Modified transport medium for the laboratory detection of *Trichomonas vaginalis* a short multi-centre study

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V1.1 Error correction. Last paragraph 'Discussion' p.4 second line now reads 'sample 3'.
V1.2 Added thank you names to credits.

Introduction:

Trichomonas vaginalis is a protozoan parasite and the cause of the most prevalent curable sexually transmitted disease globally. WHO in 2012 estimated 276.4 million cases annually worldwide (2).

In a recent BMS EQA report on the laboratory detection of *T. vaginalis* the following details were recorded by participants on methods used,

The number of participating laboratories was 29. Some laboratories reported using more than one method.

Wet Preparation = 16
TV Culture = 11
Acridine Orange = 6
PCR = 1

If we take a couple of published comments on the most prevalent methods, then we need to consider the following. Microscopy is unlikely to detect low level infection where there may be $<10^4$ cells / ml. (3). Sensitivity of the method further decreases significantly where delays occur between taking the sample and examination (4).

Culture may produce positive results within 48 hours, although evidence for incubation of at least 7 days to enable the detection from low inoculum levels has been published (3). Certainly, from our own observations there is an initial load required in culture media to allow growth of TV to accelerate more quickly.

One odd point given in
"NICE - Trichomoniasis – How should I diagnose trichomoniasis in women?"
(Revised May 2020)

- **Take a high vaginal swab from the posterior fornix (or use a self-taken low vaginal swab)** for Gram staining and to exclude other causes of symptoms.

- Place all swabs for bacterial culture in Aimes transport medium with charcoal.
- Mark the request as 'suspected trichomonas infection' if the local laboratory does not routinely perform *Trichomonas vaginalis* wet microscopy or culture.
- Transport samples to the laboratory as soon as possible. If there is a delay in transportation, the swab should be refrigerated at 4°C for no longer than 48 hours.

This sounded okay until the reader is instructed to refrigerate TV swabs for up to 48 hours. A guaranteed negative result for Wet Prep, Culture and, in my experience, probably AO.

Purpose of the Study:

To improve the preparation of EQA samples for the detection of Trichomonas by producing samples with high numbers of motile cells. This would address two issues, firstly to provide for the possibility of varying the loading of TV organisms in EQA samples. Secondly to resolve the problem of potential false negative samples due to delayed transport where transit times are extended to 48 hours rather than our anticipated 24 hours.

Two laboratories were selected to take part in the study due to their geographical locations in Jersey and Shetland. In both cases the regular delivery time for EQA samples is 48 hours.

A simple in-house pilot study had shown that a modified transport medium potentially offered advantages in both loading of TV cells and extended transit times on survival of viable cells.

Current Preparation of EQA samples for TV analysis:

The current preparation method for EQA charcoal swabs uses a high concentration of viable TV cells from a 48-hour culture. An inoculum of 0.5ml is used per swab. Higher volumes result in an unacceptable increase in the likely event of leakage from the swab during transit.

The results for detection of TV from this method are usually quite scanty with a few viable cells per x 40 field.

Method:

Four sets of samples were prepared as follows.

Sample Set 1. CPLM Medium (E & O Labs) Positive Control

Sample Set 2. CPLM Medium (E & O Labs) Modification No. 1

Sample Set 3. CPLM Medium (E & O Labs) Modification No. 2

Sample Set 4. Charcoal Transport swab (MWE) Unmodified. Current EQA sample

Each of the sample sets 1 to 3 were inoculated with 0.5 ml from a 48-hour culture of TV and then incubated for a further 48 hours at 36.0°C (+/- 1.0°C).

Sample set 4 was inoculated with 0.5ml from a 48-hour culture on the day of despatch.

Sufficient samples for each set were produced so that the test laboratories could test one of each of the four samples daily for five consecutive days.

All samples were despatched on the same day with a requested delivery by 10:00am next day.

Laboratories were asked not to incubate the samples on receipt and to store all samples at room temperature (21°C +/- 3°C) prior to testing.

Laboratories were asked to examine one of each of the samples from each set on a daily schedule from the day of receipt for five consecutive days.

Results were recorded using an empirical approach, as follows.

Direct microscopy x 40 field
1 – 10 TV cells (+),
11 – 50 TV cells (++) and
>50 TV cells (+++)

Estimate of motility
Using an approximate %, e.g.,50%

Results:

Please refer to the tables below showing results for each of the four sample sets. Where NT is recorded for two laboratories, as expected, receipt of the samples was 48 hours from despatch.

Recording of results by the laboratories begins on Day 2 (day of receipt of samples). Day 1 is taken as the day of despatch and for sample number 4 is also the day of inoculation.

