REPORT NO. 1033

Cryptosporidium and *Giardia*

(Round 39)

Proficiency Testing Program

July 2017

ACKNOWLEDGMENTS

PTA wishes to gratefully acknowledge the technical assistance provided for this program by J Smith. This assistance included providing input into the design of the program, the supply of an alternative background matrix and the technical commentary for the report. Further appreciation is extended to Ms D Oddon-Mune and the staff at BTF Pty Ltd for sample preparation, distribution and homogeneity testing.

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CONTENTS

1. FOREWORD

This report summarises the results of the thirty-ninth round of a planned series of proficiency testing rounds involving the analysis of water samples for the detection and enumeration of *Cryptosporidium* and *Giardia*.

The proficiency round was conducted in May 2017 by Proficiency Testing Australia (PTA). The Technical Adviser was J Smith. The Program Coordinators were Ms Y Christie and Mrs K Weller. This report was authorised by Mrs F Watton PTA Quality Manager.

The program aim was to assess laboratories' ability to competently detect and report levels of *Cryptosporidium* and *Giardia* (oo)cysts in water.

2. FEATURES OF THE PROGRAM

(a) A total of nine laboratories (six Australia, three New Zealand) received samples, of which all returned results for inclusion in the report.

(b) Participating laboratories were requested to report both total and confirmed count results. Participants were also requested to calculate and report an estimate of measurement uncertainty (MU) for each reported result.

(c) Results as reported by participants are presented in Appendix A.

(d) In addition to the samples, laboratories were provided with the *Instructions to Participants* and a *Results Sheet* (see Appendix C). Laboratories were instructed to perform the tests according to their routine methods (method most frequently employed). Laboratories were reminded that PTA is aware of the internal positive control ColorSeed™, developed by BTF Pty Ltd. Although PTA can see the advantage of ColorSeed™ as an internal positive control, participants were instructed to note that it is not acceptable for laboratories to adjust results obtained with the PTA proficiency testing samples on the basis of recoveries obtained using ColorSeed™. An exception to this would be if the respective laboratory's routine practice/standard operating procedure routinely uses ColorSeed™ as a true internal standard, i.e. addition to *every* sample, *and* correction of observed count using internal standard recovery during routine sample reporting.

(e) The samples for Round 39 were produced in line with EasySeed™ batch number 595, which are certified reference samples. The preparation of these certified reference samples is considered to have satisfied the homogeneity testing requirements (see Appendix B).

(f) Each laboratory was randomly allocated a unique code number for the round to ensure confidentiality of results. Reference to each laboratory in this report is by code number.

3. DESIGN OF THE PROGRAM

Participants were requested to provide quantitative results for the presence of *Cryptosporidium* and *Giardia* in five water concentrate samples. Sample design is presented below.

TABLE A: Round 39 Sample Design

Sample	***Cryptosporidium*** **(Count)**	***Giardia*** **(Count)**	***Amount of DWPFBW added***
A	60	170	50 µL
B	90	0	500 µL
C	0	120	150 µL
D	110	60	50 µL
E	50	90	150 µL
F (Trip control)	110	170	50 µL

Notes for Table A:
1. Drinking Water Plant Filter Backwash (DWPFBW) was added to samples to simulate an environmental sample.
2. One nominated laboratory (Code 9) was provided with F, as trip control.

All samples were added to Milli-Q™ water to make a final volume of approximately 3.5 mL.

Sample preparation

BTF Pty Ltd, NSW, prepared different water concentrate samples for this program, using PTA in-house method *PTPM 11.1 Sample Preparation – Cryptosporidium and Giardia (Version No. 6)*.

Seed samples were prepared on 27 April 2017. Seed samples were dispensed in IsoFlow™ and the sterilisation method was gamma irradiation.

Cryptosporidium parvum (Iowa strain) oocysts were of bovine origin, excreted on 10 March 2017. Oocysts were purified by discontinuous sucrose and caesium chloride gradient centrifugation.

