Developing a Fast and Sensitive qPCR Assay to Improve Field-Based TB Testing in Africa

Baylor College of Medicine's Andrew DiNardo depends on Quantabio's Q Cycler and PerfeCTa qPCR ToughMix to deliver high-quality data despite extreme temperature and travel conditions

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Working together in Africa.
From left to right: **Dr. Caitlyn Jasumback, Dr. Qiniso Dlamini, Dr. Tomoki Nishiguchi.**

With more than 1,674,000 related deaths around the world, including an estimated 250,000 children, Tuberculosis (TB) was the ninth leading cause of death and the leading cause from a single infectious agent in 2016. TB was also a leading killer of HIV-positive people, accounting for more than 40 percent of all HIV deaths around the world.[1] Because the mycobacterium tuberculosis infection attacks a weak immune system, individuals living with HIV or dealing with parasites, such as helminths, face up to a 20-fold increased risk of developing TB.[2] This is why African countries such as Tanzania and the Kingdom of eSwatini (formerly Swaziland) have some of the highest TB incidence rates in the world.

Andrew DiNardo, MD, Assistant Professor of Pediatrics in the sections of infectious disease and global and immigrant health at Baylor College of Medicine (BCM), has spent the past six years exploring the impact HIV and parasites have on TB epigenetic and immunology status. His research aims to better understand how parasitic helminths and HIV disturb cell-mediated immune response, and how epigenetic alterations increase the risk for developing TB.

Dr. DiNardo currently splits his time in Houston and testing for TB at the Baylor International Pediatric AIDS Initiative at Texas Children's Hospital (BIPAI) translational research laboratories across Africa. Established in 2005, BIPAI aims to provide high-quality pediatric and family-centered health care, health professional training, and clinical research, focused on HIV/AIDS, tuberculosis, malaria, malnutrition and other conditions impacting the health and well-being of children and families worldwide. BIPAI is the largest provider of pediatric HIV care in the world, treating more than 300,000 children in more than a dozen countries.[3]

Together with his BCM colleagues, Anna Maria Mandalakas, MD, PhD and Rojelio Mejia, MD, Dr. DiNardo is implementing a novel translational research method that isolates and analyzes DNA from stool samples to rapidly identify parasitic worms and TB infections by qPCR.[4] This non-invasive technique improves TB diagnosis, especially among children and people living with HIV who have trouble expectorating sputum, making it easier to screen patient samples and detect TB earlier before symptoms appear and when treatment options are more viable.

Quantabio recently caught up with Dr. DiNardo to discuss his ground-breaking translational research in Africa, the extraordinary challenges he faces in bringing molecular reagents to remote sites, and how the Q Cycler and PerfeCTa qPCR ToughMix combination is helping him maximize data quality, improve turnaround times and overcome common PCR inhibitors found in stool samples.

Developing a Stool-based qPCR Assay for Early TB Detection

Diagnosing HIV-associated or childhood TB in Africa has been challenging as sputum samples are often difficult to obtain in young children particularly in resource-limited settings. Our stool-based qPCR assay is a promising complement to the traditional sputum-based diagnosis.

The method originally came from Dr. Mejia who spent several years to optimize the conditions for DNA isolation from soil samples in order to identify helminths and protozoa. The team in Swaziland and Dr. DiNardo then adapted his method to TB and refined the protocol. With this screening method, we can isolate and amplify the DNA directly from stool samples to check for TB and 10 other gastrointestinal parasites by qPCR.

We now screen approximately 600 samples a year as part of this research study. After analyzing the data from our other clinical validation studies in Mozambique and Tanzania, we're confident that the technology is reproducible and works well. The stool qPCR assays are able to detect 95% of sputum culture-positive cases vs 40% to 60% reported in the literature by other stool techniques.

Durable to Withstand Hurricane Harvey and 38-Hour Trips to Africa

The projects in Africa require a robust qPCR solution that is portable, reliable and able to withstand temperature changes and rough travel conditions. Once I first heard about Quantabio's new portable Q qPCR Cycler and the PerfeCTa qPCR ToughMix, I wanted to bring it to Africa and test it out. However, the timing presented more challenges. We ordered the products and they were delivered to our BCM laboratory in Houston the day before Hurricane Harvey hit in August 2017. Due to the commotion caused by the storm, the packages ended up sitting on the loading dock at room temperature for more than a week. The company convinced me to still try the ToughMix, so I decided to bring the products with me on the 38-hour trip to the BIPAI TB Centre of Excellence lab in Mbabane, eSwatini, and then over to the facility in Mbeya, Tanzania (another 30-hour trip).

In the past, we have found traveling from Houston to Africa with the laboratory equipment and PCR master mixes can be a significant and expensive hurdle to our research efforts. The 30+ hour trip involves several forms of transportation and crosses several different climates. Even after packing the reagents in ice packs, we often would end up with degraded master mix due to the long distances and temperature changes.

Portable Plug-and-Play Platform Exceeds Performance Expectations

I couldn't believe how well the products traveled and performed in the field. The compact Q instrument (size of a miniature 4.5lb speaker) fit in my carry-on luggage and the Bluetooth connectivity made it extremely easy to setup and operate. The 25-minute run gave Ct curves identical to our existing, and much bulkier, real-time PCR machine that was already on site in Mbabane. I loved how we didn't have to sacrifice performance quality for speed or portability. You can really have it all in one device plus it eliminates the need for calibration, annual service and preventative maintenance. Considering its size, intuitive software, and near perfectly reproducible Ct curves, we've decided to make the Q our workhorse qPCR machine.

Meanwhile, the PerfeCTa qPCR ToughMix stood up to its name and produced beautiful Ct curves. We found it to be very stable and not sensitive to the temperature changes. Repeated freezing and thawing did not have any effect on the overall PCR performance and we have since generated high-quality reproducible data in Mozambique, Tanzania and eSwatini.

Potential to Transform TB Testing Around the World

We are now working on increasing patient access to this TB testing approach by enabling more peripheral laboratories, especially those that lack specialized equipment, to implement this same workflow anywhere in the world. The biggest hurdle to widespread adoption is the lack of an automated DNA isolation technique. We clearly need to develop an easier and higher-throughput technique to better align with the speed of the Q Cycler, which is capable of running up to 48 samples at a time in less than 25 minutes.

We're also looking at ways to detect genotypic resistance faster and easier than before. eSwatini is witnessing significant increases in drug-resistant forms of tuberculosis. The current culture and genotypic drug susceptibility testing take six to eight weeks, which is too long when dealing with deadly TB infections. When we figure out how to immediately get genotypic drug susceptibility testing straight from the sputum and stool, doctors should be able to provide patients with the most appropriate therapy in less than one week.

TB is a devastating disease that affects so many people and families around the world. We are going to keep working until we are able to provide more of them with the high-quality testing and treatment services they need.

The Quantabio products are for Research Use Only; not for use in diagnostic procedures.

References

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