

Evaluation of an in-house confirmatory assay for the detection of *Neisseria gonorrhoeae* in urogenital and extragenital specimens



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Outline of Presentation

- **Laboratory service at SJH**
- **Abbott RealTime™ CT/NG PCR Assay**
 - Specimen collection kit
 - Assay procedure
- **In-house real-time PCR *porA* assay**
 - Assay procedure
- **Assay evaluation**
 - N. gonorrhoeae* and *N. spp.* isolates
 - Analytical sensitivity
 - Clinical trial
- **Conclusion**

Introduction-Testing at SJH

Diagnostic laboratory at SJH

GUIDE, smaller clinics and general practitioners

	2007	2008
No. of patients tested for NG	12499	12580
No. of patients positive for NG	254	169
% Patients positive	2.0%	1.3%
No. of Specimens tested for NG	17000	17437
No. of Specimens positive for NG	293	205
% Specimens positive	1.7%	1.2%

Introduction-Testing at SJH

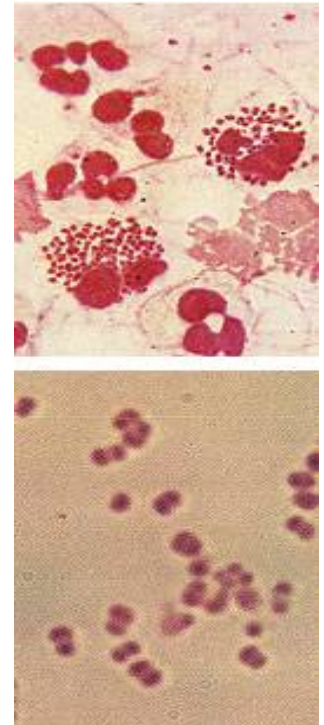
Current: Gram and Culture (GOLD STANDARD)

- Male urethral, rectal and pharyngeal
- Female endocervical
- Microscopy (gram stain)
- Culture (New York City selective media)
- Biochemical identification – Bactocard, API NH
- Serological confirmation - Phadebact

Future: Molecular detection

- Screening assay: Abbott Real-timeCT/NG assay
- Confirmatory assay: Real-time *porA* assay
 - Assay sensitivity and specificity
 - CDC guidelines

Transport and non-invasive specimen



Abbott RealTime™ CT/NG Assay

- Targets for NG = opa gene
- All strains of *N. gonorrhoeae* 11-13 copies
- *N. meningitidis* 3 - 4 copies
- *N. lactamica* 2 copies
- Common *Neisseria* spp. 1 copy
- **Target region of choice:** Promoter region upstream of the opa translational start site (Tabrizi *et al*, Geraats-Peters *et al*)
- Abbott RealTime™ CT/NG Assay – proprietary, promoter region
- Homologous real-time PCR assay – CT and NG
- Internal control monitors process from extraction to detection and detects inhibitors

Abbott *multi* - Collect Kit

- CE marked assay
- Validated sample types include
 - Male: urine & urethral swab
 - Female: urine, endocervical & vaginal swabs

Transport: Sample stable for 14 days at RT



Sterile Specimen Swab with scored shaft



Transport Tube



Transfer Pipette

Abbott CT/NG Assay Procedure

Abbott m2000sp

**Automated
extraction**

PCR setup



Abbott m2000rt

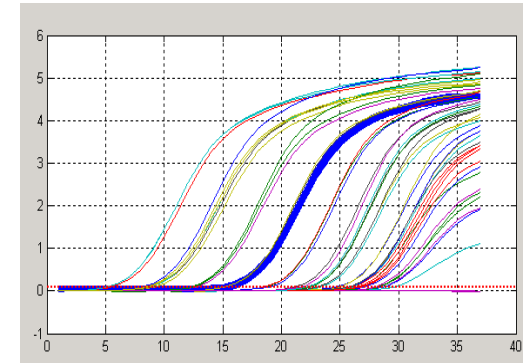
**PCR amplification
and detection**



Amplification Plot

**Positive results
expressed in DC
values**

**Proportional to the
starting copy DNA**



In-House Real-time PCR *porA* Assay Procedure

- Singleplex assay

PCR ASSAY

- TARGET: *porA* pseudogene
- Widespread in *N. gonorrhoeae* strains
- *N. meningitidis* - *porA* gene
- Absent in *Neisseria* spp.

