Editorial Article

Molecular Diagnostics for Infectious Diseases in Low or Middle-Income Countries

Prof. Dr. Andreas Nitsche

Head of the Division
Highly Pathogenic Viruses
Centre for Biological Threats and Special Pathogens
Robert Koch Institute
Seestrasse 10, 13353 Berlin,
Germany

Why do we need molecular diagnostics?

Infectious diseases remain a leading cause of morbidity and mortality in Low and Middle-Income Countries (LMICs). Indisputably, molecular diagnostics is the key to a rapid and reliable identification of an infectious agent in clinical specimens. Unlike traditional techniques, such as microscopy and cell culture, molecular diagnostics can detect extremely low levels of pathogens within a short time and can provide additional information about the pathogen by looking at the genome.
The term molecular diagnostics encompasses various laboratory techniques that directly target the DNA or RNA genome of pathogen, the proteome of a pathogen, or in an indirect manner, the immune reaction of the infected system to a specific pathogen.

In a particular setting, molecular diagnostics can replace, support or confirm a clinical diagnosis and is specifically helpful for differential diagnosis in infectious diseases, that cross-present a high similarity in clinical symptoms.

What is the state of the art in molecular diagnostics?

The dominating molecular technique in infectious diseases diagnostics is the polymerase chain reaction (PCR). With the advent of the PCR in 1985 by Kary B. Mullis, it became an unrivalled method for the identification of a pathogen in any specimen. The biomedical community has witnessed the transformation of the PCR-method into a routine application in a time span of only about 10-15 years. One cornerstone of this progression represented the advancement of the original PCR method into the real-time PCR-based methods. The unmatched sensitivity, specificity, speed and the possibility to obtain semi-quantitative results in a safe high-throughput manner, have advanced the real-time PCR to the gold standard tool in infectious diseases diagnostics that it is today. Although real-time PCR was regarded as being comparatively costly in the early years, fortunately reagent costs have dropped over the past few years and the crucial investment is no longer the reagent cost itself but the laboratory equipment and infrastructure.

Stimulated by the idea of PCR, today, numerous amplification-based technical approaches have become promising candidates to improve the portfolio of a diagnostic lab, such as isothermal amplification approaches and next generation sequencing methods (NGS). Isothermal amplification techniques (IAT) require less expensive equipment, if any, and use also less costly reagents. In comparison to real-time PCR, the recently established IATs are significantly faster but cannot easily provide quantitative results. The advent and further development of NGS technologies revolutionized molecular diagnostics by enabling the identification of sequences of millions of DNA fragments simultaneously. The most important benefit of diagnostic NGS is the open view capability, that can help to also identify nucleic acids of hitherto unknown pathogens or pathogen variants. However,

**Correspondence: NitscheA@rki.de*

although the costs per sample are comparably low, as a consequence of the extraordinary high throughput, the investments to establish an operational NGS laboratory for diagnostic purposes, including reagent costs, equipment and informatic infrastructure, are considerably higher than for a PCR laboratory.

The COVID-19 pandemic recently highlighted the importance of molecular diagnostics in global healthcare. Beside the application of various rapid and accurate real-time PCR tests for SARS-CoV-2, including the development of multiplex PCR-systems to differentiate COVID from other respiratory infections, the game changer in managing the pandemic, was the timely access to portable point-of-care (PoC) tests. These PoC tests were in the majority targeting SARS-CoV-2 viral proteins with specific antibodies in a lateral flow system (LFA). Although LFA-based PoC tests were already available prior to the pandemic, for pathogens like influenza virus, their benefit for the individual patient as well as for the global healthcare system, has never been as significant as in the COVID pandemic, helping to identify potentially contagious persons without specific skills and for acceptable costs.

Beside these LFA tests, some nucleic acid amplification based rapid tests have been proven to be also user-friendly with a usually better sensitivity, but they require at least the use of a technical device and a power supply, restricting their use to regions of intact infrastructures.

