Performance Evaluation of a New Culture Colorimetric Detection Assay

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ABSTRACT

Objective: To evaluate the performance of the culture colorimetric detection assay MYCO WELL D-ONE® (MWD-ONE), designed to detect sexually transmitted infections using real-time polymerase chain reaction (PCR) as a reference method.

Materials and Methods: One hundred and ten urogenital samples were screened for *Gardnerella vaginalis* (GV), *Trichomonas vaginalis* (TV), *Mycoplasma hominis* (MH), *Mycoplasma spp.*, and *Ureaplasma urealyticum* (UU)/*Ureaplasma parvum* (UP) using the MWD-ONE and real-time PCR assays *Gardnerella vaginalis*/*Lactobacillus* species Real-TM Quant and Anyplex II STI-7 Detection, respectively.

Results: GV was detected in 33 samples by both the MWD-ONE and real-time PCR, while 6 samples gave discordant results. TV was detected by both MWD-ONE and Anyplex II STI-7 Detection kits in 3 samples, while 107 were negative. MH was detected by both methods in 5 cases, 4 samples gave discordant results, and 101 were negative. *Mycoplasma genitalium* (MG) was detected by Anyplex II STI-7 in 2 cases, 1 of which was detected as *Mycoplasma spp.* by MWD-ONE. Ten samples were positive by MWD-ONE, and 98 were negative with both assays. With regard to *UU/UP,* 24 cases were detected by MWD-ONE and Anyplex PCR, 25 by PCR only, 4 by MWD-ONE, and 57 tested negative with both methods.

The positive predictive values (PPV) and negative predictive values (NPV) of the MWD-ONE assay for the pathogens tested were as following: GV, PPV 94.3%, NPV 94.7%; TV, PPV and NPV 100%; MH, PPV 71.4%, NPV 98.1%; *Mycoplasma spp.,* PPV 9.1%, NPV 98.9%; and *Ureaplasma spp.,* PPV 85.7 %, NPV 69.5 %. The agreement between the MWD-ONE and PCR was strong for GV and MH (k=0.8 and 0.7, respectively); perfect for TV (k=1); and moderate for UU/UP (k=0.4).

Conclusion: MWD-ONE assay appears to be suitable for routine testing of sexually transmitted infections.

Keywords: MYCO WELL D-ONE, sexually transmitted infections, colorimetric detection assay, real-time PCR

Introduction

Sexually transmitted infections (STI) are among the most common acute conditions in the human population worldwide. They have a great impact on sexual and human health and represent an important health burden [1]. There are over 30 infections that can be sexually transmitted, caused by bacteria, viruses, and parasites. Most of these infections are asymptomatic or have mild symptoms, and if unrecognized and left untreated, they may have serious consequences. In this work, we focused on some of sexually transmitted pathogens: *Gardnerella vaginalis* (GV), *Trichomonas vaginalis* (TV), *Mycoplasma hominis* (MH), *Mycoplasma genitalium* (MG), *Ureaplasma urealyticum* (UU), and *Ureaplasma parvum* (UP). These pathogens have been associated with clinical conditions such as bacterial vaginosis, pelvic inflammatory disease, urethritis, cervicitis, infertility, ectopic pregnancy, and adverse pregnancy outcomes [2–7].

Currently, real-time polymerase chain reaction (PCR) is the method of choice to detect these microorganisms because of its high sensitivity, specificity, and capability to search for multiple targets [8–11]. However, in low-resource settings, molecular methods are not always available, and less expensive methods may be required. Colorimetric culture assays based on the detection of live pathogens through enzymatic reactions may be a valid alternative [12]. In this study, we tested the performance of a new colorimetric detection assay, MYCO WELL D-ONE, which detects GV, TV, MH, MG, UU, and UP, and the results were compared with those obtained by using Anyplex II STI-7 Detection kit for TV, MH, MG, UU, and UP, and Gardnerella vaginalis/Lactobacillus species Real-TM Quant assay for GV.

Materials and Methods

Study Group

One hundred and ten outpatients with a suspected urogenital infection were admitted to the laboratory of Clinical Microbiology, Polyclinic Tor Vergata, Rome, Italy, and screened for sexually transmitted pathogens including GV, CT, NG, TV, MH, MG, UU, and UP. The number of obtained biological samples was as follows: 70 cervical, 30 vaginal, and 8 urethral swabs, and 2 seminal liquid, which were stored at +4°C until analysis. The study was carried on residual samples, otherwise destroyed, following a routine diagnostic analysis. The identification number of the patient was converted into an alphanumerical code making anonymous all information regarding the patient. Therefore, no informed consent was required. The Polyclinic Tor Vergata Foundation Ethics Committee approved the study (Protocol R.S. 152/17).

