MolecularDiagnostics.be 2014 external quality control scheme on the molecular detection of Mycoplasma genitalium and Trichomonas vaginalis

P. Descheemaeker[1*]; B. Vanmassenhove[2]; S. Pereyre[3]; M. Raymaekers[4]; I. Micalessi[5]; T. Crucitti[6]; D. Ursi[7]; O. Pontesilli[8]; N. Pinon[9]; L. Coorevits[10]; and M. Reynders[1]

AZ St-Jan Brugge-Oostende, Bruges, Belgium[1]; AZ Damiaan Oostende, Ostend, Belgium[2]; Hôpital Pellegrin, Bordeaux, France[3]; Jessa Hospital, Hasselt, Belgium[4]; Imelda Hospital, Bonheiden, Belgium[5]; Institute Tropical Medicine, Antwerp, Belgium[6]; University hospital Antwerp, Belgium[7]; Cliniques du Sud Luxembourg, Arlon, Belgium[8]; Maasstad hospital, Rotterdam, The Netherlands[9]; University Hospital, Ghent, Belgium[10]

[1*] Corresponding author: P. Descheemaeker, Department of Laboratory Medicine, AZ Sint-Jan Brugge -Oostende, Ruddershove 10, 8000 Bruges, Belgium; Tel: ++32/50/452794; Fax: ++32/50/452619; Email: patrick.descheemaeker@azsintjan.be

Introduction

Mycoplasma genitalium (MG) and *Trichomonas vaginalis* (TV) infections are considered important emerging sexually transmitted infections potentially associated with reproductive tract sequelae, PID, preterm labor, infertility, non-gonococcal urethritis, epididymitis, and prostatitis.

No external quality assessment schemes were available in 2014 for the detection of MG and/or TV. One of the goals of the non-profit organization MolecularDiagnostics.be is to provide quality control samples especially for these parameters where QC samples are not readily available

Panel composition

DNA samples 1 to 4 were composed of two Vircell DNA control samples (Amplirun *Mycoplasma genitalium* DNA control, MBC085, 13200 copies/µl and Amplirun *Trichomonas vaginalis* DNA control, MBC079, 10000 copies/µl) diluted in a Tris/EDTA/16-23S RNA buffered medium until respectively 25, 10, 5 and 2,5 copies/µl MG and TV per sample was reached.

eSwab samples 2 to 7 (eSwab sample 1 was a negative sample) were composed as follows. A TV positive urine sample was diluted 10 times in eSwab medium and a MG culture was diluted 100 times in eSwab medium. Both these diluted samples were combined in equal volumes and further diluted in eSwab medium according to the dilution factors given in the table below.

