DIAGNOSTICS

Laboratory diagnosis of *Neisseria gonorrhoeae* in St Petersburg, Russia: inventory, performance characteristics and recommended optimisations

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Objectives: To perform a comprehensive inventory of the number of samples, performance characteristics, and quality assurance of the laboratory diagnosis of *Neisseria gonorrhoeae* at five laboratories in St Petersburg and Leningradskaya Oblast, Russia, in 2004, and to recommend optimisations for an increased adherence to international evidence based recommendations of diagnostics.

Methods: Surveillance data were obtained with questionnaire and site visits. For evaluation of the culture media utilised at the laboratories, *N gonorrhoeae* reference strains (n = 29) were used.

Results: During 2004 the total numbers of *N gonorrhoeae* samples analysed at the five laboratories using microscopy of stained smears and culturing were 330 879 (407 positive) and 38 020 (420 positive), respectively. Four laboratories used a Russian non-selective culture medium—that is, Complegon, and one laboratory utilised Biocult-GC. Both media seemed suboptimal. Only two of the laboratories used any species confirmative assay. Antibiotic susceptibility testing of *N gonorrhoeae* was performed at only two of the laboratories and each year only occasional isolates were analysed. None of the laboratories comprised a complete laboratory quality assurance system.

Conclusions: According to international recommendations, the diagnosis of *N gonorrhoeae* in St Petersburg and Leningradskaya Oblast, Russia, is suboptimal. More samples need to be analysed by culturing on a highly nutritious and selective medium and, furthermore, species confirmation and antibiotic susceptibility testing should be more frequently performed. In addition, the utilised methods for culturing and antibiotic susceptibility testing, including medium and interpretative criteria used, ought to be optimised, standardised, and quality assured using systematic internal and external quality controls.

Since the mid-1990s, the incidences of gonorrhoea in several west European countries have increased.¹ In contrast, the estimated gonorrhoea incidences in several east European countries—for example, Russia, Belarus, and the Baltic countries, have declined almost annually since the mid-1990s.^{2–3} In 2003, the gonorrhoea incidences in the city St Petersburg and the surrounding region Leningradskaya Oblast, Russia, were 53 cases per 100 000 and 34.4 cases per 100 000 inhabitants, respectively.² However, reliable incidence figures in many east European countries are still lacking, which is mainly the result of suboptimal diagnostics, incomplete epidemiological surveillance, and self treatment.

Despite the development of many DNA/RNA based methods, culturing of *Neisseria gonorrhoeae* remains the gold

standard for diagnosis mainly because of the opportunity to perform antibiotic susceptibility testing. St Petersburg and Leningradskaya Oblast have populations of 4.6 million inhabitants and 1.6 million inhabitants, respectively. Nevertheless, only three municipal laboratories of the dermatovenereology dispensaries in St Petersburg and one in Leningradskaya Oblast are obliged to perform culturing of *N gonorrhoeae* routinely. In addition, one federal laboratory is confirmed to perform culture diagnostics for gonorrhoea. However, more municipal, federal, or private laboratories may perform culturing or nucleic acid amplification tests (NAATs) of *N gonorrhoeae* (under investigation). The remaining institutions exclusively use microscopy of stained smears for diagnosis.

The aims of this study were to perform a comprehensive inventory of the number of samples, performance characteristics, and quality assurance of the laboratory diagnosis of *N gonorrhoeae* at five laboratories in St Petersburg and Leningradskaya Oblast, Russia, in 2004, and to recommend optimisations for an increased adherence to international evidence based recommendations for diagnosis.

MATERIALS AND METHODS

All laboratories confirmed to perform culturing of *N gonorrhoeae* in St Petersburg and Leningradskaya Oblast, Russia, in 2004 (n = 5) were included. Surveillance data were obtained with questionnaire and site visits.

The questionnaire, comprising 28 main questions with up to four subheadings, and the site visits, focused on collecting information regarding number of samples, performance characteristics (that is, sampling procedures, types of specimens, transportation of samples, diagnostic methods utilised, etc), and quality assurance of the *N gonorrhoeae* diagnostics.

For evaluation of the culture media utilised at the laboratories, suspensions of 29 *N gonorrhoeae* reference strains were cultured on the evaluated medium and, for comparison, on non-selective and selective international reference medium.⁴

For evaluation of the *N gonorrhoeae* diagnostics at the laboratories, international evidence based recommendations,^{4–6} were used.

RESULTS AND DISCUSSION

In 2004, the total numbers of diagnosed samples regarding *N gonorrhoeae* at the five laboratories using microscopy of stained smears and culturing were 330 879 (of which 407 were positive) and 38 020 (420 positive), respectively (table 1). Consequently, most of the diagnostics was based only on microscopy because of the higher costs attributed to culturing.