Giardia lamblia (H3 strain) cysts were obtained from experimentally-infected gerbils and were excreted on 10 April 2017. Cysts were purified by sucrose and Percoll™ density gradient centrifugation, followed by water washes.

The seed samples were prepared using flow cytometry and an automated dispensing method. *Cryptosporidium* and *Giardia* (suspended in IsoFlow™ solution) were dispensed into 4 mL tubes.

Seed samples were then sealed, labelled and exposed to a controlled dose of gamma irradiation. The *Cryptosporidium* oocysts were also heat treated to prevent excystation. Quality Control was performed on the seed samples.

On 28 April each of the seed samples were spiked with DWPFBW (see 'Confounding materials' below) and then made up to approximately 3.5 mL with Milli-Q™ water to produce the water concentrate samples sent to participants on 17 May 2017.

Participating laboratories were asked to add each of the water concentrate samples to 10 L of water of their choice prior to analysis. The laboratories were also instructed to take care to ensure that the water used did not contain any cysts or oocysts and could, for example, use reverse osmosis or membrane filtered (suggested pore size ≤ 45 µm) water.

Confounding materials

Drinking Water Plant Filter Backwash (DWPFBW) was added to selected water concentrate samples at a concentration of 50, 150 or 500 µL per water concentrate sample (see Table A).

Quality assurance of DWPFBW

To ensure the DWPFBW did not contain *Cryptosporidium* oocysts or *Giardia* cysts, DWPFBW samples were analysed prior to addition to proficiency samples (2 ml packed pellet analysed by IMS-IFA in 0.5 mL aliquots), and particulates characterised and quantified using microscopic particulate analysis (USEPA 1996.)

4. FORMAT OF APPENDICES

Appendix A (A1.1 - A1.4) contains the total count and confirmed count results reported by participating laboratories for each of the five water concentrate samples. Percentage recovery rates and charts are also presented (A1.5 - A2.0). Please note that recovery rates are calculated using total counts only.

Appendix B contains details of homogeneity testing, quality control and trip control results (B1.1 - B1.2). Appendix C contains the *Instructions to Participants* and the *Results Sheet* (C1.1 – C1.3).

5. FALSE RESULTS

Results were examined for false positive and false negative results with all testing methods pooled. Laboratory code 2 received a false negative result for the analysis of *Cryptosporidium* in sample A. There were no false results for *Giardia*.

6. LOW/HIGH RECOVERIES

The acceptable range set for this program is a recovery between 10-110%. This has been determined to be an appropriate acceptability range by technical experts in this area of testing. The results were examined for low/high recoveries (recovery rates that lie outside the acceptable range of 10-110%) with all testing methods pooled.

All results (apart from the one false negative result) were within the acceptable recovery ranges.

7. PTA AND TECHNICAL ADVISER'S COMMENTS

A total of 90 Total Count results were received for this program.

Percentage Recovery Rate

- Pooled data indicated a higher range of recoveries (within the acceptable recovery rate limit range of 10% - 110%) for *Giardia* (13-103%) compared to *Cryptosporidium* (19-98%), which is different to what was seen when reviewing respective ranges observed in round 38 (*Cryptosporidium* [10-109%]; *Giardia* [12-97%]).

  Overall results are typical of recoveries obtained using the methods employed with the exceptions:
  - *Cryptosporidium* laboratory code 2 - reported a false negative for Sample A.

- Recovery range: Pooled round 39 laboratory data indicated a smaller *range of recoveries* for both *Cryptosporidium* (0-98%) for *Giardia* (13-103%) compared to round 38 (8-121% *Cryptosporidium,* 12-138% *Giardia*). With laboratory 2 false-negative result excluded, round 39 *Cryptosporidium* recovery range (19-98%) was substantially less than round 38.

