Presentation 1: “Madagascar experience with MinION”
Simon Grandjean-Lapierre

- Can you use a target-based approach with this Oxford nanopore Minion?

  Yes. The Oxford nanopore MinION, or any other nanopore-based sequencers, is a sequencing platform that’s used to generate the sequencing data. One can i) use a pre-sequencing PCR step on targeted regions ii) sequence amplicons using nanopre and, iii) perform bioinformatic analysis for genotypic DST from mutations found in the amplified resistance determining genes.

- What is the level of genome coverage you obtain with Minion? Specifically, did you have sufficient sequencing coverage across all known drug resistance mutations?

  We did. When working from culture, depth and coverage on MinION is in the same range as when sequencing on other platforms such as Illumina.

- What % NTM did you observe in the sequencing data?

  Two things;
  1) In our initial sampling, around 1% of isolates thought to be TB turned out to be NTMs. 2) For any given TB pure culture isolate, the percentage of sequencing reads which map to an NTM instead of TB reference genome is below 1-3%.

- What was the basis for retrospective sample selection for NGS and further selection for scale up plan?

  Retrospective isolates were chosen from isolates submitted to the DR-TB surveillance program. A diversity of i) drug resistance profiles ii) lineages and iii) geospatial origin in the country.

Presentation 2: “Pyrosequencing in Mumbai”
Camilla Rodriguez

- What was the cause for drug malabsorption in your case study?

  Patient had Tissue Transglutaminase IgA borderline positive, so was suspected to have malabsorption due to celiac disease.
Did you do his CYP 450 profiling to confirm the genetic mutation of the subject case of TB that did not respond to the TB drug?

No CYP profiling was done but serum levels of Rifampicin were very low.

What % in Mumbai are missed by Xpert?

This depends on the type of the sample and whether it is Pulmonary or EPTB. Culture is not part of the DRTB algorithm unless there is a discrepancy at the LPA level. At our center, we usually do both Xpert MTB/RIF & MGIT culture. Pulmonary samples that are Xpert negative & Culture Positivity is approximately 3%. This is higher for EPTB samples as paucibacillary samples may have sampling issues.

In the case of Mumbai - would it benefit to do upfront pyrosequencing from clinical specimens?

If LPA is not able to give valid results or if LPA shows absent Wild type with no corresponding mutant then PSQ is performed.

Should all re-treatment cases or treatment failures in Mumbai be sequenced immediately?

In the DRTB algorithm, all treatment failures are subjected to LPA with any discrepant being subjected to MGIT DST to second line drugs. At our center we do PSQ for the XDR defining drugs.

What is your experience with Xpert Ultra?

We worked on Xpert ultra for the initial validation studies and we found the sensitivity to be better than Xpert MTB/Rif.

Presentation 3: “UK experience with scale up of WGS at the national level”.
Derrick Crook

What is the reason for high number of NTM? - HIV coinfection?

With the decline in TB the proportion of cases yielding NTMs on culture is rising as a proportion. This is evident in many countries with an incidence of TB below 10/100000 per year. A major risk factor is chronic lung disease e.g. Cystic Fibrosis and bronchiectasis. Therefore, in resource rich countries the challenges of knowing the contribution of NTMs to disease vs contamination of cultures is growing.

When discordances between phenotypic DST and WGS are detected which is the strategy for patient management?

The standard of care for where phenotypic DST is still undertaken is to follow the accredited tests, which are phenotypic tests. In the UK we are moving to WGS only
testing of susceptibility of the 4 first line drugs based on our results reported in the the publication below. Thus, for these in the UK, having a discrepancy will be very rare i.e. < 1%, which is well below the error rate for DST and well within the FDA requirement that the major error rate is < 3%.

In practice, clinically, if there is a discrepancy, the pragmatic and precautionary step is to avoid the ‘discrepant’ drug until there is confidence in the ‘truth’ from careful investigation and retesting. This is of course only practical where there are sufficient resources to do this. It will be wholly impractical in most high incidence parts of the world.


- What is the cost per sample in PHE?

  The full ‘bottom up costing’ i.e. including the total cost – e.g. reagents, staffing, estates costs, capital costs etc is £518/sample vs £544/sample for phenotypic, line probe and MIR-VNTR costs. The raw cost of WGS only is £118/sample.


- Are you using sequencing for DR detection in other organisms HIV, AMR?

  We have research projects doing this on scale for many taxa. For viruses we are focused on HCV and Influenza A, though other groups in Oxford are doing this in research settings.

- Can the MicoTiter plate be something useful for countries? Can it be implemented instead of MGIT? How accurate is it in comparison to MGIT?

  This technique is being used in our CRyPTIC project in Peru, India, China, Brazil, South Africa, Vietnam, Italy, Germany and England; therefore, it can be used in many different geographical settings. The equivalent Trek plate is being used in many countries. Its performance is very promising and we are working with colleagues to establish robust interpretive criteria for use of the plate. This is progressing well and it is possible it will receive approvals for use in many countries. It is very much work in progress. I expect it will receive accreditation for use in UK laboratories this year. Consequently, it may become widely used over the coming years.

  It is not suitable for pyrazinamide testing, but for most other drugs it is performing very well, including in comparison to MGIT – which is being evaluated in a number of sites participating in CRyPTIC. We will be publishing results over the next two years.

  *CRyPTIC website: http://www.crypticproject.org*