PCR REACTION

- PCR reaction volume = 50µl
- Primers and hydrolysis probe of Unemo *et al*
- NG primers 900nM
- NG Taqman probe 200nM
- 10µl extracted DNA (DNA extracted on the *m2000*)
- TaqMan Universal Mastermix
- Universal Thermal Cycling Conditions
- ABI Prism® 7500 SDS
- Results expressed as cycle threshold (Ct) values

Assay Evaluation

MATERIALS

Known clinical isolates

- *N. gonorrhoeae* isolates: (n = 36)
- *N. spp.* isolates: (n = 26)
- QCMD Ng DNA 08 EQA Proficiency Panel

Analytical sensitivity: QCMD quantitated DNA sample

Clinical trial – randomly selected – GUIDE, GMHP

- Urogenital: Male urine (n = 164), endocervical samples (n = 58)
- Extragenital: Pharyngeal (n = 93), rectal (n = 91)

METHODS

- Known clinical isolates :
 - Abbott *opa* assay
 - In-house *porA* assay
- Clinical trial samples :
 - Screening assay Abbott *opa* assay
 - Positive samples confirmed *porA* in-house assay.

DEFINITION OF A TRUE POSITIVE

- A sample that is culture positive or positive with the both NAAT assays

Study Results : *N.gonorrhoeae* & *N. spp.* isolates

N. gonorrhoeae (n = 36)

N.spp (n = 26)

- *N. meningitidis* (n = 6),
- *N. lactamica* (n = 4),
- *N. polysaccharea* (n = 4),
- *N. sicca* (n = 7),
- *N .cinerea* (n = 4),
- *N. flava* (n = 1)

opa assay

		<i>porA</i>	
		Pos	Neg
opa assay	Pos	36	0
	Neg	0	26

Study Results for QCMD Ng DNA 08A Proficiency Panel

QCMD 2008	QCMD NG Results		Abbott <i>opa</i> Assay		In-House <i>porA</i> Assay	
	NG	Sample conc	NG	NG DC	NG	NG Ct
NG 08 - 01	Pos	2.0 x 10 ⁻³ Diln	Pos	2.91	Pos	38
NG 08 - 02	Weak Pos	1.0 x 10 ³ CFU/ml	Pos	1.77	Pos	40
NG 08 - 03	Pos	1.0 x 10 ⁻³ Diln	Pos	0.55	Pos	41
NG 08 - 04	Strong Pos	1.0 x 10 ⁵ CFU/ml	Pos	8.64	Pos	33
NG 08 - 05	Neg	-	Neg	-	Neg	-
NG08 - 06 (<i>N.lactamica</i>)	Neg	1.0 x 10 ⁵ CFU/ml	Neg	-	Neg	-
NG 08 - 07	Pos	1.0 x 10 ⁻² Diln	Pos	0.20	Pos	42
NG 08 - 08	Pos	1.0 x 10 ⁻³ Diln	Pos	4.50	Pos	37
NG 08 - 09 (<i>N.cinerea</i>)	Neg	1.0 x 10 ⁵ CFU/ml	Neg	-	Neg	-
NG 08 - 10	Strong Pos	1.0 x 10 ⁻² Diln	Pos	4.02	Pos	38

Sample No. 6 (*N. lactamica*): Incorrect in 95% laboratories – Roche Amplicor or the Roche COBAS Amplicor

