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► **To cite this version:**

Jens Kjølseth Møller. Culture confirmation of gonococcal infection by recall of subjects found to be positive by nucleic acid amplification tests in general practice. *Sexually Transmitted Infections*, 2010, 86 (6), pp.478. 10.1136/sti.2010.043703 . hal-00576099

HAL Id: hal-00576099

<https://hal.science/hal-00576099>

Submitted on 12 Mar 2011

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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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9 Keywords: NAAT, *Neisseria gonorrhoeae*, culture, recall samples, general practice

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11 Revised version

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18

19 **Abstract**

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

37

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
48 people. The Gen-Probe APTIMA COMBO 2[®] (AC2) and the APTIMA[®] GC (AGC) NAAT
49 assays (Gen-Probe, San Diego, USA) were used. Positive AC2 assays were repeated and
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.

61

62 RESULTS: A total of 158 subjects, 52 from Vejle and 106 from Aarhus, were included. All
63 initially positive AC2 assays were confirmed by repeat testing and the single analyte assay
64 AGC. Table 1 shows the distribution of female and male samples. Altogether, 102/158
65 (64.6%) NAAT positive subjects had swabs collected for culture by a recall examination. A
66 positive culture of *N. gonorrhoeae* was found in 54/102 (52.9%). Twenty subjects were not
67 included in the study because they had a culture for *N. gonorrhoeae* collected together with
68 the positive NAAT sample, 16/20 (80%) were culture positive. Corresponding figures during
69 a two-year period at the clinic of Genito-Urinary Medicine using similar NAAT and culture
70 assays were 40/45 = 89%.

71 Growth of *N. gonorrhoeae* was seen in 34 of 44 (77%) subjects recalled within the 1st week
72 after their positive NAAT test. Corresponding figures for the 2nd week, and the 3rd week or
73 later were 13/32 (41%) and 7/21 (33%), respectively ($\chi^2=15.1$, degrees of freedom=2,
74 $P<0.001$).

75

76 DISCUSSION:

77 Recall samples for culture of *N. gonorrhoeae* were collected from two thirds of NAAT
78 positive subjects. Among subjects with samples collected within the first week after the
79 positive NAAT test, three quarters were confirmed positive by culture. The weakness of the
80 retrospective observational design of the present study is the lack of data on antibiotic
81 treatment prior to or after the initial NAAT screening assay, which may preclude the growth
82 of *N. gonorrhoeae* in a recall culture. Furthermore that information was not available to show
83 whether failure to recall was due to subjects not attending or to GPs not routinely requesting
84 culture samples. Culture confirmation of the NAAT positive results is also challenging
85 because of the rapid loss of viability of *N. gonorrhoeae* strains during the transportation of the
86 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.

100

101 ACKNOWLEDGEMENTS, COMPETING INTERESTS, FUNDING

102 There are no competing interests.

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109 Word count: Abstract (208), Text (749).

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- 123
- 124

125 **Tables**126 Table 1. Recall of NAAT positive subjects to collect swabs for culture of *N. gonorrhoeae*

127 -----

	NAAT positive	Swabs for culture			Culture positive			
	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	-----							

139 ^a Samples from urethra, cervix and vagina

140