

Culture confirmation of gonococcal infection by recall of subjects found to be positive by nucleic acid amplification tests in general practice

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1	Culture confirmation of gonococcal infection by recall of subjects found to be positive by
2	nucleic acid amplification tests in general practice
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Abstract

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20 **Objectives:** To evaluate a routine notification of general practitioners to recall nucleic acid 21 amplification test (NAAT) positive subjects for culture of Neisseria gonorrhoeae to confirm 22 gonococcal infection in the community. 23 **Methods:** A retrospective observational study of the routine testing for *N. gonorrhoeae* by 24 analysis of test results compiled from the laboratory information system in two departments 25 of clinical microbiology. 26 **Results:** Altogether, 158 male and female subjects with NAAT positive results for *N*. 27 gonorrhoeae were included in the study. Samples for culture of N. gonorrhoeae were 28 collected from 102/158 (64.6%) subjects recalled after a NAAT assay was found positive. 29 Growth of N. gonorrhoeae was seen in the samples from 54/102 (52.9%) of the re-examined 30 NAAT positive subjects. Among subjects with samples collected within the first week after 31 the positive NAAT test, 34/44 (77%) were confirmed positive by culture. 32 **Conclusion:** This study shows that it is possible for the general practitioner to recall a 33 substantial number of NAAT positive subjects to collect swabs for culture of N. gonorrhoeae 34 to confirm gonococcal infection in the community. The majority of recall samples is culture 35 positive if collected within a week of the NAAT positive test, and may provide a sufficient

monitoring of the drug susceptibility of N. gonorrhoeae strains in the community.

38 INTRODUCTION: The gold standard of laboratory diagnosis of gonorrhoea has been the 39 culture of the causative agent, Neisseria gonorrhoeae (1). An increasing number of patients in 40 our area are primarily diagnosed with gonorrhoea by a nucleic acid amplification test (NAAT) 41 only. The aim of this study was to evaluate the recall of NAAT positive subjects to collect 42 swabs for culture confirmation of gonococcal infection in the community. 43 44 METHODS: A retrospective observational study was performed from March 2009 to March 45 2010 including all male and female subjects diagnosed by a general practitioner (GP) with a 46 NAAT positive sample for N. gonorrhoeae (NG) only. Samples were received and examined 47 in two department of clinical microbiology from their combined catchments area of 1,000,000 people. The Gen-Probe APTIMA COMBO 2[®] (AC2) and the APTIMA[®] GC (AGC) NAAT 48 assays (Gen-Probe, San Diego, USA) were used. Positive AC2 assays were repeated and 49 50 furthermore examined with the AGC assay to confirm the initial positive result. The final 51 laboratory report routinely notified the GP that the patient should be re-examined by culture 52 to obtain an antimicrobial susceptibility pattern. 53 Swabs for culture of NG (charcoal imbibed cotton swab in a Stuart medium) were transported 54 to the laboratory on the same day or mailed by ordinary postal service, and cultured on a 55 chocolate agar plate supplemented with Polymyxin B, Lincomycin, and Amphotericin B at 56 35°C and 10% CO₂ for 48 hours. N. gonorrhoeae was identified by Gram stain and 57 conventional biochemical tests. 58 The unique Danish civil registry number (CPR) was used to cross link the laboratory results 59 of all samples for NG testing of an individual, and to establish a chronological order of the 60 samples.

RESULTS: A total of 158 subjects, 52 from Veile and 106 from Aarhus, were included. All initially positive AC2 assays were confirmed by repeat testing and the single analyte assay AGC. Table 1 shows the distribution of female and male samples. Altogether, 102/158 (64.6%) NAAT positive subjects had swabs collected for culture by a recall examination. A positiv culture of N. gonorrhoeae was found in 54/102 (52.9%). Twenty subjects were not included in the study because they had a culture for N. gonorrhoeae collected together with the positive NAAT sample, 16/20 (80%) were culture positive. Corresponding figures during a two-year period at the clinic of Genito-Urinary Medicine using similar NAAT and culture assays were 40/45 = 89%. Growth of N. gonorrhoeae was seen in 34 of 44 (77%) subjects recalled within the 1st week after their positive NAAT test. Corresponding figures for the 2nd week, and the 3rd week or later were 13/32 (41%) and 7/21 (33%), respectively (χ^2 =15.1, degrees of freedom=2, P<0.001).

76 DISCUSSION:

Recall samples for culture of *N. gonorrhoeae* were collected from two thirds of NAAT positive subjects. Among subjects with samples collected within the first week after the positive NAAT test, three quarters were confirmed positive by culture. The weakness of the retrospective observational design of the present study is the lack of data on antibiotic treatment prior to or after the initial NAAT screening assay, which may preclude the growth of *N. gonorrhoeae* in a recall culture. Furthermore that information was not available to show whether failure to recall was due to subjects not attending or to GPs not routinely requesting culture samples. Culture confirmation of the NAAT positive results is also challenging because of the rapid loss of viability of *N. gonorrhoeae* strains during the transportation of the samples from the community to the laboratory (1). Previous studies also found that about two

87	thirds of the NAAT results could be confirmed by culture (2, 3). However, urgent
88	transportation and culture of samples can increase the percentage of samples with growth of
89	N. gonorrhoeae to more than 85% (3). The present study shows that it is possible for the
90	general practitioner to recall a substantial number of NAAT positive subjects to collect swabs
91	for culture of N. gonorrhoeae to confirm gonococcal infection in the community. Provided
92	that random selections of NAAT positive subjects are re-examined by culture, recall cultures
93	may provide an accurate picture of antibiotic resistance in N. gonorrhoeae strains in the
94	community. To obtain additional follow-up samples, GPs may be requested to recall subjects
95	from whom samples were not received for culture within a week after the initial positive
96	NAAT assay.
97	In conclusion, the majority of recall samples is culture positive if collected within a week of
98	the NAAT positive test, and may provide a sufficient monitoring of the drug susceptibility of
99	N. gonorrhoeae strains in the community.
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Tables

Table 1. Recall of NAAT positive subjects to colle	ect swabs for culture of N. gonorrhoeae
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126	Table 1. Recall of NAAT positive subjects to collect swabs for culture of <i>N. gonorrhoeae</i>									
127										
128		NAAT positive	Swabs for culture			Culture positive				
129		No.	No.	%	(95%CI)	No.	%	(95%CI)		
130										
131	Female subjects	69	51	74	(62 - 83)	22	43	(30 - 57)		
132	Urogenital swab ^a	65	49			21				
133	Urine	4	2			1				
134										
135	Male subjects	89	51	57	(47 - 67)	32	63	(49 - 75)		
136	Urine	70	42			28				
137	Urethral swab	19	9			4				
138										

^a Samples from urethra, cervix and vagina