Most but not all laboratories can detect the recently emerged *Neisseria gonorrhoeae porA* mutants – results from the QCMD 2013 *N. gonorrhoeae* external quality assessment programme

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We describe the results of the Quality Control for Molecular Diagnostics 2013 *Neisseria gonorrhoeae* external quality assessment programme that included an *N. gonorrhoeae* strain harbouring an *N. meningitidis porA* gene which causes false-negative results in molecular diagnostic assays targeting the gonococcal *porA* pseudogene. Enhanced awareness of the international transmission of such gonococcal strains is needed to avoid false-negative results in both in-house and commercial molecular diagnostic assays used in laboratories worldwide, but particularly in Europe.

In recent years, false-negative test results using PCRs targeting the *Neisseria gonorrhoeae porA* pseudogene have been reported from Australia, Scotland, Sweden and England [1-4]. Further investigations revealed that the gonococcal strains were not clonal, but all had replaced large segments or their entire *N. gonorrhoeae porA* pseudogene with an *N. meningitidis porA* gene.

This report describes the results of the Quality Control for Molecular Diagnostics (QCMD) 2013 *N. gonorrhoeae* External Quality Assessment (EQA) programme. It included an *N. gonorrhoeae* strain containing an *N. meningitidis porA* gene which gives rise to false-negative results in molecular diagnostic assays targeting the gonococcal *porA* pseudogene. QCMD (www.qcmd.org) is an independent international organisation which provides a wide range of molecular EQA services in the field of infectious diseases to over 2,000 participants in over 100 countries.

Quality assessment

The *N. gonorrhoeae porA* mutant strain included in the QCMD 2013 *N. gonorrhoeae* proficiency testing programme (NgDNA13) was isolated in 2011 in Sweden [3]. The strain was cultured on gonococcal agar media and diluted in an *N. gonorrhoeae*-negative urine sample to a concentration of 1.0×10^4 copies/mL. Following lyophilisation, the sample was distributed on 17 June 2013 to participating laboratories as part of the QCMD NgDNA13 proficiency testing panel, along with instructions on how the samples were to be processed. Laboratories tested the panel samples using their routine molecular diagnostic method for the detection of *N. gonorrhoeae*. Test results, together with details of the assays used, were returned to QCMD via a dedicated online data collection system. The deadline for submitting results was 19 July 2013.

Results

The NgDNA13 panel was sent to 286 laboratories in 35 countries, 23 of which are located in the World Health Organization (WHO) European Region. A total of 304 datasets with unambiguous test results (i.e. positive or negative) for the *N. gonorrhoeae porA* mutant were returned (Table 1). Some laboratories used several molecular diagnostic methods and delivered more than one dataset. Participants were also requested to specify the target gene of their assay, and 281 datasets (92%) contained this information (Table 2).

In total, 27 datasets (9%) reported the *N. gonorrhoeae porA* mutant sample as negative, and there was a highly significant association between reporting use of the *porA* pseudogene as target gene and reporting negative results in the *N. gonorrhoeae* molecular diagnostics (chi-square test, $p<0.001$). In total, 29 datasets reported the *porA* pseudogene as their sole assay target. Of these 29 datasets, 18 reported a negative result and 11 reported a positive result. The additional nine datasets that could not detect the *N. gonorrhoeae porA* mutant reported as target the *piv*NG gene (n=2), *porA* and 16S rRNA gene (n=2), *porA* and *opa* genes (n=1), *opa* genes (n=1), 16S rRNA gene (n=1), cryptic plasmid

and amino acetyltransferase gene (n=1), or did not report the gene (n=1) (Table 2).

Results in 268 datasets (88%) were from a commercially available molecular technology. In 36 datasets (12%), an in-house PCR assay had been used. Of the most frequently used commercially available *N. gonorrhoeae* molecular assays, only one manufacturer (Seegene) was reported to use the *N. gonorrhoeae porA* pseudogene as the only target gene. Fourteen datasets (5%) were generated by Seegene assays (Table 2) used in six different countries (data not shown). In addition, 14 of the 36 in-house molecular assays also used the *porA* pseudogene as the sole target gene.

The 27 datasets (9%) reporting the *N. gonorrhoeae porA* mutant sample as negative, had been created with different in-house PCR assays (n=13), Seegene assays (n=6), Sacace assays (n=2), Geneproof assay (n=2), Abbott assay (n=1), Bioneer assay (n=1), BD ProbeTec assay (n=1) and Siemens assay (n=1) (Table 2).

