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Endogenous Interferences in Clinical Laboratory Tests

Patient Safety

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Icteric, Lipemic and Turbid Samples

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To my wife Ellen and children Allison, Lauren and Jonathan.
Martin H. Kroll

To my wife Liesje and children Katie and Sam.
Christopher R. McCudden

Preface

Medicine has evolved to a new level, where not only is it expected that physicians diagnose and treat patients efficaciously, but also that all patients are protected from harm. Protecting patients from harm is part of patient safety and implies that the processes used in taking care of patients are free from error. Medical care depends on obtaining useful information from laboratory tests. Biochemical tests provide a great deal of information at relatively low cost and usually with rapid turnaround times. The achievement of low cost and rapid turnaround times depends, to a large extent, on the use of automation. The dependence on automation subsequently results in a diminution of individualized attention to each individual sample. To protect patient safety, laboratories need to establish detection systems to identify situations that could lead to biased results and rules to correct for the biased problems.

A bias occurs when the result obtained during an assay deviates from the true value of the analyte in question. A systematic bias occurs when there is an inherent problem in the measurement technique, as can occur with calibration errors and reagent deterioration. All samples are affected by a systematic bias. Interferences cause a non-systematic bias. Here, the bias occurs only for the individual sample. It is important to identify common features that occur for interferences, and to identify ways of not only identifying the interferences, but also of quantifying their impact.

For biochemical tests, especially those using serum or plasma as a matrix, a high concentration of bilirubin and turbidity can affect biochemical tests. The most common cause of turbidity is lipemia. The intent of this book is to provide a foundation for those running laboratories to identify, quantify and correct for the presence of hyperbilirubinemia and lipemia (turbidity). Because most laboratories will need to perform these processes in an automated fashion, the people working in the laboratory will need to design the appropriate procedures and to manage them.

To establish the necessary foundation to effectively design processes to manage the interferences caused by bilirubin and lipemia (turbidity), this book contains several different perspectives. The early chapters of the book provide information on the physical and chemical mechanisms involved in interferences. There is considerable emphasis on the interaction of bilirubin and lipemic particles with light, the most common form of energy used to detect clinical biochemical species. Additional chapters provide an emphasis on the clinical conditions where one might expect to encounter high concentrations of bilirubin or lipemia. The latter half of the book discusses means of detecting bilirubin or lipemia, as well as means to quantify their presence, to allow for appropriate reporting of results. Finally, the last chapter discusses means of characterizing and quantifying interferences in complex reactions,

as frequently occurs with bilirubin, where the analyte may interact with the analyte or species directly related to the concentration of the analyte. The intent of the book is to provide the laboratorian with sufficient background to deal with these interferences and protect patient safety.

November, 2012 Martin H. Kroll, MD

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1 Accuracy Goals for Laboratory Tests

It is often said that laboratory tests account for 70 % of the objective information used to diagnose and monitor patients. Even though it is true that a good history and physical examination provide a significant amount of information, physicians and clinicians, as well as nurses and other healthcare professionals, depend on laboratory test results to provide a final diagnosis, determine the degree of illness (the disease spectrum) and to monitor patients.

1.1 Accuracy and Precision

1.1.1 Definition

Accuracy of laboratory tests plays a vital role in health care, stipulating the quality and assuring patient safety [1]. Typical process steps that infringe on the quality of laboratory results and thus patient safety include patient misidentification, failure of reagents, mismanagement, and failure to communicate [2]. The accuracy of laboratory tests is critically important for achieving and maintaining quality in delivering good medical care. When the accuracy of laboratory tests is breached, the patient's safety is put at risk. Therefore, safe medical practice places a significant responsibility on the laboratory to maintain a high accuracy of test results. High accuracy of test results depends on good laboratory practice and includes such processes as Quality Control and Quality Assurance.

