Gonorrhoea Testing on the cobas 4800

Amanda Miller
Aotea Pathology

Clap clap...

1. Neisseria gonorrhoeae (NG) PCR
2. Previous studies
3. Validation of cobas 4800 NG test
4. How to improve pickup of CT and NG
5. Our recommendations

Neisseria species

• Gram-negative diplococcus
• Many non-pathogenic species
• Two pathogenic species
  – N. meningitidis and N. gonorrhoeae (NG or GC)
• Gonococcus infects genital sites, urinary tract and non-genital sites
  – Rectal, Pharyngeal, Conjunctival
• Associated with transmission of other STIs and HIV

They love to share their genes...

• Frequent genetic exchange through spontaneous mutation and/or gene acquisition from other organisms
  – Changes to gene sequence:
    • Alter target sequence of PCR assays ➔ False negatives
    • Confer antibiotic resistance
• Genetic similarities with other commensal organisms (especially other Neisseria sp)
  – Cross-reaction ➔ False positives

Australian Public Health Laboratory Network (PHLN) Meeting 2005

• All NG NAAT positives should be retested by a reliable supplementary NG NAAT
  Uro-genital specimens:
  ➔ Positive x 2 GC targets = DETECTED
  Extra-genital specimens:
  ➔ Positive x 1 GC targets = DETECTED

• Cross-reactions make PCR unsuitable for specimen sites where Neisseria spp. are ubiquitous i.e. throat, rectal.
• Any test used should have a PPV ≥ 90% in the population tested
• Assessment of assay specificity should be ongoing due to the propensity for genetic exchange in the gonococcus.

PPV in a low prevalence population

• When prevalence of the testing population is <1.0% no test is good enough
  ➔ Additional testing is required to avoid misdiagnosis

Why has it taken us so long?

- Predecessor assays required confirmatory testing
  - 2000: 411 WSHC patients tested by Amplicor showed 1.5% confirmed positives
  - Further 1.9% false positives!
  - 2005: 300 self taken swabs tested by Amplicor showed...
    - No confirmed positives!
- Funding and prescribing problems

Study design

- Audit of historical data on specimens received between 1/1/11 and 13/9/11 for CT PCR and NG culture
- Run data stored by the cobas 4800 was accessed to see NG results, and then compared culture result for the same visit (clinical specificity)
- Check analytical specificity
- Review non-genital specimen data
- What about female urines?

Discrepant results

- 37 samples were discrepant:
  - 35 PCR + Culture -
  - 2 urines PCR - Culture +
  * Historical specimens, no longer available!
- History of discrepents was analysed
- Sent 10 samples for confirmatory testing by porA/opa PCR
  We were able to rule the PCR correct in 26 cases

At first glance...

- During study period, 27,585 specimens tested for CT
- Found 187 NG positives = 0.68%
- NG cultures in the same period =0.70% NG *

*Note that only cervical and urethral swabs were cultured

2011 Clinical Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>98.5</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.8</td>
</tr>
<tr>
<td>PPV</td>
<td>91.7</td>
</tr>
<tr>
<td>NPV</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>100</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.8</td>
</tr>
<tr>
<td>PPV</td>
<td>91.7</td>
</tr>
<tr>
<td>NPV</td>
<td>100</td>
</tr>
</tbody>
</table>

* Genital swabs and urine combined
**Assay Performance**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>N</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urogenital *</td>
<td>18,206</td>
<td>98.5%</td>
<td>99.9%</td>
<td>91.7%</td>
<td>100%</td>
</tr>
<tr>
<td>Non-genital</td>
<td>610</td>
<td>100%</td>
<td>99.8%</td>
<td>91.7%</td>
<td>100%</td>
</tr>
<tr>
<td>All</td>
<td>18,816</td>
<td>98.9%</td>
<td>99.9%</td>
<td>91.7%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 1: Performance of the cobas 4800 NG assay by specimen type

Note some visits are received with multiple specimens from the same patient.

*Genital swabs and urines were grouped as "Urogenital" specimens for analytical purposes.

**Urine specimens**

- Recommended for males
- Not recommended for females
  - Low sensitivity
  - Not suitable as a sole screening specimen for females

<table>
<thead>
<tr>
<th>Specimen</th>
<th>N</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female urine</td>
<td>564</td>
<td>77.8%</td>
<td>100%</td>
<td>100%</td>
<td>99.6%</td>
</tr>
<tr>
<td>Male urine</td>
<td>950</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

**How many more by PCR?**

- 37.4% of CT PCRs are not tested for NG
  - 13,000 more NG tests / year
- 7.4% of NG cultures are not tested for CT
  - 1,750 more CT tests / year
- Extra NG infections we could project to pickup
  - 35 (discrepancies) + 43 (not requested) = 78
- Cobas NG PCR could produce:
  - 38% more positives

**They hunt in packs**

42% of NG positive patients also have CT

**Could we improve detection?**

- “FAILED” results from the cobas due to pipetting errors or clots in the specimen
- There were 97 (0.35%) repeatedly failed cobas CT results in 2011, not included in study
- Of the 97 “Failed” specimens accompanied by a culture specimen, 21% grew NG
  - All of which were male urines

**Why so many NGs in failed specimens?**

Interference from muco-purulent discharge

*Miller et al 2012*
Attempts to resolve pipetting errors

- **Vortexing**
  - All specimens already vortexed for 1 minute on a multi-vortexer...
- **Heating**
  - Specimens heated to 95°C for 5 minutes
- **Dilution**
  - Specimens diluted 1:2 with cobas PCR media
- **Dithiothreitol (1.4%)**
  - Sputasol in equal volume with specimen

- **Heating**
  - Resolved 72%
- **Dilution**
  - Resolved 82%
- **Sputasol treatment**
  - Resolved 91%, resolving the CT positive specimen missed by the other 2 resolution methods

- Both Heating and Dilution methods did not resolve one specimen that was positive for CT

Validation of Sputasol

- Treated 100 valid cobas CT specimens with equal volume Sputasol and retested
- 46 Urines and 54 genital swabs
  - 47 CT positives, 5 CT/NG positives, 50 Negative
  - 100% agreement

Sputasol in action

- Sputasol in routine use since September 2011
- 111 failed and invalid specimen's treated
- Retested on cobas 4800
- 95 resolved (86%)
- 5 invalid, 11 failed remained unresolved
- Results
  - 9 CT positive 2 NG positive 1 CT/NG positive

Analytical and Clinical Specificity
Clinical Specificity

- To ensure no cross reaction with local isolates we tested colonies from culture plates that were *?Neisseria growth (pre ID)*
- N=97 (16 NG positive)

<table>
<thead>
<tr>
<th>Site</th>
<th>Direct culture</th>
<th>Swab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throat</td>
<td>23 (1)</td>
<td>9</td>
</tr>
<tr>
<td>Rectal</td>
<td>18 (1)</td>
<td>6</td>
</tr>
<tr>
<td>Cervical</td>
<td>2 (1)</td>
<td>2</td>
</tr>
<tr>
<td>Penile</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Urethral</td>
<td>15 (1)</td>
<td>11</td>
</tr>
</tbody>
</table>

*100% agreement between PCR and culture*

*Managed to PCR CT from culture plates on 4 patients….

Summary and recommendations

- The improvement in pickup of NG offered by cobas is contributed to equally by accessibility (urines, self taken swabs) and assay sensitivity.
- Specificity and PPV is sufficient to run cobas NG without a confirmatory assay on all specimens

1. Recommend running CT and NG on all specimens
2. Recommend treating failed and invalid specimens with DTT prior to retesting to resolve

References