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On the cover this month: Solving the Rhesus (Rh) factor equation. There are two answers to the equation, one of which can have clinically important ramifications. Individuals who carry the D antigen on their red blood cells are considered Rh-positive, while those who do not carry the D antigen are Rh-negative. If a father is Rh-positive and the mother is Rh-negative, the fetus can be either Rh-positive or negative, yet only an Rh-positive fetus can create incompatibility issues between the mother and fetus. Thus, it is important to correctly solve the Rh equation so that unnecessary and expensive prophylactic therapy can be avoided. Since the discovery of cell-free fetal DNA in maternal plasma, noninvasive prenatal testing for Rh status is now possible. This issue of *Clinical Chemistry* contains the results of a study comparing digital PCR and real-time PCR for both fetal sex and Rh genotyping.

DMSO Increases Mutation Scanning Detection Sensitivity of High-Resolution Melting in Clinical Samples

By Chen Song, et al.

This paper demonstrates that simple addition of dimethyl sulfoxide within a PCR product can help boost the sensitivity of mutation detection during subsequent high-resolution melting. The authors present evidence based on serial dilutions of mutation-containing cell lines, as well as detection of mutations in clinical samples. Since high-resolution melting is a most commonly used method for mutation detection, the simple approach presented here should be widely useful in boosting mutation detection sensitivity in cancer samples.

Tandem Mass Spectrometry Has a Larger Analytical Range than Fluorescence Assays of Lysosomal Enzymes: Application to Newborn Screening and Diagnosis of Mucopolysaccharidoses Types II, IVA, and VI

By Arun Babu Kumar, et al.

This paper concerns enzyme assays for newborn screening of lysosomal storage diseases. The authors compared two methods, tandem mass spectrometry versus fluorescence with 4MU-substrates. Data showed that the tandem mass spectrometry method substantially outperformed the fluorimetric assays in terms of analytical range, which led to a lower number of false positives. The authors applied the mass spectrometry technique to the development of new assays for the newborn screening of mucopolysaccharidoses II, IVA, and VI.

Rapid, Fully Automated Digital Immunoassay for p24 Protein with the Sensitivity of Nucleic Acid Amplification for Detecting Acute HIV Infection

By Carlos Cabrera, et al.

This paper introduces automated digital immunoassay technology to infectious disease testing, specifically acute HIV detection. The authors developed a rapid immunoassay for the p24 capsid protein of HIV and demonstrated analytical and clinical efficacy by measurement of HIV in the earliest infections detectable by nucleic acid testing, the current gold standard for sensitivity. The data established the digital immunoassay to be as sensitive as nucleic acid testing for detection of acute HIV infection. A simple digital immunoassay for HIV could have important implications for HIV screening, particularly in clinical and lower resource settings where adoption of nucleic acid testing is impractical.

Obese Nondiabetic Pregnancies and High Maternal Glycated Hemoglobin at Delivery as an Indicator of Offspring and Maternal Postpartum Risks: The Prospective PEACHES Mother-Child Cohort

By Regina Ensenauer, et al.

It is unclear whether obese pregnant women negative for gestational diabetes still experience dysglycemia relevant to offspring and long-term maternal outcomes. Data analysis of 462 gestational diabetes-negative pregnancies revealed a substantial prevalence of gestational dysglycemia in obese women, as evidenced by a high hemoglobin A_{1c} at delivery. This finding was associated with high birth weights and cord-blood C-peptide concentrations as well as maternal signs of persistent dysglycemia and low-grade inflammation later postpartum. These data should raise awareness as to careful monitoring of obese pregnancies. Measurement of hemoglobin A_{1c} at delivery could help to select women who may need closer postpartum health checks.

Demonstration of the Pre-Emergency Use Authorization Path Using 3 Minor Groove Binder-Hydrolysis Probe Assays to Detect *Escherichia coli* O104:H4

By Laurie J. Hartman, et al.

This paper describes a case study documenting a pre-emergency use authorization process conceived collaboratively between the Department of Defense and Food and Drug Administration In Vitro Diagnostics for pre-disposition of validation data. Through this Department of Defense and Food and Drug Administration In Vitro Diagnostics and Radiological Health collaboration, the authors show the necessary information for the development process as well as necessary steps for validation of the assays for clinical use. The paper provides the basic steps and format for submission of pre-emergency use authorization approval to the Food and Drug Administration. This manuscript provides useful information for readers who would consider the pre-emergency use authorization or emergency use authorization process for emerging infectious disease clinical assays.

Fetal Sex and *RHD* Genotyping with Digital PCR Demonstrates Greater Sensitivity than Real-time PCR

By Kelly A. Sillence, et al.

For over a decade noninvasive prenatal diagnosis of fetal sex and RHD genotype has been routinely available using qPCR assays. However, false negative results still occur largely due to insufficient assay sensitivity. This small-cohort study compared the sensitivity of the qPCR approach compared to a digital PCR assay. The study findings show that in circumstances where qPCR was unable to detect fetal-target DNA, digital PCR performed effectively using identical samples. In addition, the study determined that the sample collection tube significantly affected the cell-free fetal DNA fraction obtained. Routine testing using digital PCR could potentially remove the risk of false-negative results.

Variant Profiling of Candidate Genes in Pancreatic Ductal Adenocarcinoma

By Jiaqi Huang, et al.

There is no reliable method for assessment of tumor variant profiling using samples with low tumor density. In this study, the authors used an Anchored Multiplex PCR method to characterize candidate gene variant profiles and implications in carcinogenesis and prognosis of pancreatic ductal adenocarcinoma. The results indicate that the Anchored Multiplex PCR-based next generation sequencing method is a reliable platform for accurate variant profiling of pancreatic ductal adenocarcinoma, and can detect mutations as low as 1% given sufficient sequencing depth. Using this method, the authors demonstrate that, in patients with pancreatic ductal adenocarcinoma, the *KRAS* mutant subtype G12V may be associated with a worse survival and transversion variants are more common among smokers.