Accuracy is a generalized term. In the vernacular it may refer to how good the quality of the test result is from an analytical perspective. Theoretically, one judges the quality of the result arising from the laboratory by comparing it to a perfect method, i.e., a method without defect, for which one has obtained a perfect specimen and the reproducibility is perfect. The term Reproducibility is an ISO term [3] and refers to the closeness of the agreement between the results of measurements of the same measurand (analyte) carried out under controlled conditions of measurement. Essentially, the term Reproducibility refers to the precision of the measurement made for a particular analyte. The laboratory easily determines the precision of an analyte by determining values for control materials. On a day to day basis, the results obtained for any particular analyte for any control material will tend to a mean or average value. The typical scatter around this value will demonstrate a normal (Gaussian) distribution, and thus have a definable standard deviation (SD). Because results for any particular analyte may take on any value across the reportable range of the analyte, a standard deviation determined at a particular value for the given quality control material may not be directly applicable. To extend the precision measurements over the reportable range, one can use the ratio of the standard deviation to the mean of the quality control value and express it as a percentage. This ratio is called the coefficient of

variation and for most tests in Chemistry it ranges between 1 % to 10 %, depending on the analyte being measured and the magnitude of the value in the quality control material. The coefficient of variation provides a measure of the precision. Ideally, the clinician would like the precision, as measured by the coefficient of variation, to be as low as possible.

1.1.2 Imprecision as a Form of Error

Another way to think of precision is that it represents the closeness of agreement between independent measurements to each other. Of course, in the laboratory, in order to put structure into the analytical process, the laboratory develops rules to stipulate the conditions for performing the assay. Clinicians assume that all the values for laboratory tests that they receive have an extremely high precision. They presume that if they took a specimen and had the laboratory run that sample today, then if they gave the laboratory the same specimen tomorrow, they would receive exactly the same result.

The laboratory has to conduct itself with the knowledge that most clinicians are not expecting that there are going to be errors in results. For this reason, laboratories, and the people who manage them, spend a lot of time and effort in controlling the processes to minimize the errors generated by running laboratory tests. Precision, or in actuality, imprecision represents a non-systematic error. A non-systematic error is not part of the designed process of deriving a value from the collection and analysis of the specimen. Even though imprecision can be measured for the process, random error causes the deviations from the central value (central tendency). Random errors, though characteristic of the process, occur independently of one another. Even though the measure of a random error allows one to predict how the population of specimens will behave, one cannot predict for each individual specimen exactly what will happen. In order to be able to predict exactly what will happen to each individual specimen, one needs to examine the systematic errors.

1.2 Types of Error

1.2.1 Bias

Systematic errors are inherent in the process. Systematic errors are part of the process of measurement, that is, they are the result of the way the sample and reagent are mixed, the amplification of the detection system, and most importantly, how values are assigned to the readings generated in the sensing process. How values are assigned to the readings generated by the sensing process relates to the calibration of the method. The calibration of the method can be biased if the standards used for

calibrating the method are not properly assigned. Most methods in the clinical laboratory use calibrators instead of standards. Standards contain purified analyte dissolved in pure water or solvent of determined composition. Calibrators contain purified analyte or measured analyte dissolved in the matrix of the naturally occurring constituents comprising the environment of the samples used for testing. The matrix often is serum, plasma, or urine. Any of these matrices contains all sorts of unidentified and unspecified materials, typically protein, lipids, and organics. Typically the laboratories making the calibrators will control the concentration of the electrolytes and some of the organics. What makes a matrix material different from a standard is the analyte of interest plus other analytes are bound or complexed with naturally occurring constituents. The naturally occurring constituents may alter the way the analytical method interacts with the analyte of interest, altering the signal from the sensor.

Testing and assigning values in the laboratory are separated into three phases: the pre-analytic, analytic and post-analytic phase. The pre-analytic phase includes preparing the patient to obtain the specimen, collecting the specimen into an appropriate container (often with an anti-coagulant for blood), labeling and transporting the specimen to the laboratory and processing of the specimen to present it to the analyzer. The post-analytical phase includes communicating the value for the test result to the clinician. The analytical phase includes physically introducing the specimen into a reaction vessel, chemically or biologically reacting the specimen with other materials, physical interaction with some form of energy to produce a signal, and translation of that signal into a number or value that can be communicated to the clinician.