Table 1

Participating laboratories in the QCMD 2013 *Neisseria gonorrhoeae* external quality assessment programme, 2013 (n=286)

Country	Number of participants	Number of datasets returned with positive or negative result[a]
Austria	3	3
Azerbaijan	1	1
Belgium	57	53
Czech Republic	5	5
Denmark	4	3
Estonia	4	6
Finland	1	1
France	18	19
Germany	1	1
Hong Kong	2	3
Hungary	1	2
Iceland	1	1
Indonesia	1	1
Ireland	4	4
Israel	6	5
Italy	5	4
Jamaica	1	1
Kenya	1	1
Luxembourg	2	7
Namibia	1	1
The Netherlands	53	63
Netherlands Antilles	1	1
New Zealand	1	1
Norway	7	5
Portugal	2	2
Slovenia	2	2
South Africa	4	4
South Korea	2	5
Spain	2	1
Sweden	11	11
Switzerland	33	34
Tanzania	1	0
Thailand	2	2
United Kingdom	43	47
United States	3	4
Total	**286**	**304**

[a] Participants may submit more than one dataset, e.g. when they have several *N. gonorrhoeae* assays available.

Discussion

Gonorrhoea remains a major public health problem globally [5], and *N. gonorrhoeae* has developed resistance to all antimicrobials used for treatment of gonorrhoea, which is of grave concern worldwide [6]. In settings with sufficient resources, molecular diagnostic methods have to a large extent replaced conventional culture diagnosis. However, a considerable number of molecular diagnostic assays for *N. gonorrhoeae* (both commercial and in-house) have shown cross-reactivity with other *Neisseria* spp. [7-9]. This suboptimal specificity has led in Europe and Australia to the recommendation that positive tests should be confirmed with another molecular detection assay targeting a different gene sequence [10,11]. The gonococcal *porA* pseudogene is often used as a target sequence in in-house PCRs and in some new commercial assays for confirmatory testing, and in some settings also for primary diagnostic examination.

In the present quality assessment for the detection of *N. gonorrhoeae*, the *opa* genes were the most commonly used individual targets (25%), followed by the DR-9 repeat sequence gene (19%), 16S rRNA (13%) and the *piv*NG gene (11%). Forty-six datasets (15%) were generated by a method targeting the *N. gonorrhoeae porA* pseudogene, either alone (n=29) or in combination with additional genes (n=17). Surprisingly, 11 laboratories using assays reported to target the *N. gonorrhoeae porA* pseudogene alone (Seegene assays and some in-house real-time PCRs), were able to detect the *N. gonorrhoeae porA* mutant sample. These results require further investigation, and it is likely that additional molecular tests and/or targets were used but were not reported. For example, although the *porA* pseudogene was reported as the sole target by all participants, the Seegene AnyplexII STI-7 Detection and Seegene Seeplex STI Master ACE Detection assays are dual-target assays (*porA* pseudogene and one additional gene) according to the manufacturer.

Most molecular diagnostic assays targeted other genetic sequences than the *N. gonorrhoeae porA* pseudogene, and accordingly detected the *porA* mutant sample correctly. However, an enhanced awareness of the potential emergence of gonococcal mutants resulting in false-negative results in the molecular diagnostic tests is essential because of the ongoing evolution

Table 2

Results for the *Neisseria gonorrhoeae porA* mutant sample NgDNA quality assessment programme, 2013 (n=304)