Detection of GV, TV, MH, MG, UU, and UP by the MYCO WELL D-ONE Assay

The urogenital samples were processed with the MYCO WELL D-ONE assay following the manufacturer's instructions (CPM SAS, Formello, Italy). Samples were suspended in sterile saline solution for 3–5 minutes until obtaining a homogeneous suspension. Then, 150 µl of the obtained suspension were added to each well plate (wells 1 to 32). All wells were covered with two drops of sterile paraffin, except wells 27 and 31, which were dedicated to the microscopic observation (40X). A series of antibiotics (levofloxacin, tetracycline, moxifloxacin, erythromycin, and clindamycin) at different concentrations were inoculated in the wells 9 through 24 according to the Clinical & Laboratory Standards Institute guidelines [13]. Then, the samples were incubated at 36±1°C for 24–48 h.

Detection of GV by the Gardnerella vaginalis/Lactobacillus Species Real-TM Quant Assay

GV was detected by real-time PCR using the Gardnerella vaginalis/Lactobacillus species Real-TM Quant assay (Sacace Biotechnologies, Como, Italy), which targets the 16S rRNA region of both GV and Lactobacillus species. The PCR amplification was performed according to the manufacturer's instructions.

Detection of TV, MH, MG, UU, and UP by Anyplex II STI-7 Detection

Anyplex II STI-7 Detection (Seegene, Seul, Korea) is a multiplex real-time PCR assay that enables detection of the seven major causative agents of sexually transmitted infections (CT, NG, TV, MH, MG, UU, and UP) in a single reaction tube. The assay is based on the Dual-Priming Oligonucleotides and Tagging Oligonucleotide Cleavage and Extension technology, as already described [11].

Statistical Analysis

The performance of MYCO WELL D-ONE test was compared with that of the real-time PCR, which was used as the gold standard. Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were calculated for GV, TV, MH, MG, UU, and UP. Confidence intervals at the 95% were estimated assuming exact binomial distribution. The agreement between the two tests was evaluated by the Cohen's kappa statistics. All analyses were performed using the STATA software version 11 (Stata Corporation, College Station, TX).

Results

Of the 110 biological samples tested, GV was detected by both methods in 33 samples (30 %); 2 samples (1.81 %) were positive with MWD-ONE, 4 samples (3.6%) with GV Real-TM Quant real-time PCR, and 71 samples were negative with both methods.

TV was detected by MWD-ONE and Anyplex PCR in 3 out of 110 (2.73%) samples. The remaining samples were all negative.

Mycoplasma hominis (MH) was detected in 2 out of 110 (1.81%) only with Anyplex PCR and in 2 out of 110 (1.81%) only with MWD-ONE. A concordant result was obtained in five cases. One hundred and one samples tested negative with both methods.

Mycoplasma genitalium (MG) was detected by Anyplex II STI-7 in 2/110 (1.81%), one of which was also detected by MWD-ONE as Mycoplasma species because the latter assay cannot discriminate between MG and Mycoplasma species. Ten more samples positive with MWD-ONE tested negative with Anyplex II STI-7 assay, suggesting the presence of mycoplasmas different from MG. The remaining 98 samples gave a negative result with both methods.

The Anyplex assay detected UU in 6 out of 110 (5.45%), UP in 29 out of 110 (26.3%) and UU+UP in 14 out of 110 (12.7%), respectively. Four samples were positive with MWD only, and 57 were negative with both assays. Results are summarized in Table 1.

Sensitivity, specificity, PPV, and NPV of MYCO WELL D-ONE assay toward GV, TV, MH, *Mspp.* and *Uspp.* were calculated using the real-time PCR as the gold standard. Results are reported in Table 2.

The level of agreement between the two tests measured by Cohen's kappa statistics was strong for GV (k=0.8) and MH (k=0.7), perfect for TV (k=1), and moderate for UU/UP (k=0.4) (Table 2).

