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Generation of Highly Biomimetic Quality Control Materials for Noninvasive Prenatal Testing Based on Enzymatic Digestion of Matched Mother–Child Cell Lines

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Guests: Drs. Jinming Li and Rui Zhang of the Department of Immunoassay and Molecular Diagnosis at Beijing Hospital.

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

Non-invasive prenatal testing based on cell-free DNA is now a widely used technique. However, quality control materials that have properties identical to clinical samples and that are applicable to a wide range of procedures are not available to support assay development, internal or external quality control, and proficiency testing.

The June 2019 issue of *Clinical Chemistry* includes a study describing the development of such quality control materials that comprise simulated human plasma and mixtures of mother cell line derived cell-free DNA based on DNA fragmentation factor digestion.

Today, we are joined by two authors of that study, Dr. Jinming Li serves as Associate Director of the National Center for Clinical Laboratories and Director of the Department of Immunoassay and Molecular Diagnosis at Beijing Hospital.

He is joined by his colleague, Dr. Rui Zhang, who is Associate Professor in the same department. And we will start with you, Dr. Li. Before we get into the generation of quality control materials, how are they usually produced and used? And how do quality control materials for non-invasive prenatal testing differ from other types of laboratory testing?

Dr. Li: Quality control materials have one or more well-established properties which are homogenous and stable and fit for measurement. For NIPT, quality control materials are homogenous and stable samples of normal chromosomal abnormalities with different fetal fraction. Quality control materials are used by clinical laboratories for assay development, assay validation, internal quality control, and proficiency testing to assure quality of their performance.

As we know, the development of a new drug usually requires *in vitro* models such as cell lines, animal models to demonstrate the effects and side effects, and then clinical

trials to approve the validity in patients. Diagnostic assays are somewhat similar. For NIPT, is the established method accurate and reliable, including the steps of blood collection, transport, storage, cfDNA extraction, and analysis? Quality control materials with known results need to be analyzed before the assays are used in clinical testing. It is essential to demonstrate the diagnostic performance, including precision, accuracy, sensitivity, and specificity. If the performances are not satisfactory, the assays should be adjusted. This process is assay development and assay validation. Quality control materials are also required for tests to monitor the reliability of each batch in daily detection. Errors may occur in all the procedures, especially during the handling of operator and the work of equipment.

Positive controls can confirm successful pipelines, such as cfDNA extraction, library construction and so on. Negative controls can identify contamination. This is the internal quality control. External quality assessment organizers distribute quality control materials to clinical laboratories, evaluate the detecting capacity of laboratories based on the degree of consistency between laboratory results and expected results, in order to promote awareness of causes and improvement. This process is also called proficiency testing.

Bob Barrett: So, the quality control is very important during clinical tests. Dr. Zhang, how did the idea of this novel quality control material come about?

Dr. Zhang: One of the main problems we encountered in NIPT standardization is the lack of proper quality control materials used for monitoring the performance over time. And apparently, a large amount of such characterized samples is needed for regular application in clinical practice. Whereas, currently the quality control materials for NIPT available include natural maternal plasma samples, and simulated samples based on fragmentation of human genomic DNA.

As we know, maternal plasma with a particular chromosomal abnormality is very limited, and shearing-based DNA products have dissimilar biological properties from those of circulating cfDNA, with differing characteristic size profile of circulating fragmented nucleosomes. Besides, the synthetic samples are not matched for mother-child relationships and therefore, do not allow single-nucleotide, polymorphism-based methods.

So, what can we do next? Many studies have revealed that the mechanism for cfDNA origins is related to apoptosis. Therefore, we envisaged if cfDNA could be generated through simulating the process of apoptosis. We then examined the mechanism of apoptosis and found that DNA

fragmentation factor prefers to cleave in the internucleosomal linker region, which generates a size distribution similar to maternal cfDNA.

Meanwhile, MNase produces mononucleosomes and then proceeds to trim the DNA of nucleosome monomers to the core particles with 146 base pair, it is similar to fetal cfDNA. By this way, we created materials based on paired mother–child cell lines, just as they would be in real NIPT samples.

Bob Barrett: Okay, that's very impressive. What quality control materials have your team designed before? For example, quality control materials and other tests related to molecular diagnostics.

Dr. Zhang: We have developed a set of cfDNA quality control materials based on MNase digestion and matched genomic DNA, which was published in *Clinical Chemistry* back in 2017. It is mainly used in liquid biopsy for cancer, aiming at somatic mutation detection, covering a wide range of oncogenic alterations ranging from single-nucleotide changes to rearrangements. Because MNase can digest the DNA between nucleosomes, it can produce a size profile analogous to cfDNA in patients with cancer.

The quality control materials meet the requirements of various ctDNA testing methods, including NGS. Using the quality control materials, we have provided 3 runs of proficiency testing in recent 3 years, and around 400 laboratories have participated in this scheme, which would be challenging if we depend on clinical plasma samples from patients with tumor. Laboratories have validated their methods and implemented internal quality control procedures with the materials, which is critical for ensuring laboratory quality and patient safety.

This exploration to create cfDNA quality control materials have laid a foundation for us to prepare simulated maternal plasma free DNA in this research. In addition, our team have generated quality control products for molecular testing. These include molecular oncology for solid tumors, hematologic oncology, pharmacogenetics, genetic testing, and microbiology. The materials cover biological samples and bioinformatics files for NGS. Accordingly, proficiency testing programs are also provided for clinical laboratories to improve their performance.

Bob Barrett: Finally, Dr. Li, what do you see in the future for your biomimetric quality control materials?

Dr Li: As for future applications, we think their potentials are not limited in fetal aneuploidy detection of NIPT, they're going to shine in other non-invasive areas as well. In a similar

approach, as previously described, the quality control product can also be applied to other NIPT-related fields, such as single genetic disease. We're not just talking about envisioning. We're now working in this direction and believe there will be new progress. Considering the scarce positive samples of noninvasive prenatal diagnostics for single gene disorders, the quality control materials created will play important role in promoting the detection in this field. Therefore, we hope to find solutions to the problems in this area as soon as possible to promote its faster clinical application, for the benefit of people's health.

Bob Barrett: That was Dr. Xiu-Min Li, Associate Director of the National Center for Clinical Laboratories and Director of the Department of Immunoassay and Molecular Diagnosis at Beijing Hospital. He was joined by his colleague, Dr. Ri Zhang, from the same institution. They have been our guests in this podcast on the development of a new type of quality control material for cell-free DNA testing. Their paper describing that approach appears in the June 2019 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening!