An exciting and ambitious path to progress

Clinical virologist Professor Judith Breuer of the PATHSEEK project discusses an international collaborative consortium she is leading that is working towards rapid whole-genome sequencing of pathogens, such as HIV and Mycobacterium tuberculosis, directly from clinical samples.

Can you briefly introduce the PATHSEEK project and describe how it has progressed to date?

PATHSEEK aims to develop a next-generation sequencing platform for use in diagnostic microbiology. Within a turnaround time of less than two days the platform aims to generate deep sequencing data, in a clinically useful format, directly from a clinical specimen.

So far, we have implemented our concept of sequencing the whole pathogen genome directly from clinical material as a semi-automated pipeline. We have successfully sequenced the complete genomes of several hard-to-culture pathogens including Mycobacterium tuberculosis, chlamydia, influenza, HIV, the hepatitis B and C viruses (HBV and HCV), other herpesviruses, norovirus and enteroviruses directly from clinical samples within a five-day timeframe depending on the photogene. In some cases, we have also been able to sequence multiple viruses from the same sample.

PATHSEEK’s consortium is made up of four collaborators from across Europe – University College London, Oxford Gene Technology (OGT), QIAGEN Aarhus and Erasmus Medical Centre (EMC). What part does each collaborator play?

UCL leads the project. In this role, it has sourced clinical samples, contributed its genomics expertise and developed the assay and the bioinformatics analysis of the sequencing data, and is helping with the commercialisation of the project. With the support of UCL, OGT has developed new reagents that allow the process to be performed more quickly. QIAGEN Aarhus is developing a user-friendly software platform for the analysis of sequencing data by clinicians. EMC is testing whether the methodology can be applied in an alternative lab and is providing valuable clinical specimens.

What makes PATHSEEK unique in comparison to existing platforms of its kind?

PATHSEEK does not require either culture or pre-amplication of a pathogen template prior to sequencing from clinical material. This is a major breakthrough as without this step in other methods, direct sequencing of pathogens in clinical material is too insensitive to be able to identify resistance. The unique advantage of PATHSEEK is that, by enriching pathogen genomes directly from clinical samples, it improves the quality of sequence to the extent that it can be used to identify outbreaks and resistance.

Unlike the other methods for pre-amplication, we can carry out the PATHSEEK method in a single tube; this means that the process can be automated, saving time and money. Moreover, the sensitivity of the method is such that resistance occurring at low level can be identified. This is particularly important for HIV, HCV and cytomegalovirus, where low levels of resistant mutations can be signs of clinical resistance.

Can you expand on the project’s bioinformatics software, which has been described as flexible and user-friendly?

Yes, we have created a semi-automated platform capable of sequencing full genomes within five days. We created it so that it would be accessible to people of varying degrees of expertise. The software being developed will generate a summary report that can be understood by novice bioinformaticists users – such as clinicians and clinical scientists – along with more detailed quality information and visualisation of data directed towards experienced bioinformaticians.

As you have mentioned, the PATHSEEK consortium has successfully sequenced the whole genomes of several infectious diseases. What new horizons have dawned from these activities?

There were two main opportunities which we hoped to develop: first, the potential to identify all the resistance mutations in a single test – even where these occur in widely spaced genes. Second, the chance to generate genomes faster than other methods for slow-growing or hard-to-grow pathogens, such as M. tuberculosis or chlamydia, as well as some viruses and intracellular bacteria. The method will allow faster linking of infections in hospital outbreaks and identify emerging epidemics in addition to the personalisation of the treatment to combat antimicrobial resistance.

How is PATHSEEK anticipated to direct clinical treatment in the prevention of antimicrobial resistance?

PATHSEEK gives a comprehensive readout of all known antibiotic resistance with a single test; because of this, the results are available faster than those achieved with conventional methods for infection agents such as M. tuberculosis or hard-to-culture pathogens. This allows antimicrobial resistance to be determined in a timeframe suitable for treatment. Our system also makes the rapid analysis of outbreaks for nosocomial infections possible.

Can you highlight the expected outcomes and applications of PATHSEEK?

We have three main priorities for PATHSEEK over the next year in terms of development and outcomes. The first is to provide reference diagnostic testing for slow- and hard-to-grow pathogens. The second is to support clinical trials of new antimicrobials or other de novo solutions by identifying emerging resistance. Finally, we hope to source further funding to improve the speed and implementation of the method.
Speed and sequence

A dedicated team from University College London, Oxford Gene Technology, QIAGEN Aarhus and Erasmus Medical Centre has developed a new method for rapidly sequencing whole pathogen genomes within the PATHSEEK project.

NEXT-GENERATION, HIGH-THROUGHPUT gene sequencing has presented life scientists with a great range of opportunities over the last few years. Between the mid-1970s and the early 2000s, manual sequencing methods such as Maxam-Gilbert and Sanger sequencing were the most expedient routes towards genome analysis on the small-scale – but with the advent of high-throughput, automated methods, it has become possible to take a broader view of the genome, or even study it in its entirety. In practice, this whole genome approach has usually been applied to microbes, as their genomes are relatively manageable and they often have great relevance to human health.

In particular, whole-genome sequencing has great potential for battling the growing problem of drug-resistant pathogens. When a doctor diagnoses Mycobacterium tuberculosis in a patient, for example, the challenge is only just beginning; the treatment then has to be personalised based on the exact strain of mycobacteria infecting the patient, which could be resistant to any combination of the available drugs.

It is a difficult situation, as well as one that is becoming increasingly common across many diseases based on bacterial and viral pathogens – hepatitis, chlamydia, herpes, enterovirus infection and influenza. Hospital-acquired infections such as norovirus are also becoming more difficult to treat because they quickly acquire resistance.

RESISTANCE IS FERTILE Whole-genome sequencing reveals these features for the benefit of clinicians, but unfortunately, current methods can take weeks to provide useful results. This delay is not down to the methods themselves, however – in fact, the sequencing process is comparatively quick. What takes time is culturing samples of the pathogen to the point where they are sufficient to be used in the sequencing process. This can take weeks, and by the time the results are available to clarify the best treatment in an individual case, the disease has already progressed.

A new solution from the PATHSEEK consortium, led by Professor Judy Breuer at University College London (UCL), may change the field for good. The consortium has been at the forefront of work on an innovative technology project, an endeavour centred on designing a new and eponymous platform for whole-genome sequencing from clinical samples with results available in not weeks, but in days. Initiated in autumn 2012, PATHSEEK is pursued by an international consortium of scientists with funding from the European Commission’s Seventh Framework Programme (FP7) – and, following an extension earlier this year, will come to a close in February 2016. By that time, the team expects to have completed not only an optimised platform for whole-genome sequencing, but also software to improve its usability and accessibility.

CULTURE SHOCK Part of the secret of PATHSEEK’s dramatic reduction in timescales over other whole-genome sequencing platforms lies in the fact that it does not rely on culturing bacteria. Usually, once a sample is collected from the patient, the genetic material within that sample then needs to be ‘amplified’, usually by culturing (growing more of the bacteria) or employing polymerase chain reaction (PCR), a process whereby selected

It might be possible for doctors and scientists to use the rapid sequencing method to trace an infection back through different hosts, and even identify those patients most at risk of passing the disease on to others.
genetic sequences of interest can be repeatedly ‘copied’. Using target enrichment methods directed at the pathogens within the patient samples, PATHSEEK improves the quality of the pathogen sequences to the point where they can be used directly from the sample without needing culture or PCR. This considerably speeds up the process. The system makes use of RNA/DNA probes that bind with the DNA of the pathogen and help the researchers to extract the most salient information from the sequences. This innovation will be useful for all whole-genome sequencing applications, but Breuer and the PATHSEEK team see a particular value for it in dealing with pathogens that are either hard to culture – such as viruses and intracellular bacteria – or derived from samples which are hard to culture, such as cerebrospinal fluid. ‘For example, in the context of M. tuberculosis diagnostics, which we have tested using PATHSEEK platform, this technique could be particularly advantageous because current approaches are based on culture which takes a long time (in excess of weeks) or are gene-specific molecular tests, which lack the breadth and depth of information that whole genomes can provide,’ Breuer explains.

What is more, the faster processing times will allow clinicians to combat antimicrobial resistance in a new way, with increasingly personalised and targeted medicine.

A HISTORIC FIRST
Within the PATHSEEK project as a whole, the new high-speed sequencing method is being applied to a variety of infectious diseases – including chlamydia, HIV, hepatitis B virus and hepatitis C virus, Herpes simplex, influenza A, cytomegalovirus and the hospital-acquired infection norovirus. Many of these conditions are becoming increasingly associated with complications due to antimicrobial resistance.

The PATHSEEK researchers focused on M. tuberculosis in their most recent and promising demonstration of the system, which was published in PubMed in a paper entitled ‘Rapid Whole Genome Sequencing of M. tuberculosis directly from clinical samples’ in July 2015. In this influential study, the team took 24 M. tuberculosis-positive sputum samples from patients in the UK and Lithuania, as well as two samples that had failed to grow in culture, and applied their method to sequencing the genetic information contained within.

The results were very encouraging; M. tuberculosis sequencing data was recovered from all 24 positive samples, and 20 of the datasets acquired were of a very high quality, with 90 per cent of the genome or more covered by the results. But the researchers did not stop there – they also compared the outcomes of their assay with those generated by genome-wide sequencing with prior culturing, and found that the clinical significance of both sets of results was very similar.

That was the first time that M. tuberculosis had been sequenced in full without first culturing the bug – but since then, the UCL group has also initiated two further studies to assess the diagnostic potential of their new method more fully. As well as having an impact on the treatment of individual patients, this novel way of determining the exact characteristics of pathogens may be able to assist researchers and clinicians in discovering more about the transmission of infectious diseases. In theory, it might be possible for doctors and scientists to use the rapid sequencing method to trace an infection back through different hosts, and even identify those patients most at risk of passing the disease on to others.

FURTHER STUDIES
Going forward, Breuer and her colleagues at UCL and further afield will continue to probe the applications of their invention in a diagnostic setting. The problem of antimicrobial resistance is a global challenge and persists in the UK. Using the PATHSEEK methodology for whole genome sequencing of pathogens, the researchers see a bright future ahead, one where their methodology could be used in a range of scenarios, including to combat antimicrobial resistance and improve surveillance for public health – all in one test.

PATHSEEK
OBJECTIVES
• To develop and set up an automated, user-friendly next-generation sequencing platform for use in diagnostic microbiology
• To utilise target enrichment methods to specifically sequence the genetic material of single or multiple pathogens, and relevant host biomarkers, from a single specimen

PARTNERS
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is Clinical Director of the Medical Research Council (MRC)’s CMMV at University College London, where she heads the viral genome sequencing programme, which was the first to use targeted enrichment for next-generation sequencing of viruses. She is also a clinical consultant virologist at Great Ormond Street for Children. She has over 20 years of experience in research into molecular epidemiology and sequencing of herpes and other viruses. Before moving to UCL in 2009, Breuer was head of St Bartholomew’s Hospital and the London regional diagnostic virology lab.

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