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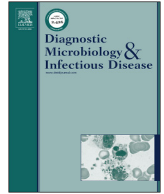

Evaluation of the APTIMA Combo 2 Assay using self-administered vaginal swabs for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

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ABSTRACT

We report on the performance of the APTIMA Combo 2 assay for the detection of chlamydial infection and gonorrhea using self-administered vaginal specimens in specimen transport medium and specimen kept dry. The results are compared with the results using the Amplicor assay and the BD ProbeTec assay™.

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Nucleic acid amplification tests (NAATs) are widely used for the diagnosis of chlamydial infection and gonorrhea. However, there are concerns about the specificity of NAATs and, in particular, of those used to detect *Neisseria gonorrhoeae* (NG) (Geraats-Peters et al., 2005; Palmer et al., 2003; Tabrizi et al., 2011). False-positive test results are reported, and it is therefore recommended to confirm positive NG results with a second NAAT in a different format amplifying a different target (Bignell, 2009; Bignell and FitzGerald, 2011; Johnson et al., 2002; Smith et al., 2005). This should be performed on the original specimen, which is not always feasible due to eventual incompatibility of the transport system. The majority of the manufacturers recommend a stabilization medium to store and transport the specimens. The media are often specific for the assay. Besides the media need to be available at the site of collection, it increases the costs and the risk of spillage during transport.

We assessed in the past the performance of the Amplicor *Chlamydia trachomatis* (CT)/NG test and the BD ProbeTec assay™ ET CT and NG amplified DNA assay using genital swab specimens transported in dry tubes and stored at −20 °C until processing (Van Dyck et al., 2001). We report here the evaluation of the APTIMA Combo 2 assay using self-administered vaginal specimens stored in dry tubes and compare it with the results obtained for the specimens stored in the APTIMA specimen transport medium (STM).

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Specimens from cross sectional surveys conducted among female sex workers in Ivory Coast were used (Vuylsteke et al., 2012). The participants collected 2 self-administered vaginal specimens, 1 with a culturette EZ swab (Becton-Dickinson, Sparks, MD, USA) and 1 with the APTIMA collection kit including the STM (Gen-Probe, San Diego, CA, USA). The EZ swabs were kept in dry tubes (dry swabs), the APTIMA swabs were kept in STM (STM swabs), and both were stored at −20 °C until shipment on dry ice to the Institute of Tropical Medicine.

The dry swabs were thawed and suspended in 1500 μL of diluted phosphate-buffered saline (PBS) (9 parts saline and 1 part PBS). Aliquots of eluted specimens were extracted as described elsewhere (Van Dyck et al., 2001) and tested with the Amplicor assay (Roche, Molecular Systems, Branchburg, NJ, USA) and the BD ProbeTec assay™ (Becton-Dickinson) according to the manufacturer's instruction. An additional aliquot was tested with the APTIMA Combo 2 assay (AC2) (Gen-Probe). The aliquot of 250-μL elute was first added to the APTIMA swab specimen collection tube, incubated for 5 min at room temperature, and from there on, testing was resumed according to the instructions provided by Gen-Probe.

The STM swabs were tested with the AC2 according to the manufacturer's instruction.

All results were interpreted per the manufacturer's recommendations. Specimens were considered to be true positive according to the expanded gold standard and defined by a positive result for CT or NG in at least 2 independent assays.

A total of 1106 vaginal dry swabs and 1092 vaginal STM swabs were tested. The prevalence of CT and NG was 6.6% and 6.0%, respectively. The results obtained for the detection of CT and GC using the different assays are presented in Tables 1 and 2, respectively. We

Table 1
Results obtained with the different molecular amplification assays detecting CT on the same sample, as well as with the APTIMA assay performed on the swabs stored in the APTIMA transport medium.

Results	Amplicor $n = 1106$	ProbeTec $n = 1106$	APTIMA $n = 1106$	APTIMA + transport medium $n = 1092$
True positive[a]	63	65	65	64
True negative	1026	1033	1019	998
False positive	6	0	13	21
False negative	10	8	8	9
Inhibition/no result	1	0	1	0

[a] A specimen was defined to be true positive if a positive result was obtained in at least 2 independent assays.

Table 2
Results obtained with the different molecular amplification assays detecting NG on the same sample, as well as with the APTIMA assay performed on the swabs stored in the APTIMA transport medium.

Results	Amplicor $n = 1106$	ProbeTec $n = 1106$	APTIMA $n = 1106$	APTIMA + transport medium $n = 1092$
True positive[a]	58	64	41	52
True negative	997	1022	1037	1020
False positive	43	18	1	7
False negative	8	2	24	11
Inhibition/no result	0	0	3	2

[a] A specimen was defined to be true positive if a positive result was obtained in at least 2 independent assays.

obtained comparable sensitivity and negative predictive values for the detection of CT using the 3 assays. There was also no difference between the use of dry swabs and STM in the AC2. However, for this assay, the specificity and positive predictive value were lower compared to the 2 other assays, and this was independent of the swab storage condition (Table 3).

