13no4/130401002.htm>.
12. Lippi G et. al. Abstract: Influence of hemolysis on routine clinical chemistry testing. Clin Chem & Lab Med. 2006 44(3):311-316.
13. Cook SL, Bruns DE. Persistent hemolysis in a patient with pancreatitis. Clin Chem 2012; 58:974-978.
14. CLSI, INTERFERENCE TESTING IN CLINICAL CHEMISTRY; APPROVED GUIDELINE, 2ND ED, CLSI DOCUMENT EP07-A2, WAYNE, PA, 2005.
15. Bornhorst JA, Roberts RF, Roberts WL. Assay-specific differences in lipemic interference in native and intralipid-supplemented samples. Clin Chem 2004; 50:2197-2201.
16. Nanji AA, Poon R, Hinberg I. Lipaemic interference: effects of lipaemic serum and intralipid. J Clin Pathol 1988; 41:1026-1027.
17. Spain MA, Wu AHB. Bilirubin interference with determination of uric acid, cholesterol, and triglycerides in commercial peroxidase-coupled assays, and the effect of ferrocyanide. Clin Chem 1986; 32(3):518-521.
18. Artiss JD, McEnroe RJ, Zak B. Bilirubin interference in a peroxidase-coupled procedure for creatinine eliminated by bilirubin oxidase. Clin Chem 1984; 30(8):1389-1392.
19. Artiss JD, Zak B. Problems with measurements caused by high concentrations of serum solids. Crit Rev Clin Lab Sci 1987; 25: 19-41.
20. www.westgard.com/basic-method-validation-3rd-edition-faqs.htm
21. Sun Diagnostics, LLC: www.sundiagnosics.us and www.sundiagnosics.us/assurance