- Recovery variability**:**

  -*Intra-sample*: The greatest recovery variability occurred for sample A (50 µL matrix, 60 *Cryptosporidium* oocysts, 170 *Giardia* cysts; MU = 140% - *Cryptosporidium*, 98% - *Giardia.*) The least recovery variability occurred for sample E (150 µL matrix, 50 *Cryptosporidium* oocysts, 90 *Giardia* cysts; MU 50% - *Cryptosporidium*, 62% - *Giardia.*)

-*Intra-laboratory*: Laboratory 2 had the greatest variability in *Cryptosporidium* recovery (77% RSD) and lowest mean recovery (20%). Laboratory 8 had the least *Cryptosporidium* recovery variability (7% RSD). Laboratory 9 had the greatest *Giardia* recovery variability (50% RSD) and lowest mean recovery (22%). Laboratories 1 and 8 had the least variability in *Cryptosporidium* recovery (11% RSD).

- Recovery medians: Median recoveries were generally similar to those reported for other proficiency schemes and published literature (45-73% *Cryptosporidium*; 49-72% *Giardia*).
- Recovery maxima: Maximum recoveries were generally lower for *Cryptosporidium* (86-98%) and *Giardia* (81-103%) than those in PTA round 38 (*Cryptosporidium* 96-109%: *Giardia* 95-97%).
- Recovery minima: Laboratory 2 *Cryptosporidium* recoveries were the lowest (mean recovery 20%) compared to all other laboratories for all samples (A1.5). Laboratory 9 had the lowest *Giardia* recoveries (mean 22%) with the exception of sample E (A1.8). Laboratory 6 also reported generally lower *Giardia* recoveries (mean 30%) than other laboratories; with the lowest recovery of *Giardia* for sample E.
- Control Samples: Counts of trip control samples (F_T) were lower than those of the sample kept on premises (F_{NoT}) for both *Cryptosporidium* and *Giardia* (B1.2.) Considering typical measurement uncertainties associated with analysis of these measurands, however, these recoveries were not significantly different (F_{NoT} and F_T; B1.2). Control sample recoveries were also similar to their respective samples analysed by participant laboratories in terms of (oo)cysts per unit matrix; D for *Cryptosporidium* (controls 66-68%; participant laboratories median 69%) and A for *Giardia* (controls 56-63%; participant laboratories median 49%).

Impact of Matrix

- Considering test measurement uncertainty, median and average recoveries of *Cryptosporidium* and *Giardia* were generally similar regardless of matrix amount or type (A1.5, A1.8).
- Lower mean *Cryptosporidium* recoveries were generally associated with lower matrix material levels (Fig. 2B). A similar impact was not observed for *Giardia* (Fig. 3B).

Impact of Reference Count

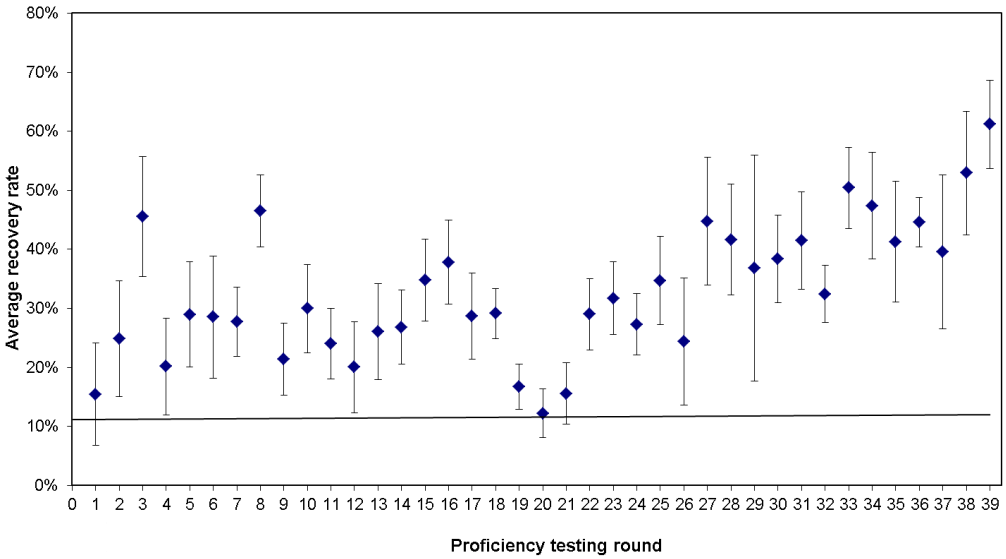
- No impact of reference count on mean recovery of *Cryptosporidium* was observed, however, increased *Giardia* reference count produced progressively lower mean recoveries (Fig. 3A).
- With the exception of sample D, higher reference counts of *Cryptosporidium* and *Giardia per-unit-matrix* produced lower mean recoveries (Figs. 2C, 3C).

