The use of next generation sequencing technology to detect integrase inhibitor resistance in treatment naïve patients with HIV in Wales.

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Background

- Use of integrase inhibitors (INI) is increasing
- Estimates of INI resistance are incomplete
- In July 2018, Public Health Wales was first UKAS laboratory to switch to Next Generation Sequencing (NGS) for routine detection of HIV resistance

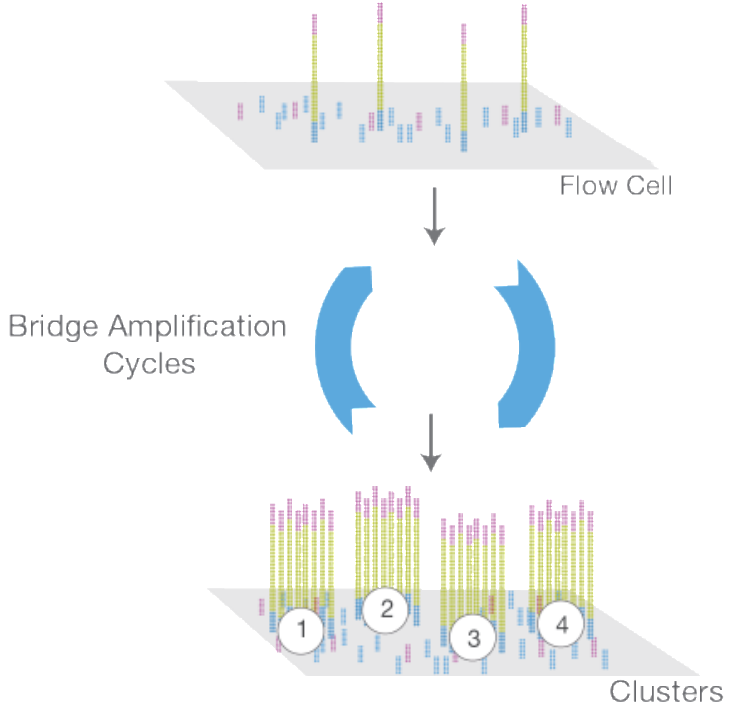
Methods

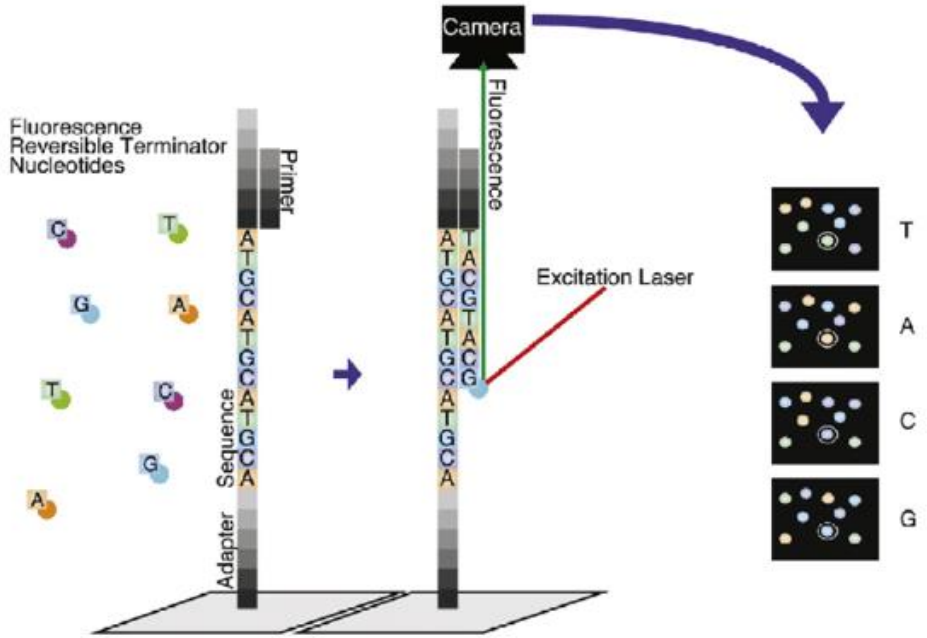
- Retrospective analysis of HIV resistance tests performed by NGS from July 2018 - January 2020

- Only baseline tests on ART naïve patients were included

Technology
Next Generation Sequencing

Sample Extraction & Real-time PCR → RT-Amplification → Clean-up and Quantification → Library Preparation → Quantification and Pooling → Cluster Generation and Sequencing → Bioinformatics pipeline





