Raphael Nyaruaba, PhD

Diagnostic Microbiology

Wuhan Institute of Virology, Center for Bio-Safety Mega-Science

Category: Respiratory Disease

About Dr. Raphael Nyaruaba

Raphael Nyaruaba, PhD in Microbiology, is a research fellow at the Wuhan Institute of Virology and serves as the Senior Medical and Technical Advisor to InVitro Vista. His research focuses on developing rapid molecular diagnostic tools for diagnosing infectious diseases like SARS-CoV-2 and tuberculosis, with a special focus on the applications of digital PCR for such diseases. He aims to introduce these techniques in Africa and other low-resource countries

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Dr. Raphael Nyaruaba's Key Publications

- Developing multiplex ddPCR assays for SARS-CoV-2 detection based on probe mix and amplitude based multiplexing
- Development and evaluation of a single dye duplex droplet digital PCR assay for the rapid detection and quantification of Mycobacterium tuberculosis
- Digital PCR applications in the SARS-CoV-2/COVID-19 era: a roadmap for future outbreaks

Impact of Droplet Digital PCR on Dr. Raphael Nyaruaba's Research

Droplet Digital PCR (ddPCR) has been a valuable asset in my research. In the beginning, we were looking for ways to sensitively detect small changes in Mycobacterium tuberculosis (MTB) DNA after drug sensitivity tests (DST). Tuberculosis diagnosis takes weeks to months to determine drug sensitivity due to slow mycobacterial growth. The often-used molecular diagnostic methods like qPCR, Gene X-pert, etc. are qualitative and cannot rapidly detect small changes in bacterial concentration after drug sensitivity tests. However, with Droplet Digital PCR, one can quantitatively detect small bacterial amounts in any organism. Additionally, prior researchers have already shown that this method could sensitively detect low bacterial loads compared to commonly used mycobacterial detection methods (as summarized in our review droplet digital PCR applications in the tuberculosis world). Hence, we conceptualized that the mycobacterial slow growth can be determined by a sensitive method like Droplet Digital PCR. In a proof-of-concept article, we used Droplet Digital PCR to reduce drug sensitivity detection time from weeks to days (4 days). In addition, we also leveraged Droplet Digital PCR's higher-order multiplexing approach and developed an assay that could detect two stable MTB biomarkers (i.e., IS6110 and IS1081) within a single channel. The developed assay was more sensitive than qPCR in detection of patient samples. We are currently in the process of developing commercial assays for this purpose.

The SARS-CoV-2 outbreak highlighted the potential of Droplet Digital PCR in managing future pandemics as summarized in our review "Digital PCR Applications in the SARS-CoV-2/COVID-19 Era: A Roadmap for Future Outbreaks." When the SARS-CoV-2 outbreak started in Wuhan, there were no reference standards to evaluate our own in-house assays. Since we had a Droplet Digital PCR system, we used it to quantify viral loads from representative patient and cell samples. These reference samples quantified using Bio-Rad's QX200-Auto DG ddPCR system were used for relative quantification in RT-qPCR to determine viral loads in both patient and wastewater samples. In addition, we also showed that an inactivated whole-virus SARS-CoV-2 RNA reference standard quantified by RT-ddPCR can be used to evaluate the performance and detection limits of commercial RT-qPCR assays. Majority research also highlighted that RT-ddPCR was superior to RT-qPCR in detecting SARS-CoV-2 in patients with low viral loads. Most of them were mainly simplex to duplex assays (if no commercial multiplex assay was used). We sought to bridge this gap by publishing protocols on how to develop higher-order multiplex assays for SARS-CoV-2 detection using Bio-Rad's two-color ddPCR system. The multiplex assays were superior to RT-qPCR in detection of clinical samples with an extended application spectrum to drug sensitivity tests.

We continue to push the application spectrum of Droplet Digital PCR in the detection and diagnosis of infectious disease pathogens. This is important as Droplet Digital PCR's specificity, sensitivity, reproducibility, detection limits, and tolerance to inhibitors are generally unaffected by the common factors affecting most molecular diagnostic assays. Once the technique is adapted by many laboratories around the world, we believe that future pandemic monitoring would be performed using Droplet Digital PCR and the technique could eventually replace the currently used RT-qPCR as the gold standard for diagnosis.