Extreme PCR:
A Breakthrough Innovation for Outbreaks?

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The latest Ebola outbreak is the diagnostics grand challenge of the moment. Groups across the world are racing to build the “ideal test” that according to the WHO should work without laboratory infrastructure, take fewer than 3 steps, report results in under 30 min, and have minimum biosafety requirements (1). Leo Poon, head of Public Health Laboratory Sciences at the University of Hong Kong School of Public Health, who was one of the first to decode the SARS (severe acute respiratory syndrome) virus and develop a test for H1N1 influenza, would add “affordable” to that list. “What we need in the field is something simple; and it must be cheap,” he says.

What is the most radical innovation with the potential to deal with viral outbreaks? “The portable PCR machine,” Poon shares. Seven thousand miles away a pathologist in Utah may have hit on an innovation that will change PCR forever. Dr. Carl Wittwer, professor of pathology at the University of Utah Medical School, has been recognized by the inventor of PCR, Kary Mullis, as “someone who has thought about PCR in a way that very few others have” (2). Wittwer invented the LightCycler system, which was licensed to Roche with over 10000 units placed across the world. He spoke with this journal about his latest innovation, “extreme PCR.”

Why Is This Innovation Important?

“Once a technology becomes established, further innovation is still possible.” says Wittwer, referring to PCR.

The dependence on central laboratory testing constrains the ability for a community to deal with an outbreak such as Ebola. The motivation for decentralization is to obtain faster results to provide medical action in the field. “That is a place where historically PCR has not been very competitive because of the time required just to amplify—an hour or two. When you show you can amplify in less than a minute, now you can do nucleic acid testing in a very short time,” explains Wittwer, whose rapid-cycle PCR technology that brought PCR time down to 10–30 min dates back to the 1990s (3).

“A number of advances including microfluidics showed that PCR in less than 10 minutes was feasible, but the quality of amplification became poor when limited with time,” says Wittwer. “We changed the chemistry of the reaction.”

How Does It Work?

“This is pretty obvious, and I kick myself for not doing it 20 years ago. The information was all there,” he shares.

PCR has 3 steps: annealing, extension, and denaturation. The “critical thing” in PCR is getting the solutions to the required temperatures. Conventional instruments do this poorly, and have to add additional holding times in order for the sample to reach temperature targets. But according to Wittwer, a trick was needed in chemistry, not instrumentation (Fig. 1).

Annealing occurs between primers and single-stranded DNA targets. Because there are 2 oligonucleotides binding, it is a “second-order issue.” However, “for most of PCR you have millions of copies of primers per microliter. When the primer concentrations are much greater than the concentration of target, second-order dynamics reduce to pseudo first order. The percentage of annealed template at any time only depends on the primer concentration. So if you double the primer concentration, the required annealing time is halved.” As an added benefit, shorter annealing times increase specificity (4).

Similar logic goes for extension. You increase the concentration of polymerase, which otherwise becomes...
rate limiting. And with the cost of polymerase falling like crude oil in early 2015, adding a lot of the enzyme becomes very feasible.

In a recent report, Wittwer shows efficient and specific DNA amplification of a 45-bp sample with a 26-fold reduction in time when the primer concentration is increased 20-fold and the polymerase concentration is increased 16-fold (5). His most aggressive result is a 60-bp amplification with 35 cycles in 14.7 seconds.

What about nonspecific binding? The prevailing idea is that high concentrations of primers and polymerase befuddle the reaction because the excess reactants produce nonspecific products. Not true when the reaction runs at extreme speeds, says Wittwer.

“Just hit the temperatures precisely.” When the reaction is really fast, there is no time for nonspecific binding or extra polymerase extension. He accomplishes the required speeds by using 2 large water baths to rapidly change the temperature. As noted in this journal, this design is “harking back to the early days of PCR” (6).

Finally, denaturation “is so fast that it is hard to measure and does not require any chemistry modification.” Wittwer estimates that denaturation occurs in less than 100 milliseconds and is using stopped-flow instrumentation to further pin down denaturation rates.

**Where Can This Technology Fit In the Field?**

Wittwer’s vision is a less than 5-minute, end-to-end test that includes sample preparation and detection. “That is what interests me and excites me. You have to wait a bit, but certainly not the hour. If we can push it down to this time range, more people will use it. I have 100% conviction that the PCR part can be done in under a minute for targets of less than 100 base pairs.”

Also, some analysis such as gel electrophoresis or sequencing is required after the PCR. “What I like the best is simple melting analysis. The advantage is that you do the analysis on the same instrument,” he says, having recently demonstrated serial PCR and high-speed melting analysis (7). At a melting rate of 0.5 °C per second, “if we need to cover a 30 °C range, that’d require 60 seconds.”

“The harder piece and the reason you don’t see 5-minute sample-to-answer PCR systems out on the market yet is that the sample preparation piece depends on the sample matrix; it is hard to provide a very fast one-stop solution that will process every sample type.” However, Wittwer believes, “you can have different front ends that handle different samples. For Ebola you would run blood; a complex matrix that is nevertheless doable.” He admits, “Integrating very fast sample preparation, amplification and analysis in a convenient and user friendly system is not there yet.”

“Microfluidic approaches are promising, and perhaps with augmented chemistry, will succeed,” says Wit-
Although he has shown 90%–100% PCR efficiency, concerns about the robustness of extreme PCR and whether it can quantify as well as slower systems remain. “The instruments we have developed are not yet practical—we are using large water baths to change temperature quickly.” Poon shares these reservations. “The main concern for this approach is this is still a PCR test, the prototype in the manuscript has 2 water baths only. So this might not be able to do a 1-step RT-PCR [reverse-transcription PCR] for emerging RNA viruses.”

But Poon is optimistic about Wittwer’s latest breakthrough. “Clearly, this is going in the right direction,” he says. It turns out that Wittwer has already made a 4–water bath system (Fig. 2), so at least this concern may already be solved.

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**References**