Abbreviations: NAATs, nucleic acid amplification tests; PCR, polymerase chain reaction

Table 1 Number of samples, gender of the patients, and type of specimens analysed using microscopy of stained smears and culturing of *N gonorrhoeae* at five laboratories in St Petersburg and Leningradskaya Oblast, Russia, in 2004

Lab*	Number of samples analysed using microscopy† (positive)										Number of samples analysed using culturing (positive)									
	Male					Female					Male					Female				
	In total	Urethra	Rectum	Semen	Cervix	Urethra	Rectum	Semen	Cervix	Vulva (girls)	In total	Urethra	Rectum	Semen	Cervix	Urethra	Rectum	Semen	Cervix	Vulva (girls)
A	184 980 (10)	12 756 (3)	—	—	100 464 (5)	71 760 (2)	—	—	—	—	1558 (3)	1294 (1)	—	50 (1)	214 (1)	—	—	—	—	—
B	28 151 (190)	2901 (52)	—	—	14 620 (90)	10 630 (48)	—	—	—	—	1502 (58)	174 (6)	2 (1)	—	915 (36)	—	—	—	—	—
C	14 101 (182)	2387 (29)	—	—	8493 (92)	3077 (61)	—	—	—	144 (—)	14 548 (194)	2313 (34)	36 (—)	4 (—)	11 789 (91)	411 (15)	—	—	—	—
D	101 579 (6)	1016 (—)	—	—	75 680 (6)	24 883 (—)	—	—	—	—	20 381 (165)	1728 (32)	106 (—)	—	17 342 (132)	120 (68)	—	—	—	286 (1)
E	2068 (19)	820 (7)	—	—	849 (12)	399 (—)	—	—	—	—	31 (—)	10 (—)	—	—	21 (—)	1012 (—)	—	—	—	193 (1)
In total	330 879 (407)	19 880 (91)	—	—	200 106 (205)	110 749 (111)	—	—	—	144 (—)	38 020 (420)	5519 (73)	144 (1)	54 (1)	30 281 (260)	1543 (83)	—	—	—	479 (2)

*All the laboratories performed bedside microscopy and culturing.
†All smears were stained with methylene blue and Gram staining.

Urethral specimens (all the male samples and 36% of the samples from women), cervical specimens, and vulval specimens from girls were analysed using microscopy (table 1). However, according to the instructions of the laboratories, specimens from vagina, rectum, prostate, and conjunctiva may also be used. Microscopy of stained urethral exudates from symptomatic men may comprise a high sensitivity (95%) and specificity (97%).^{4,7} Consequently, according to international guidelines,^{4,6} these samples do not have to be cultured for routine laboratory diagnosis. However, microscopy of stained cervical smears comprises only 40–60% sensitivity and the specificity is lower (80–95%).^{4,7} Accordingly, cervical samples, as well as samples for definitive diagnosis of rectal and pharyngeal gonorrhoea, asymptomatic infection, or for test of cure ought to be cultured,^{4,5,7} or at least antigen or nucleic acid of *N gonorrhoeae* should be detected.^{5,6} The small proportion of positive samples in the microscopy at the laboratories may indicate that the performance can be optimised. However, no obvious shortcomings were identified and the results may reflect that many patients belonged to a low risk population. Nevertheless, microscopy of stained urethral, cervical, and rectal exudates may facilitate an immediate provisional diagnosis in most symptomatic cases.⁵ For confirmation, however, culturing, amplified antigen detection tests or NAATs, should be performed on all samples.⁵ Most importantly, culturing allows antibiotic susceptibility testing.⁵

Urethral specimens (97% of the male samples and 5% of the samples from women), cervical specimens, rectal specimens (2% of the male samples), semen (1% of the male samples), and vulval specimens from girls were cultured (table 1). However, according to the instructions of the laboratories, vaginal, pharyngeal, and conjunctival specimens may also be cultured. The four municipal laboratories mainly used the non-selective Russian culture medium Complegon and the federal laboratory utilised Biocult-GC (table 2).

A limited evaluation of four different batches of Complegon and one batch of Biocult-GC indicated a suboptimal quality—that is, interbatch variation, lack of selectiveness (Complegon), and insufficient growth ability (time for identifiable growth, number, and size of colonies), of both media. Larger volumes of a more nutritious and selective medium, which is crucial for extragenital specimens but also for cervical and urethral specimens, should be considered.⁴ This is optimally combined with a non-selective medium in order also to isolate occasional *N gonorrhoeae* strains that are susceptible to the selective antibiotics used. However, such strains are rare in most countries.⁴ Furthermore, inoculated plates should be examined after 18–24 hours' incubation and, if negative, also after 48 hours.⁴

Only two of the laboratories utilised any species confirmative assay—that is, *Neisseria* 4H for extragenital isolates (laboratory B) and API NH and AmpliSens *Neisseria gonorrhoeae* PCR kit for all isolates (laboratory A) (table 2). Consequently, only these laboratories could provide a definitive diagnose of *N gonorrhoeae* in clinical specimens from all examined body sites,⁴ (table 2).