Portable PCR devices that combine nucleic acid extraction and amplification in closed systems have been developed. They can be useful in replacing LFA rapid diagnostic tests in outbreak settings, nonetheless, they should be operated in a lab environment and are rarely suitable for bed-side testing. Until now, NGS applications have not been further improved to a level that allows fully portable routine analysis of clinical specimens. In addition, the analysis of NGS data requires different levels of computational power and bioinformatic skills, depending on the sequencing platform chosen, but still require sound knowledge in infectious disease to interpret the huge amount of data accordingly. Additional molecular diagnostic techniques will continue to evolve, with promising approaches like single cell sequencing, CRISPR-based tools and better portable devices for on-site testing.

Importance of molecular diagnostics in LMIC

It seems to be a law of nature that newly developed technical approaches are first evaluated and included into routine workflows in those regions of the world that can afford to offer a well-established healthcare infrastructure with trained personnel and well-equipped laboratories, but unfortunately not in those regions of the world, e.g. LMIC, that need the diagnostic improvements the most. These regions can face unique challenges, with an increased risk of zoonotic-spillover events that may lead to outbreaks, as seen in Western Africa in 2014 with Ebola virus, but also with a higher burden of common infectious diseases, like hepatitis or tuberculosis. In combination with a less developed healthcare infrastructure and constrained financial resources, in economic systems of sometimes low stability, the application of modern molecular diagnostic techniques in LMICs requires well-tailored approaches to ensure accessibility, affordability, and sustainability.

Despite the above-mentioned technical advantages of molecular diagnostics techniques, their widespread implementation faces several additional barriers in LMICs. The high costs compared to traditional techniques are associated with specific equipment, additional reagents, and maintenance costs, which often put molecular diagnostics out of reach for many healthcare systems. Supply chains may be a challenge in LMIC when regulatory hurdles delay or even prevent import of reagents and consumables, disrupting diagnostic workflows. The COVID-19 pandemic has shown dramatically that there was a lack of consumables and reagents to perform PCR diagnostics for SARS-CoV-2, in high income countries as well, which is a situation most LMIC have to routinely deal with. Limited infrastructure can be another challenge. Molecular diagnostics often requires stable power supply, access to clean water and controlled laboratory environments. Additionally, there is a shortage of trained personnel capable of operating and maintaining these devices, but also to interpret results accurately.

**Correspondence: NitscheA@rki.de*

What to expect from the future?

To further support the implementation of molecular diagnostics in LMICs, there are several starting points. Technically thinking, we need more and cheaper diagnostic tests with performance characteristics that are ideally as good as the gold standard technique, the real-time PCR. They need to be reliable, sensitive, easy to handle and safe. PoC test may be a promising tool, but there is a clear need to improve sensitivity and specificity, to reach beyond being a screening test, as evident from the COVID-19 pandemic. The combination of rapid and sensitive nucleic acid amplification techniques like IATs, with LFA readout systems or other battery-operated handheld systems, are promising candidates that evolved recently.

As balancing the assay performance and the ease of use, further cost reduction is another area to focus on, since a widely applicable test should cost no more than 5-6 USD. The COVID-19 pandemic has taught us, that in an emergency case, there is certainly potential to develop and promote diagnostic tools; however, the availability of these tests in LMICs was unfortunately limited in comparison to high income countries. Hence, availability of reliable diagnostics will be essential in the future.

Capacity-building initiatives are essential for the sustainability of modern diagnostics in LMICs, including training programs for students, nurses, physicians and other scientists to run accurate molecular diagnostics and to maintain the laboratory infrastructure. Although automated systems can reduce the need for specialized training, they still do not eliminate the requirement for skilled personnel evaluate and interpret results. While generally the financial investment in laboratory infrastructure is regarded as the most crucial hurdle to take, the education and training of dedicated personnel is the fundament of any healthcare system and depends, in this case, on the integration of molecular diagnostics into national health policies, therefore reducing further the risk of brain drain from LMICs.

Conclusion

Molecular diagnostics has the potential to enhance healthcare in LMICs by enabling accurate and timely detection of new and emerging pathogens. Its implementation must not only address technical improvements, but also the financial, infrastructural, and logistical hurdles, which are beyond the scope of biomedical science. The challenge for the biomedical community will be to provide and ensure availability: availability of achievable tests systems for various infrastructural settings and the availability of motivated, trained personnel in LMICs, to keep a high level of expertise in the field of molecular diagnostics in infectious diseases.

**Correspondence: NitscheA@rki.de*