Confirmation

- Percent confirmed (DAPI[+]) *Cryptosporidium* oocysts in F_T and F_{NoT} samples (100%) were higher than those of round 38 (90-94%). Percentages of DAPI(+) *Giardia* cysts in F_T and F_{NoT} samples (94-96%) were also higher than those of round 37 (77-82%) or 38 (85-87%).

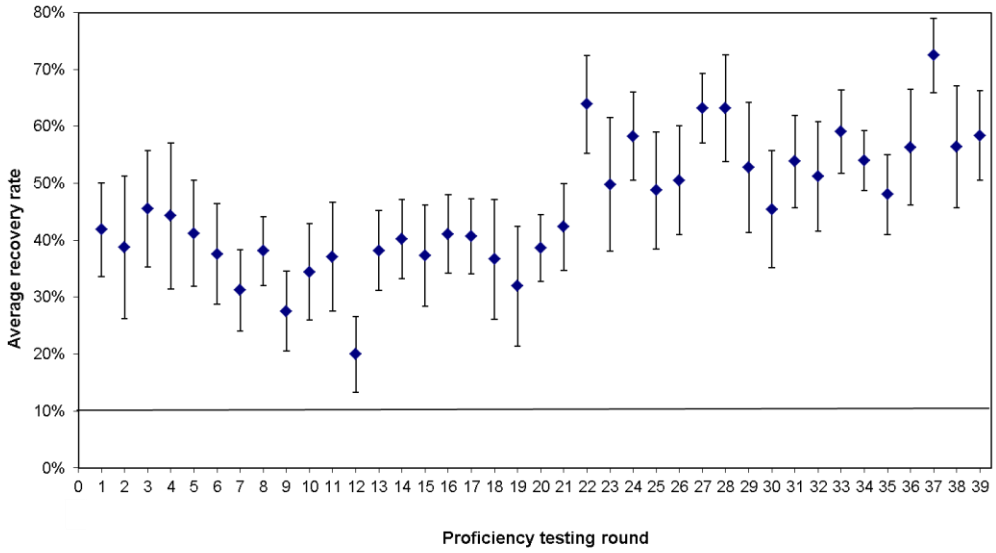
Total average *Cryptosporidium* recovery rate (61.2%) has increased compared to the previous round. Figure 1A shows the average percent recovery rate for *Cryptosporidium* for each round (refer to notes below).

Figure 1A: Comparison of total average recovery rates for *Cryptosporidium*



Total average *Giardia* recovery rate (58.4%) has increased compared to the previous round. The graph below displays this (refer to notes below figure).

