Clinical Chemistry

Effect of Dried Blood Spot Quality on Newborn Screening Analyte Concentrations and Recommendations for Minimum Acceptance Criteria for Sample Analysis

Article: Roanna S. George and Stuart J. Moat.
Effect of Dried Blood Spot Quality on Newborn Screening Analyte Concentrations and Recommendations for Minimum Acceptance Criteria for Sample Analysis.
Clin Chem 2016;62:466-475.
http://www.clinchem.org/content/62/3/466.abstract

Guest: Dr. Roanna S. George is from the Wales Newborn Screening Laboratory and the Depts of Medical Biochemistry, Immunology, and Toxicology at the University Hospital of Wales in Cardiff.

Bob Barrett: This is a podcast from *Clinical Chemistry* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett.

Newborn screening is performed in many countries to allow early detection of conditions that are either life threatening or can cause a clinically significant adverse outcome if left untreated. The number of disorders screened has also expanded substantially in recent years. Dried blood spots on paper collection devices have been used for this purpose since the early 1970s because the sample is easy to collect and transport and the sample volume required is relatively small. However, there is a lack of evidence regarding minimum blood spot quality acceptance criteria for sample analysis.

The March 2016 issue of *Clinical Chemistry* published the paper titled "Effect of Dried Blood Spot Quality on Newborn Screening Analyte Concentrations and Recommendations for Minimum Acceptance Criteria for Sample Analysis." The lead author of that paper is Dr. Roanna George from the Wales Newborn Screening Laboratory and the Departments of Medical Biochemistry, Immunology, and Toxicology at the University Hospital of Wales in Cardiff. She is our guest in this podcast.

So, Dr. George, why did you undertake this study? Why was this an important topic for you?

Dr. Roanna George: In the UK, the performance of newborn screening programs are monitored using what we call avoidable repeat rates. What this means is effectively cards that aren't of acceptable quality or have problems with the cards such as incorrect demographics or details on the card, then we would reject these. However, these avoidable repeat rates are termed as avoidable repeats because they are not routinely required, so there's some form of error that has happened with the card.

During discussions at UK level, we did note that actually, newborn screening laboratories are accepting and rejecting samples of very different quality. The reason for this was

that there is lack of scientific evidence that define what criteria was actually acceptable. This study therefore was carried out in order to determine if sample quality does indeed have an effect on newborn screening results. And if it did, to provide an evidence base to set the acceptance criteria that could be used at the UK level.

So this is important in order for us to set defined criteria to allow standardization of practice. And the standardization of practice therefore will prevent confusion amongst sample takers as to what constitutes an acceptable sample, because midwives at transfer areas, samples taken at transfer areas, often found that they were having samples of different quality accepted and rejected. And more importantly, it showed avoidable repeat rates can be comparable between UK Laboratory which allows us to fully compare performance within the laboratories in the UK Screening Program and hopefully we'll get defined, and the correct, outcomes from the babies that are being screened according to the sample quality that is now deemed to be acceptable.

Bob Barrett: Screening for PKU in the UK started in the late 1960s and CHT in the 1980s. Would there have been an effect on screening outcomes for these conditions?

Dr. Roanna George: Okay. In classical PKU, the phenylalanine concentration is generally significantly higher than the cutoff that we have set. So our referral cutoff in our current newborn screening program in the UK is 240 micromoles per liter. But in children with the classical condition, they tend to have results which are around a thousand micromoles per liter. And because these results are significantly higher than the cutoff, the biases I found with different sample qualities in the study would not have an effect to cause those results to fall below the screening cutoff. So it's highly unlikely that a case of PKU in the UK would have been missed.

In terms of congenital hyperthyroidism, we currently have two cutoffs in use in the UK Screening Program. Here in Wales, if the TSH concentration is between 10 and 20 milliunits a liter, then a repeat sample is requested. In this case, the case will be defined as borderline positive. A repeat sample is then analyzed and the child will be referred as THT positive if the result is greater than 10 milliunits per liter. Because a repeat sample is being requested, it's quite unlikely that you'll have two poor quality samples previously. So therefore, the chances of a case would not have been missed.

