Mobile Nucleic Acid Amplification/Detection Systems for Pathogens

H. Unger, IAEA*, G. Mair, VUW, M.Leletna, IAEA, G. Vlijoen, IAEA, Vienna, Austria

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Gene-based techniques have revolutionised disease diagnostics in terms of sensitivity, specificity and time to result. The major obstacles to their widespread application – cost of equipment, multitude of process steps and advanced training requirements – were addressed by researchers and industry to present cost-effective tools for the application in marginal settings or under field conditions.

Systems based on qPCR technology with cartridge systems for nucleic acids extraction, meet the performance and quality parameters or requirements but are costlier. An innovation is "convection" qPCR, where the extraction step and the amplification are performed in a small portable reader.

With the re-discovery of isothermal amplification (IA) procedures, competition for the cheapest and most efficient system started. The limiting step, double-strand separation, was addressed by strand-displacing polymerases using a nicking enzyme, a recombinase or a helicase. The primer design is relatively straightforward for modifying enzyme systems with two primers. Loop-mediated amplification (LAMP) depends on a sophisticated design requiring 6–8 primers. Intercalating dyes are the mainstay; labelled probes are vital for nicking enzyme systems to avoid background and add quality. Commercial companies are developing assays for different pathogens and thus instruments capable of performing these assays in the field are increasingly being developed. A wide array of new molecular tests is available on the market, making difficult the selection of the system that fit the end-users needs. Owing to their similar sensitivities, the selection of nucleic acid detection tools should rather be based on the ease of performance in the field, the quality control procedure and the rational design for qualifying a result as positive, negative or indecisive. Overall, the transfer of these technologies to field conditions requires the simplification of the sample processing steps, interpretation of the results and the QC of the assay. We evaluated the possibility of performing QC of IA diagnostic tests on crude samples.

For field applications, the ease of performance mostly depends on the extraction step. The requirement of full-fledged bench-top centrifuge during the extraction steps makes the test unsuitable for field conditions. We demonstrated that boiling the sample followed by sedimentation of coagulates using a 12V mini-spin yields a good quality sample for IA. Regarding the stability of the reagents, lyophilised or room temperature stabilised reagents were chosen to overcome the constraints of maintaining cold chain during transport and field conditions.

Most isothermal techniques only perform an amplification reaction without a specific QA procedure. It was felt that this is a drawback when reporting a result. For LAMP only a hybridisation analysis is published that allows a second determining data point to substantiate the results even with crude samples.

For the evaluation of IA results, we identified and addressed some critical parameters. Because all isothermal processes continuously multiply the target of interest, the reaction temperature and the interfering factors from the crude samples influences the efficiency of the assay. Similarly, mutation in the target can also influence the efficiency. In the IA, the time to detection is used to qualify a sample as positive or negative; absolute quantification may be performed only in research setting. As crude samples often present a high background, relative quantification is not reliable. For our runs, we decided to work only on an absolute scale (mV) and defined a positive result through a minimum increase of fluorescence in time (<30mV/min). A sample was considered positive, when this value was reached twice consecutively and the hybridisation temperature matched with the expected value by ± 0.5°C. As most of the IA platform manufacturers still use a threshold, as in qPCR, in their system, it would be valuable to rethink their practises when using IA.

Regarding the validation procedures, it is important to consider that assays abrogating a sample extraction step might suffer from matrix effects. On the other hand, straightforward procedures reduce human error and thus increase repeatability and confidence in the results. The newly proposed evaluation protocol for these tests certainly needs a wider evaluation to become standard.

We show that IA diagnostic tests using crude samples performed well in combination with QC. Instant messaging these results allows a rapid response to disease outbreaks in field condition.