Kit/Assay typea	Assay target[a]	Results on *porA* mutant sample	
		Positive	Negative
Bioneer AccuPower CT and NG Real-Time PCR Kit	*porA* pseudogene	0	1
Seegene Anyplex CT/NG Real time detection	*porA* pseudogene	1	0
Seegene AnyplexII STI-7 Detection	*porA* pseudogene	4	0
Seegene Seeplex STD6 ACE Detection	*porA* pseudogene	0	4
Seegene Seeplex STD6B ACE Detection	*porA* pseudogene	1	1
Seegene Seeplex STI Master ACE Detection	*porA* pseudogene	2	1
Real time in-house PCR	*porA* pseudogene	3	10
Conventional in-house PCR	*porA* pseudogene	0	1
Geneproof Neisseria gonorrhoeae PCR kit	*porA* pseudogene and 16S rRNA gene	0	2
Diagenode C. trachomatis and N. gonorrhoeae Real-Time PCR	*porA* pseudogene and *opa* genes	3	0
Diagenode Neisseria gonorrhoeae Real-Time PCR	*porA* pseudogene and *opa* genes	11	0
Real time in-house PCR	*porA* pseudogene and *opa* genes	0	1
Abbott RealTime CT/NG	*opa* genes	58	1
Goffin Presto CT-NG assay	*opa* genes	3	0
TestLine Clinical Diagnostics Real-time Neisseria gonorrhoeae	*opa* genes	1	0
Real time in-house PCR	*opa* genes	13	0
Roche COBAS 4800 System CT/NG assay	DR-9 repeat sequence	59	0
Roche COBAS Amplicor CT/NG	M-Ngo PII	3	0
Gen-Probe APTIMA Combo 2 Assay	16S rRNA gene	33	0
Gen-Probe APTIMA GC Assay	16S rRNA gene	4	0
Institute of Applied Biotechnologies as Neisseria gonorrhoeae RG detect	16S rRNA gene	1	0
Real time in-house PCR	16S rRNA gene	1	1
BD Probetec ET CT/GC Amplified DNA Assay	pivNG gene	8	0
BD Probetec ET CT/GC/AC Amplified DNA Assay	pivNG gene	13	0
BD ProbeTec GC Qx Amplified DNA Assay	pivNG gene	6	1
Bio-Rad Dx CT/NG/MG assay	pivNG gene	1	0
Siemens VERSANT CT/GC DNA 1.0 Assay (kPCR)	pivNG gene	3	1
Fast-track Diagnostics FTD Gonorrhoea confirmation	*opa* genes and pivNG gene	1	0
Fast-track Diagnostics FTD STD9	*opa* genes and pivNG gene	1	0
Fast-track Diagnostics FTD Urethritis	*opa* genes and pivNG gene	1	0
Fast-track Diagnostics FTD Urethritis basic	*opa* genes and pivNG gene	2	0
Fast-track Diagnostics FTD Urethritis plus	*opa* genes and pivNG gene	1	0
Fast-track Diagnostics FTD Vaginal swab	*opa* genes and pivNG gene	1	0
TIB MOLBIOL NG TaqMan 48	*opaD* gene	1	0
TIB MOLBIOL NG TaqMan 48	90 bp fragment	1	0
TIB MOLBIOL LightMix Kit 480HT CT and NG	*opaD* gene	3	0
TIB MOLBIOL LightMix Kit 480HT CT and NG	90 bp fragment	2	0
TIB MOLBIOL LightMix Kit 480HT CT and NG	gyrA gene	4	0
TIB MOLBIOL LightMix Kit 480HT CT and NG	nvt gene	1	0
Sacace Neisseria gonorrhoeae Real-TM	CCp + Methyltransferase gene	1	0
Sacace Neisseria gonorrhoeae 370/660 IC	Cryptic plasmid and amino acyltransferase gene	0	1
EuroClone Duplica Real Time Neisseria gonorrhoeae	Cryptic plasmid pJD1 and 16S rRNA gene	1	0
Conventional in-house PCR	cppB gene and 16S rRNA gene	1	0
Real-time in-house PCR	cppB gene	1	0
Cepheid GeneXpert CT/NG assay	not reported	16	0
Ecoli s.r.o. Amplisens Neisseria gonorrhoeae-test	not reported	1	0
Sacace C. trachomatis/N. gonorrhoeae/M. genitalium Real-TM	not reported	1	0
Sacace N. gonorrhoeae/C. trachomatis/T. vaginalis/ M. genitalium Real-TM	not reported	0	1
Real-time in-house PCR	not reported	3	0
Conventional in-house PCR	not reported	1	0
Total		**277**	**27**

[a] As reported by 2013 external quality assessment participants

and genetic diversity of *N. gonorrhoeae* and other bacteria. For example, the new variant of *Chlamydia trachomatis* reported from Sweden had a 377 bp deletion in the cryptic plasmid, which contained the target sequences for the *C. trachomatis* molecular assays from two main manufacturers and resulted in many thousands of false-negative tests in Sweden [12-14]. With the replacement of conventional culture methods, laboratories need to be aware of the risk of emergence of these mutant strains that cannot be detected by molecular assays.

In conclusion, enhanced awareness of the international transmission of *N. gonorrhoeae porA* mutant strains is needed to avoid false-negative results in several molecular diagnostic assays, both in-house and commercial. The opportunities to use combinations of different diagnostic methods such as several molecular methods, molecular methods and culture, and multi-target methods, remain exceedingly valuable.

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Conflict of interest

None declared.

Authors' contributions

DL wrote the initial draft. All authors were involved in the analysis of the results and preparations of the final draft of the paper and tables.

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