There was more variation in the performance of the 3 assays in the detection of NG. A very low sensitivity was observed for the AC2 using dry swabs. The sensitivity of the assay improved for the STM swabs but was still lower compared to the Amplicor and the ProbeTec. The specificity of the AC2 was superior to 99% for dry swabs and for STM swabs. The highest sensitivity was observed with the ProbeTec, and the lowest specificity was obtained with the Amplicor (Table 4).

Each of the assays detects a different target in a different format or amplification method. The Amplicor is based on a regular end-point polymerase chain reaction and targets a sequence of the cryptic plasmid of CT and of the M Ngo PII gene of NG. The ProbeTec amplifies another sequence of the cryptic plasmid of CT and the pilin-inverting gene of NG in a strand displacement amplification method. The AC2 is a transcription-mediated amplification assay and replicates specific regions within the 23 rRNA of CT and the 16S rRNA of NG.

Table 3
Sensitivity, specificity, positive predictive value, and negative predictive value obtained with the different molecular amplification assays detecting CT on the same sample, as well as with the APTIMA assay performed on the swabs stored in the APTIMA transport medium.

	Amplicor	ProbeTec	APTIMA	APTIMA + transport medium
Sensitivity, % (95% CI)	86.3 (75.8–92.9)	89.0 (79.0–94.8)	89.0 (79.0–94.8)	87.7 (77.4–93.9)
Specificity, % (95% CI)	99.4 (98.7–99.7)	100 (99.5–100)	98.7 (97.8–99.3)	97.9 (96.8–98.7)
PPV, % (95% CI)	91.3 (81.4–96.4)	100 (93.0–100)	83.3 (72.8–90.5)	75.3 (64.5–83.7)
NPV, % (95% CI)	99.0 (98.2–99.5)	99.2 (98.4–99.6)	99.2 (98.4–99.6)	99.1 (98.2–99.6)

PPV = positive predictive value; NPV = negative predictive value; CI, confidence interval.

Table 4
Sensitivity, specificity, positive predictive value, and negative predictive value obtained with the different molecular amplification assays detecting NG on the same sample, as well as with the APTIMA assay performed on the swabs stored in the APTIMA transport medium.

	Amplicor	ProbeTec	APTIMA	APTIMA + transport medium
Sensitivity, % (95% CI)	87.9 (77.0–94.3)	97.0 (88.5–99.5)	63.1 (50.2–74.4)	82.5 (70.5–90.6)
Specificity, % (95% CI)	95.9 (94.4–97.0)	98.3 (97.2–98.9)	99.9 (99.4–100)	99.3 (98.5–99.7)
PPV, % (95% CI)	57.4 (47.2–67.1)	78.0 (67.3–86.1)	97.6 (86.0–99.9)	88.1 (76.4–94.7)
NPV, % (95% CI)	99.2 (98.4–99.6)	99.8 (99.2–100)	97.7 (96.6–98.5)	98.9 (98.0–99.4)

The 3 NAATs showed almost identical sensitivities in the detection of CT. The false-positive results obtained with the AC2 could be true positives, which were not detected by any of the 2 other assays and which explains the lower specificity of the AC2. Theoretically, the AC2 could be more sensitive as it targets ribosomal RNA, which is present in high copy number in bacteria and infected cells (Gaydos et al., 2010). The use of the APTIMA STM had no impact on the performance of the AC2 in the detection of CT.

The AC2 failed to detect a remarkable number of NG among the dry swabs but not among the STM swabs. This effect was observed for NG and not for CT and may be attributable to several factors. The first factor is a possible overall lower concentration of the NG target in the infected specimens. The second factor is a degradation of the RNA in the absence of protective STM. It is currently unknown whether different RNA species may have different stabilities (Tsui et al., 2002), but a less stable NG RNA compared to CT RNA could explain our observation. However, the specificity of the AC2 to detect NG was remarkably better compared to Amplicor and SDA (Tabrizi et al., 2011).

In conclusion, the performance of the AC2 for the diagnosis of chlamydial infection on self-administered vaginal specimens stored dry or in STM is comparable to the AMPLICOR and the ProbeTec. The specificity of the AC2 for the detection of gonorrhea is considerably better compared to the AMPLICOR and the ProbeTec, albeit that the swabs have to be stored in STM to ensure the stability of the NG RNA and the sensitivity of the AC2.

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