Results

Extraction and amplifcation												
Lab		1a	1b	2	3	4	5	6	7	8	9	10
Extraction												
Amplification												
Equivalent of the sample in the reaction (µl)		4,6	4,6	20,0	100,0	11,1	16,7	40,0	41,7	27,7	20,0	83,3
Assay		In house	In house	In house	In house	Sacace Real-time PCR	S-DiaMGTV v1,0, Diagenode	In house	BioRad Dx CT/NG/MG assay	In house	S-DiaMGTV v1,0, Diagenode	S-DiaMGTV v1,0, Diagenode
NAAT results for *Mycoplasma genitalium*												
Target gene		pdhD	MgPa	MgPa	MgPa		MgPa & m219	pdhD	MgPa	MgPa	MgPa & m219	MgPa & m219
Direct amplification												
Sample ID	**Concentration**	Ct	Ct	Ct	Ct		Ct	Ct	Ct	Ct	Ct	Ct
DNA sample 1	25 copies/µl	32,30	32,28	31,58	32,30	--	32,55	29,47	35,80	34,10	35,00	34,61
DNA sample 2	10 copies/µl	32,05	33,00	32,62	32,70	--	33,48	30,54	37,80	35,00	35,00	36,54
DNA sample 3	5 copies/µl	34,14	34,36	33,97	34,53	--	35,10	31,93	36,10	35,60	35,00	37,72
DNA sample 4	2,5 copies/µl	neg	40,78	34,97	34,68	--	36,32	32,76	38,20	37,50	35,00	38,30
Extraction and amplification												
Sample ID	**Dilution factor**	Ct	Ct	Ct	Ct		Ct	Ct	Ct	Ct	Ct	Ct
eSwab sample 1	negative	neg	neg	neg	neg	--	neg	neg	neg	neg	neg	neg
eSwab sample 2	10^{-3}	23,29	23,88	28,71	28,30	--	25,59	23,70	27,00	25,50	27,50	32,77
eSwab sample 3	$10^{-3.5}$	25,39	25,90	32,77	28,40	--	28,00	25,80	29,50	28,50	28,70	30,97
eSwab sample 4	10^{-4}	26,53	28,21	**neg**	31,72	--	30,06	29,18	31,00	30,20	31,90	32,18
eSwab sample 5	$10^{-4.5}$	30,81	31,00	37,21	34,46	--	33,63	26,96	34,00	34,10	34,30	33,80
eSwab sample 6	10^{-5}	33,36	34,67	38,00	**neg**	--	36,37	**neg**	36,80	37,90	31,40	36,95
eSwab sample 7	$10^{-5.5}$	**neg**	**neg**	**neg**	36,46	--	37,94	**neg**	**neg**	39,10	**neg**	39,56
NAAT results for *Trichomonas vaginalis*												
Target gene		Btub	repeated seq	2kb repetitive seq		G3 hypothetical protein	repeated seq			2kb repeat	repeated seq	repeated seq
Direct amplification												
Sample ID	**Concentration**	Ct	Ct	Ct		Ct	Ct			Ct	Ct	Ct
DNA sample 1	25 copies/µl	29,51	24,95	21,4,	--	18,60	25,34	--	--	27,00	27,80	28,48
DNA sample 2	10 copies/µl	30,69	26,31	22,61	--	19,82	26,80	--	--	28,30	29,90	29,89
DNA sample 3	5 copies/µl	31,08	27,04	23,58	--	21,02	27,74	--	--	29,30	30,30	31
DNA sample 4	2,5 copies/µl	32,05	28,49	24,63	--	22,00	28,74	--	--	30,20	31,20	31,89
Extraction and amplification												
Sample ID	**Dilution factor**	Ct	Ct	Ct		Ct	Ct			Ct	Ct	Ct
eSwab sample 1	negative	neg	neg	neg	--	neg	neg	--	--	neg	neg	neg
eSwab sample 2	10^{-3}	29,36	24,57	28,73	--	19,39	26,43	--	--	24,50	28,00	32,7
eSwab sample 3	$10^{-3.5}$	30,90	26,54	31,14	--	20,81	28,35	--	--	26,10	29,60	31,33
eSwab sample 4	10^{-4}	33,62	29,12	**neg**	--	23,27	30,33	--	--	28,80	33,00	32,99
eSwab sample 5	$10^{-4.5}$	35,32	31,21	**neg**	--	25,64	34,25	--	--	32,20	35,00	35,44
eSwab sample 6	10^{-5}	**neg**	33,96	34,58	--	28,10	34,27	--	--	39,10	35,00	36,39
eSwab sample 7	$10^{-5.5}$	**neg**	34,03	**neg**	--	32,5,	37,49	--	--	40,20	35,00	**neg**

Conclusions

All participating laboratories performed equally well in detecting 2,5 copies TV-MG DNA/µl indicating an efficient amplification process. However, considering the simulated clinical samples, where pre-analytical factors and extraction efficiency play an additional but important role, some laboratories missed a few positive samples which is not correlated to the analytical sensitivity of the assay.

Acknowledgement

Besides all participating laboratories we especially thank Prof. Bébéar, Bordeaux, France for providing us the MG culture.