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Technology
Next Generation Sequencing vs Sanger Sequencing

- Sanger sequencing's lower limit of detection of amino acid variants (AAV) is circa 20% vs NGS of circa 2%

- The presence of minority resistance variants (MRVs) below this threshold would have previously been undetectable

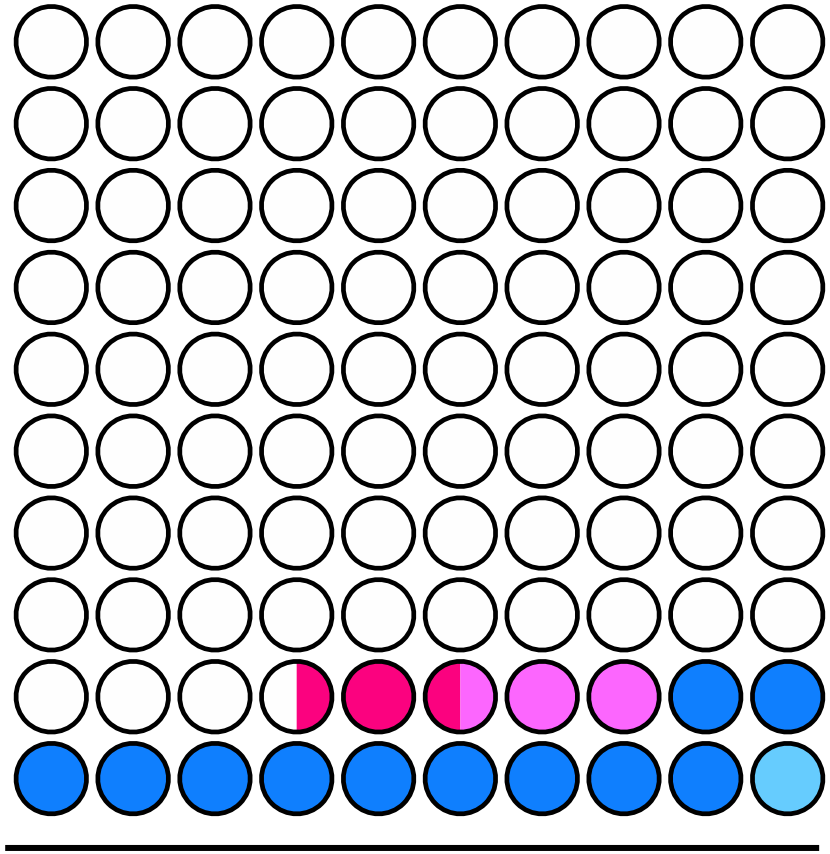
Results
Turnaround Time (TAT) & Cost

- Standard TAT pre-NGS is 14 days

- TAT decreased by 43% to 8 days, N=62 range (7.1-8.6)

Results

Detection of Major resistance mutations in Rx naïve patients using NGS



N = (110)

- None: 83.64% (92)
- PI: 1.82% (2)
  (V82A & I50L)
- NRTI: 2.73% (3)
  (T215S, K219N & M41L/L210W/T215S)
- NNRTI: 10.91% (12)
  (E138A x8, K103N, P225H & M230L)
- Dual (N)NRTI: 0.91% (1)
  (M41L/L210W/T215S w/ E138A)

- Major TDR in 16.36% (18)
- No Major INI detected
- Minor E157Q found in 2.73% (3)

Results
E157Q INI mutation

- Polymorphic mutation present in 2.7% of ART naïve patients

- Though observed in cases of virological failure, phenotypic analysis suggests no decrease in susceptibility to INI, including raltegravir

Saladini, Francesco, et al. "The HIV-1 integrase E157Q polymorphism per se does not alter susceptibility to raltegravir and dolutegravir in vitro." Aids 31.16 (2017): 2307-2309.
Charpentier, Charlotte, and Diane Descamps. "Resistance to HIV integrase inhibitors: about R263K and E157Q mutations." Viruses 10.1 (2018): 41.

Discussion/Future

Todays polymorphism is tomorrows mutation

- Prevalence of INI resistance is low
  - Use of INI in increasing
- Introduction of NGS resulted in decreased TAT
  - Clinical service improvement
  - Economic benefits
- Closer to individualized medicine

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