

Validation of the Roche cobas® 4800 CT/NG assay on eSwab samples

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Introduction and purpose

Chlamydia trachomatis and *Neisseria gonorrhoeae* are two of the most prevalent sexually transmitted pathogens. Nucleic acid amplification tests (NAAT) are used extensively for their diagnosis.

The Roche cobas® 4800 system combines a fully automated nucleic acid extraction with real-time PCR technology. The cobas® 4800 CT/NG assay for amplification and detection of *C. trachomatis* (CT) and *N. gonorrhoeae* (NG) is validated by the manufacturer to be performed on swabs submitted in cobas® PCR medium. As we use eSwab (Copan) in our lab, our aim was to validate the cobas® 4800 CT/NG assay on genital specimens submitted in eSwab medium.

Methods

Cellysis was performed by adding 500 µL cobas® medium to 500µL of the eSwab sample. Extraction was done automatically on the cobas® x480, followed by amplification and detection on the cobas® z480. The cobas® 4800 CT/NG assay was checked for analytical sensitivity, accuracy, specificity, precision and stability following international publications on molecular validation methods (Raymaekers *et al.* 2011, Rabenau *et al.* 2007).



QCMD	Correct result		Result cobas 4800	
NG10-08	NG	5x10 ⁶ copies/mL	NG	Ct 27.2
NG10-09	Neg		Neg	
NG10-10	NG	5x10 ⁶ copies/mL	NG	Ct 31.9
NG11-07	NG	1000 CFU/mL	NG	Ct 35.3
NG11-08	NG	1x10 ⁴ CFU/mL	NG	Ct 32.3
NG11-09	NG	100 CFU/mL	NG	Ct 39.1
NG11-10	Neg		Neg	

Table 1 NG

QCMD	Correct result		Result cobas 4800	
CT11-07	CT	3x10 ⁴ copies/mL	CT	Ct 32.9
CT11-08	CT	1500 copies/mL	CT	Ct 36.4
CT11-09	Neg		Neg	
CT11-10	CT	250 copies/mL	CT	Ct 42
CT12-06	CT	156 copies/mL	CT	Ct 37.5
CT12-07	CT	781 copies/mL	CT	Ct 34.8
CT12-08	CT	31 copies/mL	Neg	
CT12-09	Neg		Neg	
CT12-10	CT	6 copies/mL	CT	Ct 40

Table 2 CT

Results

Analytical sensitivity: To determine the limit of detection (LOD with a 95% hit rate), Vircell CT and NG DNA controls were used. The LOD on eSwab samples was 1200 copies/mL for CT and 520 copies/mL for NG. Based on literature (Wiggins *et al.* 2009), the mean CT load on vaginal swabs is 10405 copies/mL. We didn't find any data about the mean NG load.

Accuracy: QCMD (Quality Control for Molecular Diagnostics) panels (swab samples) were used to check accuracy (Table 1-2). QCMD NG 2010 and 2011 were used for NG and showed 100% accuracy. QCMD CTA 2011 and 2012 were used for CT and showed 90% accuracy: 1 sample with a CT load of 31 copies/mL was not detected. Based on literature, this CT load is clinically not relevant.

Specificity: The assay was tested on a broad range of bacteria, fungi and viruses by the manufacturer, including other species of the genus *Neisseria*. No cross-reactivity was found. We tested two samples containing *Neisseria cinerea* (QCMD) which had a negative result.

Precision: Samples were tested in triplicate on three different days. The standard deviations (SD) for weak positive samples were 0.7 Ct (threshold cycle) for CT and 0.4 Ct for NG, meeting our validation criterion of SD <1 Ct.

Stability: One positive CT and one positive NG swab were stored during 5 days at 2-8°C. After five days, no significant change in Ct-value was noted.

Conclusions

The cobas 4800 CT/NG assay performed well on eSwab samples and was implemented in our daily routine. Using eSwab has the advantage that in addition to molecular tests, also bacterial and yeast cultures can be performed on the same sample.