Sample 1 was intended to provide a positive control being simply the culture medium with no modification. Sample 4 showed a rapid decline in TV cells seen and this began to fail by day 4 where three laboratories reported some cells present with no motility whilst two laboratories recorded an occasional motile cell.

Discussion:

The study added confirmation that the current EQA swab represents a challenge for participating laboratories in the EQA scheme. Low numbers of TV cells are evident by day 3 and failure of the swab to show motile TV cells by day 4.

Sample 2 showed a general trend towards fewer TV cells seen and lower motility from Day 4.

Sample 3 showed exceptional results. No endpoint being demonstrated up to Day 6 in the study.

The key recommendation from the study that a partial move towards the modification given in sample 3 for the TV EQA scheme should be made. This should enable more variation within the scheme in relation to loading of TV cells in EQA samples and provide greater stability where transport delays are experienced.

Results: Estimates of TV cells seen, approximate motility, over five consecutive days

Sample No. 1		Day 2 19.07.22	Day 3 20.07.22	Day 4 21.07.22	Day 5 22.07.22	Day 6 23.07.22	Day 7 24.07.22
Lab 1	TV Seen	++	++	++	+	+	-
	Motility	90%	90%	70%	30%	10%	-
Lab 2	TV Seen	++	+++	+++	+++	++	-
	Motility	80%	40%	50%	40%	<10%	-
Lab 3	TV Seen	++	+++	++	++	++	-
	Motility	100%	100%	90%	10%	1%	-
Lab 4	TV Seen	NT	++	++	+	+	-
	Motility	NT	60%	60%	50%	10%	-
Lab 5	TV Seen	NT	+++	+++	++	+	++
	Motility	NT	90%	90%	60%	10%	2%

Sample No. 2		Day 2 19.07.22	Day 3 20.07.22	Day 4 21.07.22	Day 5 22.07.22	Day 6 23.07.22	Day 7 24.07.22
Lab 1	TV Seen	++	++	++	+	+/-	-
	Motility	100%	90%	20%	10%	0%	-
Lab 2	TV Seen	+++	++	++	++	<+	-
	Motility	80%	40%	30%	10%	0%	-
Lab 3	TV Seen	++	++	+	++	++	-
	Motility	70%	40%	10%	0%	0%	-
Lab 4	TV Seen	NT	+	+	+	+	-
	Motility	NT	50%	20%	10%	10%	-
Lab 5	TV Seen	NT	+++	+++	+++	+	+
	Motility	NT	80%	80%	65%	5%	1%

Sample No. 3		Day 2 19.07.22	Day 3 20.07.22	Day 4 21.07.22	Day 5 22.07.22	Day 6 23.07.22	Day 7 24.07.22
Lab 1	TV Seen	++	++	++	++	+	-
	Motility	80%	80%	70%	50%	30%	-
Lab 2	TV Seen	++	+++	+++	+++	+++	-
	Motility	80%	80%	70%	60%	50%	-
Lab 3	TV Seen	++	++	++	+++	++	-
	Motility	100%	70%	70%	30%	15%	-
Lab 4	TV Seen	NT	++	++	+++	+++	-
	Motility	NT	80%	60%	70%	60%	-
Lab 5	TV Seen	NT	++	++	++	++	++
	Motility	NT	80%	85%	60%	50%	50%

Sample No. 4		Day 2 19.07.22	Day 3 20.07.22	Day 4 21.07.22	Day 5 22.07.22	Day 6 23.07.22	Day 7 24.07.22
Lab 1	TV Seen	+	+	+	+	-	-
	Motility	80%	60%	0%	0%	0%	-
Lab 2	TV Seen	++	++	<+	+	-	-
	Motility	70%	30%	50%	10%	0%	-
Lab 3	TV Seen	+++	++	+	+	+	-
	Motility	95%	40%	0%	0%	0%	-
Lab 4	TV Seen	NT	+	<+	<+	<+	-
	Motility	NT	60%	0%	0%	0%	-
Lab 5	TV Seen	NT	+	+	+	+	<+
	Motility	NT	75%	50%	20%	5%	0%

Key: NT = Not Tested

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References:

For a detailed comprehensive review please refer to (1),

1. Edwards, T., Burke, P., Smalley, H. and Hobbs, G. (2014) *Trichomonas vaginalis*: Clinical relevance, pathogenicity and diagnosis. Critical Reviews in Microbiology, 42 (3).

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3. Garber, G. (2005) The laboratory diagnosis of *Trichomonas vaginalis*. Can J Infect Dis Med Microbiol, 16, 35 – 38.

4. Kingston, M.A., Bansal, D. & Carlin, E.M. (2003) 'Shelf life' of *Trichomonas vaginalis*. Int J STD AIDS, 14, 28 – 29.

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