Furthermore, there is debate regarding the clinical significance of the cases with results near to these cutoffs because at the classical PKU, the classical congenital

hyperthyroid cases tend to have TSHs that are much, much higher than this cutoff.

Screening has expanded in the UK since 2009 and these additional disorders are what prompted the investigation of sample quality because these conditions, particularly MCADs, MSUD, and homocystinuria may have results that are closer to cutoff in babies with these disorders.

One such case was outlined in my paper. A case that was picked up in Wales where the C8 in the MCAD was not 0.55 micromoles per liter, which is only just above of the clinical referral cutoff of 0.5 micromoles per liter. And therefore with these expanded conditions, you are at risk of missing a child with a condition if the sample is not of appropriate sample quality.

Bob Barrett: Many countries screen for a larger number of disorders than the UK. Does the defined criteria apply to all screening tests?

Dr. Roanna George: This criteria will apply to all screening tests for which a screen positive result is one that is defined as being above a particular cutoff. So in the UK, the conditions that we screened for and the conditions that were tested for in the study, all of the results are screened positive if they are above a certain cutoff. However, some screening tests that are used worldwide, such as that for biotinidase deficiency will have a screen positive result as one that is below a designated cutoff.

In this case, the criteria outlined in this study cannot be applied because in these cases, you would need to avoid false-positive results to ensure that cases are not missed which is the opposite from results where you're looking for a raised—a result above a defined cutoff. Therefore, it's likely that studies would show that layered samples which we would accept in our current UK program would not be accepted because you have a risk of missing cases in those scenarios. However, this would need to be backed up using scientific data for these conditions.

Bob Barrett: Well, let me ask you this. Do you believe any future disorders should be added to the UK program? And if so, would this study need to be repeated for each additional disorder?

Dr. Roanna George: As alluded to in the editorial, if anyone analyzed in a panel is affected by poor sample quality, then the sample should be rejected for all tests. So, as is the case, all the results are very similar for all the conditions that we screen for in the UK that if we added on a sample, a new condition to a screening program, then we now have to find criteria that

sets out how exactly what sample quality we require. Because pilot studies are carried out before any new disorders are added to a newborn screening panel of tests, which includes setting to find analytical and screening cutoffs, these studies will be carried out using the defined quality of a full filled circle evenly saturated by one drop of blood that are now in place secondary to the introduction of UK sample quality guidelines that we have in place. So the method would then be validated using samples of this quality. Therefore, you wouldn't need to particularly validate every single test that came in because this will be done as part of the pilot study.

The exception would be for layering effects, if a layering effects are the result. For example, if a condition was introduced where result is below the defined cutoff I've described earlier, if this was added to the panel, then this would need to be potentially validated.

Bob Barrett: Finally, are there any wider implications to this study?

Dr. Roanna George: Yes. This study has implications for any assay that uses dried blood spots as the sample type, this will include assays that are for monitoring and diagnostic purposes where the criteria of acceptable blood spots will be different to that for screening. As such, each individual assay should be validated to determine the effect of sample quality on these assay results, and hence, which sample quality is therefore acceptable for the assay in question. As an example, we monitor phenylketonuria and maple syrup urine disease patients using dried blood spots that they take at home and then send them to the laboratory themselves.

We would follow our screening criteria, but we would also need to reject layered samples which are those samples that we found to cause a false positive result. So those would not be rejected for screening, but they would be rejected for monitoring for these purposes. Because a positive bias may cause the patient's diet to be more strict than it needs to be based on the dietician trying to restrict the phenylalanine intake further on the basis of this incorrect result.

So therefore, any assays that use dried blood spot needs to be validated individually to determine the effect of different sample quality on the analytical results. From there, assay-specific criteria can be set which will take into account the required assay performance or the acceptable assay performance for these tests that use dried blood spots.

Bob Barrett: That was Dr. Roanna George from the Wales Newborn Screening Laboratory at the University Hospital of Wales in Cardiff. She's been our guest in this podcast from *Clinical*

Chemistry on Acceptance Criteria for Dried Blood Spot Quality in Newborn Screening.

I'm Bob Barrett, thanks for listening!