Two of the laboratories performed antibiotic susceptibility testing of *N gonorrhoeae* (table 2). However, this assay was rarely requested by the treating physician and a few isolates were analysed each year. The disc diffusion method was utilised on Complegon and, notably, the utilised interpretative criterion—that is, zone diameter break points, had been determined on a Mueller Hinton agar based medium. Nowadays, the antibiotic resistance of *N gonorrhoeae* is widespread globally and is increasing in most countries.^{7–12} However, there are marked geographical variations and therapy should be informed also by local surveillance of susceptibility.^{5,8,10,11} The knowledge regarding the antibiotic

Table 2 Performance characteristics of *N gonorrhoeae* culturing at five laboratories in St Petersburg and Leningradskaya Oblast, Russia in 2004

Lab*	Culture medium	Quality control of the medium growth (sterility)	Incubation time	Species confirmation	Antibiotic susceptibility testing†
A	BioCult-GC‡	Every new batch, <i>N gonorrhoeae</i> reference strains are cultured (36–38°C, 48 hours)	48 hours	Oxidase test, Microscopy (Gram staining, 1000× magnification), API-NH ₂ , PCR¶	–
B	Complegon** (60 mm plates, 10–12 ml per plate) or BioCult-GC‡ (for extragenital specimens)	If recognised problem, <i>N gonorrhoeae</i> clinical isolate is cultured (36–38°C, 48 hours)	48 hours (examined also after 24 hours)	Oxidase test, Microscopy (Gram, 900×), Neisseria-4H††	Rarely
C	Complegon** (40 mm plates, 5 ml per plate)	Every new batch, <i>N gonorrhoeae</i> clinical isolate is cultured (36–38°C, 48 hours)	48 hours (examined also after 24 hours)	Oxidase test, Microscopy (Gram and 1000×)	–
D	Complegon** (3–3.5 ml slanted in glass tubes or, for children, 60 mm plates, 10–15 ml per plate)	If recognised problem, <i>N gonorrhoeae</i> clinical isolate is cultured (37°C, 24 hours)	24 hours	Oxidase test, Microscopy (Gram and 1000×)	Rarely
E	Complegon** (3–3.5 ml slanted in glass tubes)	– (37°C, 24 hours)	72 hours (examined also after 24 and 48 hours)	Oxidase test, Microscopy (Gram and 1000×)	–

*All the laboratories performed bedside microscopy and culturing.

†Disc diffusion method was utilised on Complegon. The discs were purchased from Research Centrum for Pharmacotherapy (St Petersburg, Russia) or HiMedia Laboratories Limited (Mumbai, India). The utilised interpretative criteria – that is, zone diameter break points, were from HiMedia Laboratories Limited and had been determined on a Mueller Hinton agar based medium.

‡Slides (5.0×1.5 cm) with a thin layer (1–2 mm) of selective culture medium – that is, modified Thayer-Martin medium, which are incubated in accompanying tubes with sterile water in the bottom and carbon dioxide (CO₂) generating tablets at 36–38°C. BioCult-GC and accessories, including oxidase reagent, are manufactured by Orion Diagnostica, Espoo, Finland.

¶API NH (bioMérieux, Lyon, France) for biochemical species confirmation.

¶AmpliSens *Neisseria gonorrhoeae* PCR kit (InterlabService, Moscow, Russia), which is a Russian PCR assay that separately targets both the *oppB* gene and the *cytB* gene.

**Non-selective Russian culture medium manufactured by St Petersburg Institute of Vaccines and Sera, Russia, which contains agar, rabbit meat extract, Bacto-pepton, NaCl, autolysate of yeasts, orotic acid (6-carboxyuracil), bovine serum, and hydrolysate of casein. The inoculated medium was incubated in candle jars with a small volume of water in the bottom at 36–38°C.

††Neisseria 4H (Bio-Rad, Marnes-la-Coquette, France) for biochemical species confirmation.

susceptibility of *N gonorrhoeae* in Russia is incomplete,^{11 12} and in St Petersburg and Leningradskaya Oblast, reliable information concerning the level of antibiotic resistance of *N gonorrhoeae* is entirely lacking.

None of the laboratories had a complete, implemented laboratory quality assurance system. However, comprehensive Russian guidelines from 1986, which certainly need to be updated, were available at all the laboratories.

In conclusion, this study provides unique information regarding a suboptimal diagnosis of *N gonorrhoeae* in St Petersburg and Leningradskaya Oblast, Russia, which may reflect the situation in several other geographic areas of Russia. For adherence to international evidence based recommendations, more samples need to be analysed by culturing on a highly nutritious and selective medium and, furthermore, species confirmation and antibiotic susceptibility testing should be more frequently performed. In addition, the utilised methods for culturing and antibiotic susceptibility testing, including medium and interpretative criteria used, ought to be optimised, standardised, and quality assured using systematic internal and external quality controls. For achievement of such crucial improvements, the results of the present study, including recommendations of concrete optimisations, have been reported to the surveyed laboratories and an evaluation of a selective culture medium as well as a local sentinel study concerning the antibiotic susceptibility of *N gonorrhoeae* are in progress.

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CONTRIBUTORS

MU, MD, ES, and AS designed the study as well as analysed all the data; MU wrote the manuscript in collaboration with all co-authors; ML performed the laboratory evaluation of the culture media; MD and OB translated all written material provided by the Russian laboratories.

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