

## Advances in POC and laboratory testing and the challenges that remain

For many indications, especially bacterial or viral infections, next generation sequencing, which is becoming ever cheaper and more accessible in central laboratories will soon become the technique of choice as the tester will not need to know which infection they are testing for.

Today, the majority of molecular diagnostic central laboratory tests still look for a single target molecule, or a low number in a multiplexed test. However, standard DNA amplification techniques such as PCR require continuous power and careful control of solution conditions, often making them unsuited to point-of-care applications, where simplicity and short timescales are crucial if products are to fit into a practitioner's workflow.

### ▸ Rolling circle amplification embedded on paper

Recently, researchers at McMaster University demonstrated a novel implementation of an alternative DNA amplification technique which could point towards routes for new PoC diagnostics: the reagents necessary for sample amplification were embedded on a paper test strip, and a selective colour change reaction was used to show the presence of the Hepatitis C virus. The test can be run with just a drop of the right biological fluid: blood, sweat or saliva; and can even give quantitative information on concentration of the infectious agents.



### ▸ Isothermal DNA amplification

The test works by using rolling-circle amplification, a room-temperature alternative to PCR, which does not require thermal cycling or such careful control of conditions. Utilising a mixture of enzymes, the technique “nicks” into the circular DNA of the virus and repeatedly copies the strands by passing a DNA polymerase unit around the loop. Although this technique is fairly well established in the solution phase, the innovative steps to adapt this technique have included demonstration of the ability to deposit the important reagents by inkjet deposition, and immobilisation of the necessary enzymes to increase the shelf life of the test strip. As clinicians are familiar with paper-based tests like lateral flow assays, this technology should integrate quickly into the PoC environment. The authors also propose reading the results of the test by radiotracing, fluorescence assays or colorimetric assays, e.g. using gold nanoparticles.

### ▸ Challenges to overcome

Clearly there are challenges to overcome in putting this process into practice in the PoC setting. For one,

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rolling-circle amplification only works with circular DNA, which is found in plasmids, bacteriophages and some viroids; this will limit the applicability of the approach, but also helps to avoid the need for many purification techniques – other DNA sources should not be amplified and detected. Further, using a colorimetric reading in biological matrices can be complicated by the many other sources of colour and light absorbance, so future implementations may need simple sample handling techniques to filter off red-blood cells (as in many lateral flow assays). Whichever of the proposed detection methods are used, it is likely to require a robust measuring device to perform the reading.

#### ↳ Look to the future

Rolling-circle amplification is just one of a number of promising iso-thermal DNA amplification technologies being explored academically with the PoC setting in

mind. Using better and more optimised enzymes may eliminate the need for the classical “melt, anneal, extend” temperature cycling of PCR, opening up applications outside the laboratory environment, with implications in healthcare, especially in less developed countries. However, in light of the challenges and potential concerns over sample complexity, in the short term these techniques may better find use as an early indicator; allowing a clinician to order a targeted set of laboratory tests and supporting practitioners’ decision making processes.

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