Article:
Rajeevan Selvaratnan and Amy B. Karger.
The Importance of Verifying Reference Intervals for Calculated Results.
J Appl Lab Med 2017;2:466-8.
http://jalm.aaccjnls.org/content/2/3/466

Guest: Dr. Rajeevan Selvaratnam is a clinical chemist in the Department of Laboratory Services at BayCare Health System in Clearwater, Florida.

Randye Kaye: Hello, and welcome to this edition of "JALM Talk" from *The Journal of Applied Laboratory Medicine*, a publication of the American Association for Clinical Chemistry. I'm your host, Randye Kaye.

In clinical laboratories, reported results have reference intervals appended to them in order to aid the clinician in interpreting the result in the context of a healthy population. If an assay is purchased from a manufacturer, the manufacturer establishes reference intervals during method validation and clinical laboratories verify that these ranges are applicable in their patient population. However, if a result is derived from a calculation, manufacturers are not required to provide a reference interval. Therefore, laboratories that report calculated results have to establish their own reference intervals.

A Technical Tips article titled "The Importance of Verifying Reference Intervals for Calculated Results" published in the November 2017 issue of JALM, discusses the common ways in which laboratories establish reference intervals for calculated results. Further, it contains recommendations as to the best practice for verifying these reference intervals in the patient population that they serve. The first author of this article is Dr. Rajeevan Selvaratnam, clinical chemist in the Department of Laboratory Services at BayCare Health System in Clearwater, Florida, and he is our guest for today's podcast.

Welcome, Dr. Selvaratnam. Your article sheds light on the importance of verifying reference intervals of calculated results such as the anion gap or globulin fraction. How can labs ensure that the reference intervals of calculated parameters are verified or validated?

Dr. Selvaratnam: Before we can address this question, I think as laboratorians, we need to first understand what calculated parameters or results are being reported in patient charts, and where these calculations are being performed. For example, it could be that your middleware performs the anion gap calculation and your laboratory information system, LIS, performs various other calculations such as

iron saturation. So, when we have a mix of both the middleware and LIS performing various calculations that are ultimately reported to patient charts. My recommendation is that one should work with the information services department and the manufacturer of the chemistry analyzer to extract, identify, and review all calculations that are charted in patient reports.

For each of those calculations that are reported to patient charts, the lab needs to ask at least two questions. One, are those reference intervals valid? And two, have they been verified? There are of course some calculations where the reference intervals are medical decision limits established by national organization such as for estimated glomerular filtration rate derived from creatinine measurements. However, for the anion gap, albumin to globulin ratio, A/G ratio, calculated serum osmolality and other calculations, there are no guideline-based values. Therefore, the clinical laboratory has the responsibility to verify or establish appropriate reference intervals for these calculated parameters specific to the method that the laboratory employees and the demographics that the laboratory serves.

It's easy to overlook the importance of verifying reference intervals of calculated results. But if these results are being reported in patient charts, we must provide the end users with an accurate and meaningful interpretation.

Randye Kaye: So, I noticed you said it's easy to overlook the reference intervals of calculated results. So, why is it that clinical laboratories may overlook the need to verify or establish the reference intervals of calculated values?

Dr. Selvaratnam: I think there are several reasons why reference intervals for calculated parameters may be overlooked. One, when labs transition to a new chemistry analyzer, they are well aware that all new tests must have reference intervals verified or established. In terms of new tests, laboratories often focus only on measurable analytes and not calculated derivations such as the anion gap or globulin fraction. So, during the transition to a new analyzer, the laboratories strive to meet the regulatory requirements for reference intervals of measurable analytes but overlook or may not recognize the need for verification of calculated results.

Additionally, it may be incorrectly assumed that a successful verification of reference intervals of the individual components is the same as the verification of the reference interval for the calculated result itself. However, our experience told us otherwise. We found that errors for individual analytes may be small enough to allow them to pass each individual analyte's reference interval verification

but these small errors may propagate into a large, clinically significant error in the final calculation due to the added of effect.