Sample No. 9 (*N. cinerea*): Incorrect in 74% laboratories – BD ProbeTec assay

Abbott assay (n =5) - three samples negative

Study Results for Analytical Sensitivity

- **Limit of Detection (LOD):** Lowest conc. to give a reproducible positive result
- *N. gonorrhoeae* : Quantitated QCMD Sample No. 4: 1×10^5 CFU/mL.
- Marshall *et al* : LOD of 20 copies DNA / PCR reaction or 2 organisms / reaction
- Unemo *et al* : LOD of 7.5 Geq / PCR reaction

Dilution (Pre-sample preparation)	NG DNA copies / PCR reaction	No. of dilutions per series	Abbott <i>opa</i> Assay LOD	In-house <i>porA</i> Assay LOD
1	10 - 90	8	30	40

Overview of Clinical Trial Results – Urogenital Specimens

		NAATs			
		<i>opa & porA</i>			
NG Culture			Positive	Negative	Total
		Male Urine	Positive	2	0
	Negative	0	162	162	
	Total	2	164	164	
Endocervical Swab	Positive	1	0	1	
	Negative	0	57	57	
	Total	1	57	58	

Male Urine ($n = 164$)

% Positivity = 1.2%

Endocervical Swab ($n = 58$)

% Positivity = 1.7%

Overview of Clinical Trial Results – Extragenital Specimens

		NAATs			
NG Culture			Positive	Negative	Total
		Pharyngeal Swab	Positive		4
Negative			2	87	89
Total			6	87	93
Positive			6	0	6
Negative			1	84	85
Total			7	84	91

Pharyngeal Swab ($n = 93$)

Rectal Swab ($n = 91$)

Analysis of Discrepant Results

Specimen Type	NG culture	Other sites culture pos	Abbott <i>opa</i> original & repeat	In-house <i>porA</i>	Reported as
Pharyngeal Swab	Neg	Rectal +	Pos (DC = 3.41) (DC = 6.26)	Pos (Ct = 33.8)	Pos
Pharyngeal Swab	Neg	Rectal +	Pos (DC = 0.97) (DC = 2.74)	Pos (Ct = 40.13)	Pos
Rectal Swab	Neg	Rectal +, but one month post Rx	Pos (DC = 17.29) N/T	Pos (Ct = 24.8)	Pos
Male Urine	Neg	Neg	Pos (DC = 0.04) (Neg)	Neg	Indeterminate

REPORTING

***opa* + *porA* + :** *N. gonorrhoeae* DNA (PCR) = Detected - Interpretative comment
***opa* + *porA* - :** *N. gonorrhoeae* DNA (PCR) = Indeterminate, please repeat
***opa* - *porA* - :** *N. gonorrhoeae* DNA (PCR) = Not Detected

Sensitivity, Specificity, PPV & NPV

Specimen Type	Assay Type	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Male Urine	NG Culture	100%	100%	100%	100%
	NAATs	100%	100%	100%	100%
Endocervical Swab	NG Culture	100%	100%	100%	100%
	NAATs	100%	100%	100%	100%
Pharyngeal Swab	NG Culture	66%	100%	100%	97.7%
	NAATs	100%	100%	100%	100%
Rectal Swab	NG Culture	85.7%	100%	100%	98.8%
	NAATs	100%	100%	100%	100%

Conclusions

- Automated extraction combined with the real-time PCR *opa* and *porA* assays are **sensitive and specific for detection of *N.gonorrhoeae***
- Abbott *opa* assay has greater sensitivity than the in-house *porA* assay
- **Urogenital:** NAAT assays suitable to replace detection by culture
- **Extragenital:**
 - NAAT not validated, test extragenital samples in parallel with culture
 - NAATs more sensitive for detection of NG from pharyngeal swabs
- High-throughput, minimum labour, sample stability, non-invasive specimens

Susceptibility testing

- Symptomatic patients:
 - 1st visit: Culture, Abbott tube and Rx
- Asymptomatic patients:
 - 1st visit: Abbott Tube
 - 2nd visit (if positive): Culture and Rx



Thank you

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