

Infection detection

Testing device uses an RPA technique to determine the presence of the MRSA virus in patients

THE SPREAD of MRSA in hospitals could be curtailed with a quick test from a portable device according to its developer, Cambridge-based TwistDx.

The technology, which employs recombinase polymerase amplification (RPA), tests swabs from patients' noses and returns results within 10-15 minutes indicating if the infection is present.

The battery-powered device, about the size of a VHS case, is expected to be easy to use, eliminating the need for trained technicians to conduct tests.

MRSA is particularly virulent in hospitals. Patients with open wounds or a weakened immune system are particularly susceptible to acquiring infection from the so-called 'super bug'.

Niall Armes, chief executive of TwistDx, said the current, dominant technology for testing MRSA involves a polymerase chain reaction (PCR), a technique that can take up to 48 hours using expensive equipment housed in a hospital laboratory away from patients.

PCR works on the principle that DNA is a two-strand double helix, with each strand perfectly matched to the information on the other side.

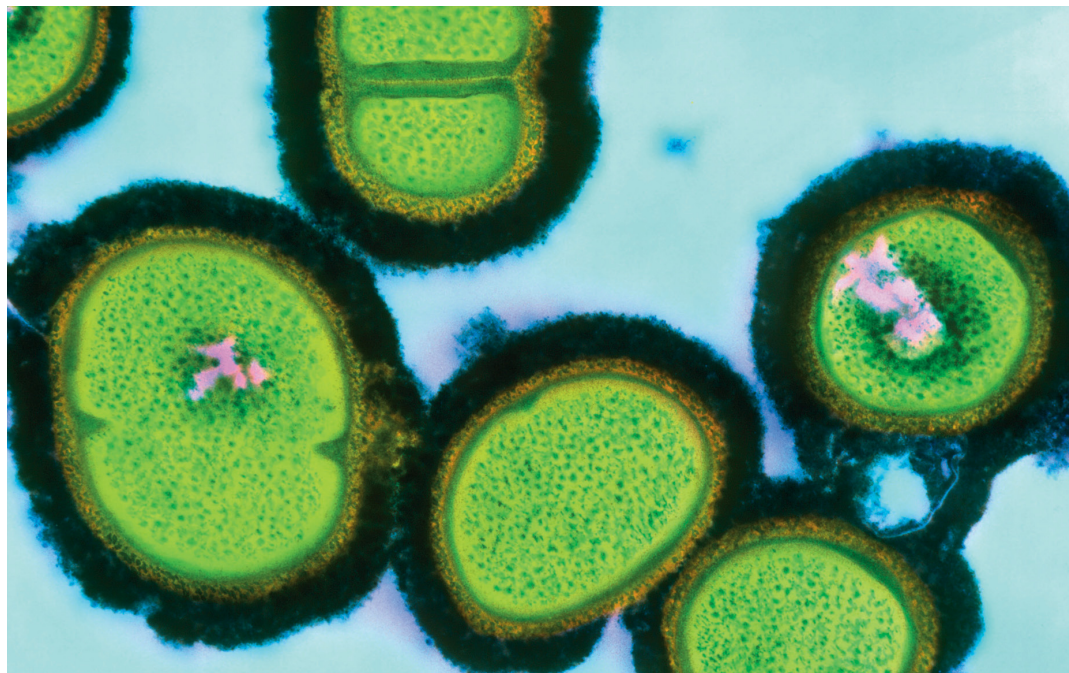
'If you pull the two strands apart, it is possible to rebuild the missing information on either side,' added Armes.

The sample is placed inside a reaction mixture that is doped with a large quantity of small synthetic pieces of DNA that are matched to the sequence of the target. In this case, it would match MRSA.

With the PCR technique, the DNA strands are separated by boiling and cooling the sample.

'When you carry out the boiling and cooling, the very high excesses of those small synthetic pieces of DNA always find their target,' he said. 'This will form back into a small duplex if the target is present.'

An enzyme will then recognise the end of the small double-stranded DNA and continue rebuilding the rest of the information.



Coloured transmission electron micrograph (TEM) of a deadly cluster of MRSA Staphylococcus aureus bacteria

'If the target is present it will go from one DNA duplex to two,' added Armes. 'The process then repeats from two to four, four to eight, and within 35 iterations it produces very large amounts of the DNA sequence that was the MRSA DNA.'

The PCR method is very effective at amplifying and detecting MRSA DNA, but Armes believes it is too time consuming and the process cannot be used in point-of-care applications.

The problem, he said, is boiling, cooling and otherwise adjusting the temperature of the sample requires sophisticated instrumentation to keep the thermal characteristics extremely accurate. PCR devices are expensive, have a large footprint and are not at all portable.

The TwistDx device, however, uses much less instrumentation. The secret behind the innovation, added Armes, is a twist on the PCR biochemistry.

'We amplify DNA in a fashion that is somewhat similar to PCR,' he said. 'We use these small synthetic pieces of DNA that will target themselves to their matching

sequences in the sample if it is present, and lead to DNA synthesis and the doubling of the target DNA from two to four to eight.

'The important difference is that we do not use cycles of thermal melting and annealing of the DNA substrates to achieve this amplification cycle.'

Instead, TwistDx uses RPA. Recombinases are enzymes that physically bind to the synthetic pieces of DNA and then search for identical sequences in double-stranded DNA. The process is very similar to those that happen naturally within living cells to match up identical sequences.

When the enzymes find identical sequences, they will then place a synthetic piece opposite its target and allow DNA synthesis.

'Our process will operate at physiological temperatures ideally around body temperature,' added Armes. 'This allows us to develop instruments that are much simpler and ultimately, the process may require no instruments at all.'

The system will still require a detector to determine whether a reaction occurred. The TwistDx

method includes a biochemical system that releases fluorescent light if DNA amplification and synthesis occurs in a sample. LED-driven sensors that are embedded within the TwistDx device measure the fluorescence.

Beyond the advantages of more compact instrumentation is time saving. 'We can detect single DNA molecules in as little as six to eight minutes,' said Armes.

The system will also be simpler to use. 'A member of staff will just take a swab from the nose and dunk the swab into a liquid that we supply,' he added. 'They will then transfer the liquid onto a freeze-dried powder that contains all of our active reagents, quickly mix it and then put it into a device that will measure fluorescence.'

Armes said that the TwistDx is likely to go into production in April and be on the market in a year.

'This will be our first-generation device,' he added. 'We anticipate improvements going forward to make it even more suitable for use by non-expert technicians in medical settings.'

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