Another reason for overlooking these calculated results is that regulatory requirements by accrediting agencies such as the College of American Pathologists, CAP, which recently released the revised checklist requirement in August 2017, still do not explicitly indicate the need to verify or establish reference intervals for calculated parameters. Therefore, by default, the master activity menu does not list common calculated results such as the anion gap. Laboratories relying strictly on the activity menu for documentation requirements, may only have documents of established or verified reference intervals for measurable analytes and may overlook the documentation needed for calculated results. This has been my experience during inspections at labs accredited by CAP. A further reason a lot may overlook this calculated results is that manufacturers may not provide reference intervals such as for globulin fraction. Therefore, the lab is left to establish or verify an adopted reference interval from reference textbooks or other sources.

Randye Kaye: Thank you, doctor. Now, your article also brought attention to globulin fraction and A/G ratio. So, what can you tell us about these calculated results?

Dr. Selvaratnam: The globulin fraction and albumin to globulin ratio, A/G ratio, are essentially free results that the laboratory can provide when both total protein and albumin are ordered, for example with the comprehensive metabolic panel. The globulin fraction which is an estimated difference between serum total protein and albumin measurements is not universally reported by labs, but can be useful in identifying the presence of paraproteins. Additionally, with an appropriate cutoff, globulin fraction can be used to detect primary and secondary antibody deficiency. The calculated globulin fraction is also used for secondary calculations to determine the A/G ratio, a nonspecific indicator of a disease state based on normalization of the albumin by globulin content.

What's important to remember in these calculated results is that serum albumin and total protein methods are not harmonized or standardized. Therefore, reference intervals will differ depending on methodology. For example, we know that there are significant differences in serum albumin results measured with immunochemical methods, methods using bromocresol green dye, and bromocresol purple dye. A change in albumin method would be expected to impact a reference interval for the calculated globulin fraction and A/G ratio. Similarly, given the variation in total protein methods, a change in total protein quantitation methodology

would impact the reference interval and interpretation for the calculated globulin fraction in A/G ratio.

Randye Kaye: So, let's talk about a more familiar calculation that you mentioned in the publication. The anion gap isn't old but a useful calculated result. Clinical laboratories can refer to reference intervals from various textbooks and literature sources. What should labs know about the reference intervals of anion gaps?

Dr. Selvaratnam: You are correct on the usefulness of the anion gap. However, as with any test, the usefulness or clinical utility is dependent on having accurate and appropriate reference intervals. Otherwise, you may hear from savvy clinicians as a result of flagging patients incorrectly at an unusual frequency. In fact, a call from one of our key nephrologists alerting us to an increase in low anion gaps is what prompted us to reevaluate the reference interval for anion gap.

As an initial first step, we simply looked retrospectively at large data sets covering the 95th percentile distribution before and after implementing a new analyzer. Of note, our old analyzer measured electrolytes with direct ion selective electrode whereas our new analyzer was equipped with an indirect ion selective electrode. So, there was a change in methodology. This retrospective analysis was useful in telling us that within a month of transitioning to a new analyzer, the median anion gap values had fallen.

So, labs can use retrospective analysis using a large patient population to compare patient distribution between methods as an initial assessment of reference intervals. Due to our investigation and validation of the anion gap reference interval, we lowered the anion gap reference interval from 6 to 17 millimole per liter to 3 to 14 millimole per liter. The 6 to 17 millimoles per liter reference interval worked well with the direct ion selective electrode method we had previously, and is similar to what was found in common reference textbooks. However, anion gap reference intervals adopted from textbooks or literature sources may not address the variability and methodology.

For example, consider the most recent 2016 publication of "Henry's Clinical Diagnosis and Management by Laboratory Methods, 23rd edition" and "Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 5th Edition" published in 2012.

These reference textbooks list the reference interval for anion gap as 8 to 16 millimoles per liter. The most recently released 6th edition of "Tietz Textbook of Clinical Chemistry Molecular Diagnostics" in 2017 suggests the limits of anion

gap should be lowered to 6 to 14 millimoles per liter based on new indirect ISC methods for electrolyte measurement. However, these proposed reference intervals may still not be ideal for your laboratory. Our experience and recommendation is independent verification of the anion gap reference interval, optimized for your methodology and the population that the laboratory serves.

Randye Kaye: Fantastic! Thank you so much for joining us today, doctor.

Dr. Selvaratnam: You're very welcome. Thank you for having me.

Randye Kaye: That was Dr. Rajeevan Selvaratnam from BayCare Health System, talking about the JALM Technical Tips article, "The Importance of Verifying Reference Intervals for Calculated Results" for this podcast. Thanks for tuning in for "JALM Talk." See you next time and don't forget to submit something for us to talk about!