Comparing the technical performance of two real-time PCR kits for the detection of *Trichomonas vaginalis* in the laboratory

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Introduction and Purpose

- *Trichomonas vaginalis* (TV) is the most common non-viral cause of sexually transmitted diseases worldwide. The global prevalence of trichomoniasis is most likely underestimated since conventional laboratory tests lack sensitivity. The development of commercially available TV PCR kits offers an opportunity to improve the detection of this genital parasite in the laboratory.
- In the present study, the technical performance of the Trichomonas vaginalis Real-Time® PCR kit of Diagenode (Liège, Belgium) and the Trichomonas vaginalis Real-TM® kit of Sacace Biotechnologies (Como, Italy) is compared.

Methods

- **DNA extraction** was performed using a QIAsymphony® SP extraction robot (Qiagen inc., Hilden, Germany). All PCR analyses were performed according to the manufacturer's instructions. The extracted DNA was analyzed with both PCR kits using the Rotor-gene® Q analyzer (Qiagen inc., Hilden, Germany). The technical validation was performed in accordance with the Checklist for Optimization and Validation of Real-Time PCR Assays, published by Raymaekers et al. in 2009.[1]
- Fifteen TV positive and ten TV negative samples, detected with an in-house validated PCR method in the AZ Damiaan hospital, were reanalyzed with both PCR kits in order to evaluate **sensitivity** and **specificity**.
- **Specificity** was further evaluated by analyzing American Type Culture Collection strains and external quality controls of seven different genital pathogens, other than Trichomonas species: *Neisseria gonorrhoeae* (ATCC 43069), *Gardnerella vaginalis* (ATCC 14108), *Streptococcus agalactiae* (ATCC 13813), *Candida albicans* (ATCC 90028), *Chlamydia trachomatis* (Quality Control for Molecular Diagnostics (QCMD) CTB 2013-01), herpes simplex virus (QCMD HSV DNA 2013-02) and human papillomavirus (QCMD HPV 2013-06). In addition, four patient samples from the collection of the Institute of Tropical Medicine (ITG), positive with Trichomonas species other than *Trichomonas vaginalis,* were reanalyzed with both PCR kits. The analyzed Trichomonas species were: *T. foetus*, *T. gallinae* (two samples) and *T. gallinarum*.
- In order to evaluate linearity, a serial dilution of a quantified TV positive patient sample from the ITG collection was analyzed with both PCR kits. Linearity was evaluated with TV DNA concentrations ranging from 6,5 x 10^6 copies/mL (c/mL) to 5000 c/mL, 500 c/ml and 25 c/mL.
- To determine the **limit of detection** (LoD_{95}), a quantified TV positive patient sample from the ITG collection was diluted near the expected limit of detection and each dilution step was analyzed in tenfold, spread over eight days. Prior to PCR analysis, aliquots of the diluted TV DNA were stored at -20°C.

Results

- The PCR kits of Diagenode and Sacace detected TV DNA in 14 (93%) and 15 (100%) out of 15 TV positive samples, respectively. Quantification Cycle (Cq) values are listed in Table 1. In accordance with the manufacturer's instructions and in contrast to the PCR of Diagenode, fluorescence was not collected during the first five cycles of the PCR of Sacace. This results in lower Cq values obtained with the PCR kit of Sacace compared to the PCR kit of Diagenode.
- With neither PCR kit, false positive results were obtained.
- Both PCR kits showed best results for linearity with TV DNA concentrations ranging from 6,5 x 10^6 c/mL to 5000 c/mL. Even when considering this concentration range, not all criteria for efficiency, slope and coefficient of correlation, as suggested by Raymaekers et al.[1], were met. (Table 2)
- The LOD_{95} of the PCR kit of Diagenode and Sacace were 25 c/mL and 50 c/mL, respectively. (Table 3)

Table 1: Cq values of 15 TV positive samples reported with an in-house validated PCR test in the AZ Damiaan hospital and with the PCR kits of Diagenode and Sacace in the Jessa hospital

Sample number	Cq values		
	AZ Damiaan hospital	Jessa hospital	
		Diagenode	Sacace
1	20.24	19.81	13.07
2	21.75	20.34	12.84
3	17.92	20.12	14.25
4	17.71	21.28	14.29
5	25.00	28.06	23.66
6	27.97	32.53	29.93
7	18.80	22.20	15.05
8	23.34	21.53	15.15
9	18.80	22.17	16.02
10	16.32	18.64	12.37
11	24.76	31.42	25.07
12	25.66	29.27	23.26
13	19.75	21.29	14.80
14	28.06	Negative	27.35
15	21.74	20.42	13.85

In accordance with the manufacturer's instructions and in contrast to the PCR of Diagenode, fluorescence was not collected during the first five cycles of the PCR of Sacace. This results in lower Cq values obtained with the PCR kit of Sacace compared to the PCR kit of Diagenode.

Table 2: Efficiency, slope and coefficient of correlation detected with both PCR kits for TV DNA concentrations ranging from from 6,5 x 106 c/mL to 5000 c/mL

PCR kit	Parameter	Result	Reference values
Diagenode	Efficiency	0.99	≥ 0.90
	Slope	-3.6	-3.6 > m > -3.1
	Coefficient of correlation	0.89	≥ 0.99
Sacace	Efficiency	0.99	≥ 0.90
	Slope	-3.4	-3.6 > m > -3.1
	Coefficient of correlation	0.98	≥ 0.99

Results for linearity parameters, which do not meet de reference values as suggested by Raymaekers et al., are indicated in red.

Table 4: Number of aliquots of each dilution step, in which TV DNA was detected with the PCR kits of Diagenode and Sacace

Dilution step (c/mL)	Number of TV positive aliquots (n=10)	
	Diagenode	Sacace
500	10	10
100	10	10
50	10	10
25	10	7
10	7	6
Negative sample	0	0

Conclusions

- **Neither PCR kit showed good linearity.**
- **The LOD_{95} of the PCR kit of Diagenode and Sacace was 25 c/mL and 50 c/mL, respectively.**
- **In this laboratory evaluation, we found no big differences in technical performance of both PCR kits for the detection of *Trichomonas vaginalis*.**

References

[1] Raymaekers, M., Smets, R., Maes, B. and Cartuyvels, R. (2009), Checklist for optimization and validation of real-time PCR assays. J. Clin. Lab. Anal., 23: 145–151.

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