In the analytical phase, calibrators do not always translate the signal into exactly the same set of values that a purified standard would. The mistranslation results in a systematic error. Systematic errors can be separated into two types of error, based on how they relate to the underlying true concentration. If the error, for example for creatinine, were high or low and did not depend on the value for creatinine over the entire range of results, then the error is *constant*. To illustrate the *constant* error, take a value of 115 μmol/L of creatinine. If there is a constant error or bias of 27 μmol/L, then the reported value would be 88 μmol/L instead of 115 μmol/L. Further, if the true value of creatinine were 71 μmol/L, then the reported value would be 44 μmol/L; and if the true value of creatinine were 398 μmol/L, then the reported value would be 371 μmol/L. The deviation from the true value would always be the same. What differs in the error for each of these examples is the percentage of error that occurs. For the 115 μmol/L the percentage error is a negative 23 %, for the 71 μmol/L, the percentage error is a negative 37 % and for the 398 μmol/L of creatinine, the percentage error is a negative 7 %. The impact of a constant bias decreases with an increasing true value of the analyte. More important is the effect that the error has on the interpretation of the laboratory result. If the bias is negative and the true value falls within the reference interval and values below the reference interval have no clinical impact, then

the negative bias itself has no clinical impact. For a true value that exceeds the upper limit of the reference interval, if the negative bias causes the reported value to fall within the reference interval, then the interpretation would indicate that the patient does not have the condition implied by abnormal values. Thus, if the upper limit for creatinine in the reference interval were 106 μmol/L and the true value of the analyte was 115 μmol/L, a constant bias of –27 μmol/L would cause the reported value to be 88 μmol/L, which falls within the reference interval. The reported result would indicate that there is not a condition of renal dysfunction or impairment, which is classified as a false negative. At a creatinine concentration of 398 μmol/L, the clinician is already aware that the patient has renal dysfunction. If the physician receives a result of 371 μmol/L instead of 398 μmol/L, it would not change the assessment by the physician, because the interpretation of the test is that the patient has renal dysfunction and the interpretation of the test is unchanged by the creatinine result. These examples are typical of those used for the purpose of making a diagnosis.

1.2.2 Impact of Bias

In addition to making a diagnosis, clinicians use laboratory tests to monitor the disease or condition that the patient is experiencing. Here the situation is different, because the clinician has already made a diagnosis for the patient's disease or condition. The clinician is interested in whether the patient is getting better or worse, how well the therapy is working or predicting the course of the disease and giving a prognosis. The clinician may be observing the patient to follow the natural course of the disease, waiting until the patient crosses a particular threshold of disease severity or demonstrates enough change in their condition to indicate a time to institute therapy. If the clinician is waiting for the values reported from the laboratory to indicate that the patient has crossed into a more severe degree of their disease, then a constant bias may disturb the proper conclusion. If the constant bias is negative, and the clinician is waiting for the laboratory values to exceed a reference interval limit, then the patient's condition will exceed the limit before the reported laboratory values do. In such a case, the clinician may not institute therapy soon enough and may inadvertently postpone therapy. If the constant bias is positive, and the clinician is waiting for the laboratory values to exceed the reference interval limit, then the reported laboratory values will exceed the limit before the patient's condition truly does, and the clinician may institute therapy too early, potentially exposing the patient to risk from the therapy. If the institution of therapy is not warranted, because it is a false positive, then in addition to exposing the patient to the risk of therapy, the clinician may cause valuable resources to be expended when they are not needed. In a cost-conscious world, expending resources when they are not required results in a waste of resources, which potentially can risk the safety of the entire patient population, because abuse of resources may prevent the use of resources for another patient.