Discussion

Laboratory diagnosis of STI is crucial for the choice of appropriate therapy and for preventing the sequelae induced by the transmitted pathogen. Over the years, several diagnostic tests have been developed and used to detect STI: culture methods, serological and hybridization assays, and antigen detection [14–18]. In this study, we evaluated the performance of a new culture colorimetric detection assay named MYCO WELL D-ONE, that detects some of the sexually transmitted pathogens, namely: GV, TV, MH, MG, UU, and UP. The last three pathogens (MG,

Table 1. Pathogens Detection by PCR and MYCO WELL D-ONE

	*M+/PCR+	M+/PCR-	M-/PCR+	M-/PCR-	Total	Cohen's kappa
Gardnerella vaginalis	33	2	4	71	110	0.8
Trichomonas vaginalis	3	-	-	107	110	1
Mycoplasma hominis	5	2	2	101	110	0.7
Mycoplasma genitalium	1	-	1	98	-	
†*Mycoplasma spp.*	-	10	-	-	110	
Ureaplasma urealyticum	3	-	3	-	-	
Ureaplasma parvum	8	-	21	-	-	
††*Ureaplasma urealyticum* + *Ureaplasma parvum*	13	4	1	57		
Total	24	4	25	57	110	0.4

*M: MYCO WELL D-ONE
†Mspp: Sector *Mycoplasma spp.* of MYCO WELL D-ONE (including eventually growth of *M.genitalium, M.fermentans, M.penetrans*, *M.pirum*, and other *M.spp.*, except *M.hominis*).
††Uspp: Sector of *Ureaplasma spp.* of MYCO WELL D-ONE (including *U.urealyticum* and *U.parvum*).

Table 2. Sensitivity, Specificity, Positive Predictive Values (PPV), and Negative Predictive Values (NPV) of the MYCO WELL D-ONE Assay

Sexually Transmitted Pathogens	Prevalence	Sensitivity	Specificity	Positive Predictive Value (NPV)	Negative Predictive Value (NPV)
Gardnerella vaginalis (N=110)	33.6%	89.2% (74.6–97.0)	97.3% (90.4–99.7)	94.3% (80.7–98.5)	94.7% (87.5–97.8)
Trichomonas vaginalis (N=110)	2.7%	100% (29.2–100)	100% (96.6–100)	100%	100%
Mycoplasma hominis (N=110)	6.4%	71.4% (29–96.3)	98.1% (92.4–99.7)	71.4% (29.0–96.3)	98.1% (92.4–99.7)
Mycoplasma spp. (N=110)	1.8%	50.0% (1.3–98.8)	90.7% (83.6–95.5)	9.1% (2.17–31.1)	99.0% (96.1–99.7)
Ureaplasma urealyticum + *Ureaplasma parvum* (N=110)	44.55%	48.98% (34.4–63.7)	93.4% (84.0–98.18)	85.71% (65–94.1)	69.5% (62.2–75.1)

UU, and UP) are detected, but not discriminated by this assay. Therefore, a colorimetric change toward the positivity in the well containing selective medium for the growth of mycoplasmas can be attributed not only to MG, but also to other *Mycoplasma spp.* such as *Mycoplasma fermentans, Mycoplasma penetrans,* or others. This would explain the discrepant results observed between the colorimetric assay and the real-time PCR. The same could apply to some discordant results observed in the case of GV (2) and UU/UP (4) detection. It can be envisaged that microorganisms present in the sample analyzed and characterized by similar biochemical requirements may cause a pH change in the well that is misinterpreted as positivity. This is a limitation of the assay because it requires further molecular testing to identify the pathogen involved.

Although PCR is currently the method of choice in the most-advanced microbiology laboratories, it is not always available in low-resource settings. In this contest, colorimetric culture methods can be a valid alternative [12]. Actually, despite the limitations reported above, the overall performance of the test was quite good. Cohen's kappa statistics revealed a strong agreement between the MWD-ONE and PCR for GV and MH, a perfect agreement for TV, and a moderate agreement for UU/UP. Furthermore, MWD-ONE showed a high NPV for all the microorganisms investigated, except UU/UP, making it suitable for routine testing of sexually transmitted pathogens.

In conclusion, although PCR is more sensitive and specific, MWD-ONE is an economical and practical system that could be appealing for laboratories equipped for general microbiology purposes, especially in low-resource settings. In addition, the assay offers the possibility to perform antimicrobial susceptibility testing in the same plate.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Polyclinic Tor Vergata Foundation.

Informed Consent: N/A

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