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Prevalence of *por A* pseudogene deletion amongst *N. gonorrhoeae* isolates referred to GRASP

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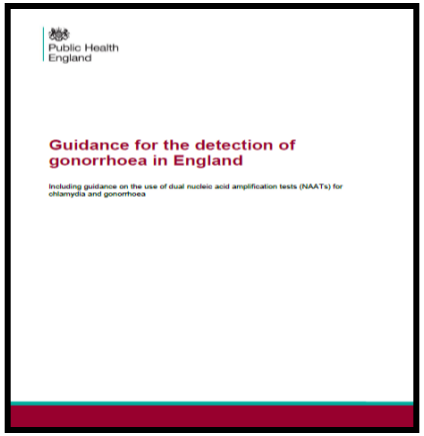
Introduction



- PHE recommends confirmatory testing - GC NAAT Positives >90%
- Can be problematic to confirm using commercial platforms
- Several In-house assays available: *opa* gene, 16s rRNA, *porA* pseudogene
- porA pseudogene – conserved in NG & significant different in NM – ideal sensitivity & specificity
- porA negative GC have been reported – Australia, Sweden, Scotland & England
- **Aim:** Determine the prevalence of *porA* negative GC isolates in England & Wales using isolates referred to GRASP

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Methods

- GRASP – sentinel surveillance study
- Regional representation of GUM clinic patients
- DNA lysates were prepared from 533 *N. gonorrhoeae* isolates 2011
    - 20 centres around England & Wales
- Tested using an in house RT-PCR for the *porA* and *opa* gene
- DNA sequencing of discrepant strains

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PCR Results

No Isolates	*PorA* PCR results	*opa* PCR Results
531	+	+
2	-	+

99.6% - GC isolates *porA* positive

0.4% - GC isolates *porA* negative

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DNA Sequencing Results

```
MC          ACTCTCCGGACTTTTCCGGTTTCAGCGGCAGCGTCCAATTCGTTCCGGCTCAAAACAGCA
GC          ATTCCCCCGGATTTTCCGGTTTCAGCGGCAGCATTCAATTTGTTCCGAGTCAAAACAGCA
            * ** ** *  ********************** * ***** ******  ***********

MC          AGTCCGCCTATACGCCGGCT-----TATG-TGGATAAGGAGCAGGTG---TCTCATGCGG
GC          AGTCCGCCTATACGCCTGCTACTTTCACGCTGGAAAGTAATCAGATGAAACCAGTTCCGG
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- Primers and probes – target gonococcal specific regions
- 2 *porA* PCR negative GC Isolates – *N. mengitidies porA gene*
- False negative GC *por*A PCR results

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Conclusions

- *por*A pseudogene is a popular target for confirmatory assays
- 0.4% (2) gonococcal isolates were identified as *porA* negative in England
- Both GC Isolates have incorporated MC *por*A gene into their genome
- Front line and confirmatory strategies remain a challenge for GC NAATs
- Neisseria are genetically fluid and competent – all stages of their life cycle
- *porA* pseudogene is a nationally accepted target for a supplementary test
- Microbiologists must be vigilant to decreases in numbers of confirmatory tests

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