Figure 1B: Comparison of total average recovery rates for *Giardia*



Notes to Average Recovery Rates Graphs:

1. The vertical bars in the graphs represent 95% confidence intervals.
2. All rounds up to Round 37, except rounds 1, 2, 3 and 8, contain QC mud (see table on pages 11 through to 14). For Round 5, one sample (Sample type 4); for Round 14, one sample (Sample C); for Round 15, one sample (Sample D); and for Round 34, one sample (Sample C) out of the five samples analysed by each laboratory did not contain QC mud.
3. From Rounds 14-21, average recovery rates are calculated on confirmed counts only. For rounds excluding Rounds 14-21, participants reported either total or confirmed counts, and therefore the average recovery rates presented in this table prior to Round 14 may include both total and confirmed counts. From Round 22 onwards, only total counts are presented.
4. Regarding Round 37, selected samples contained QC mud or Drinking Water Plant Filter Backwash (DWPFBW).
5. Round 38 samples contained DWPFBW only.

Figure 2A: Reference Count vs % Recovery for *Cryptosporidium*

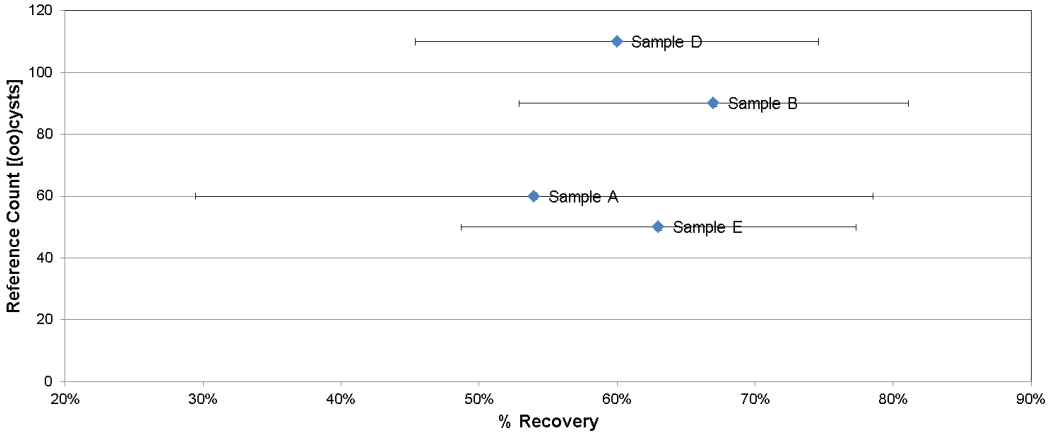


Figure 2B: Added Matrix vs % Recovery for *Cryptosporidium*

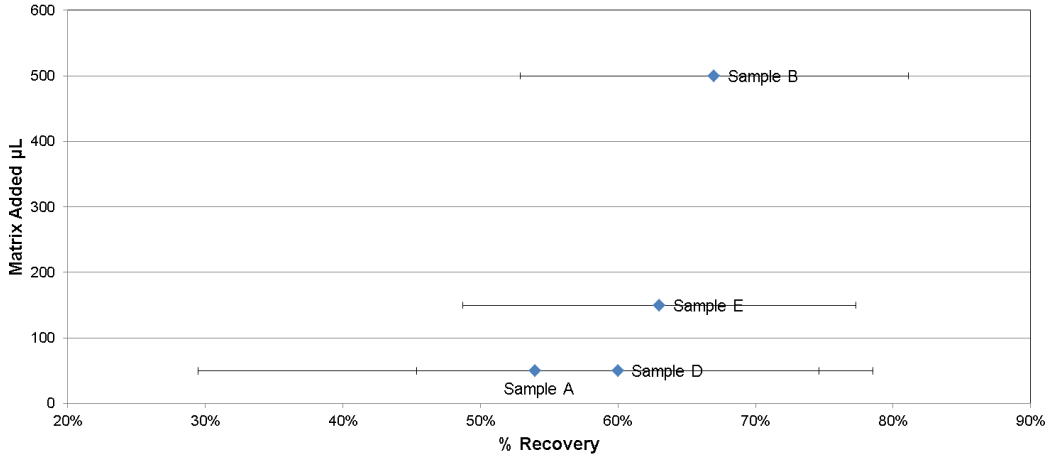
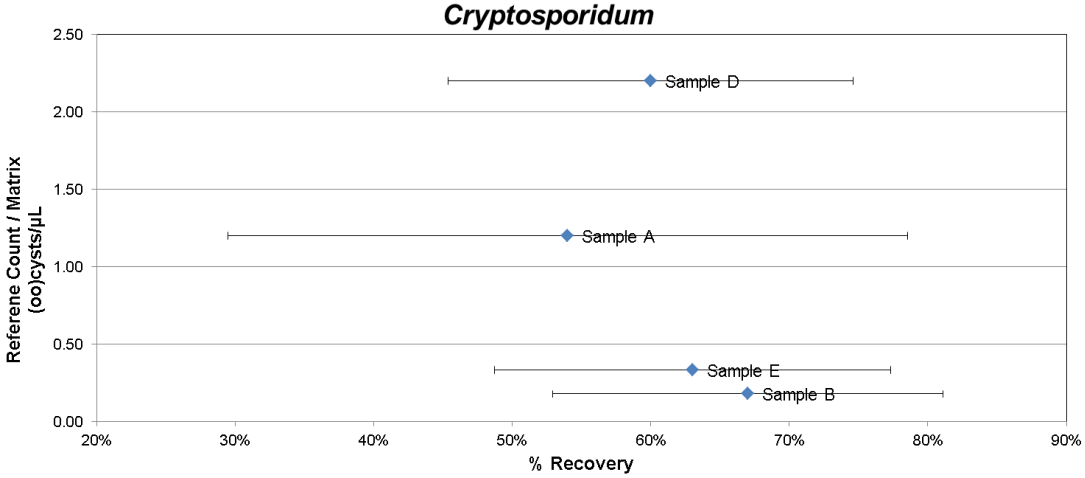


Figure 2C: Reference Count/Matrix vs % Recovery for *Cryptosporidum*



1. The blue diamonds represent the mean recoveries for each sample.
2. The horizontal bars (error bars) represent measurement uncertainties.

Figure 3A: Reference Count vs % Recovery for *Giardia*

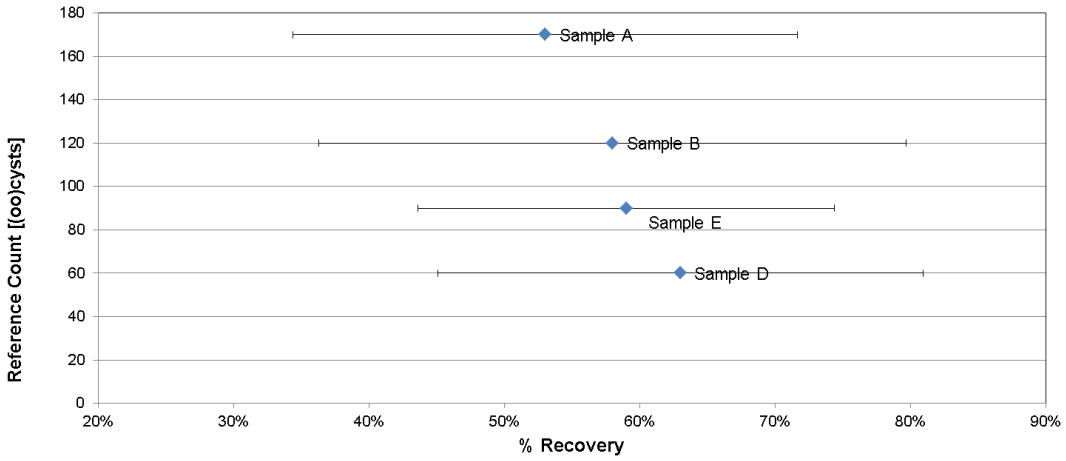


Figure 3B: Added Matrix vs % Recovery for *Giardia*

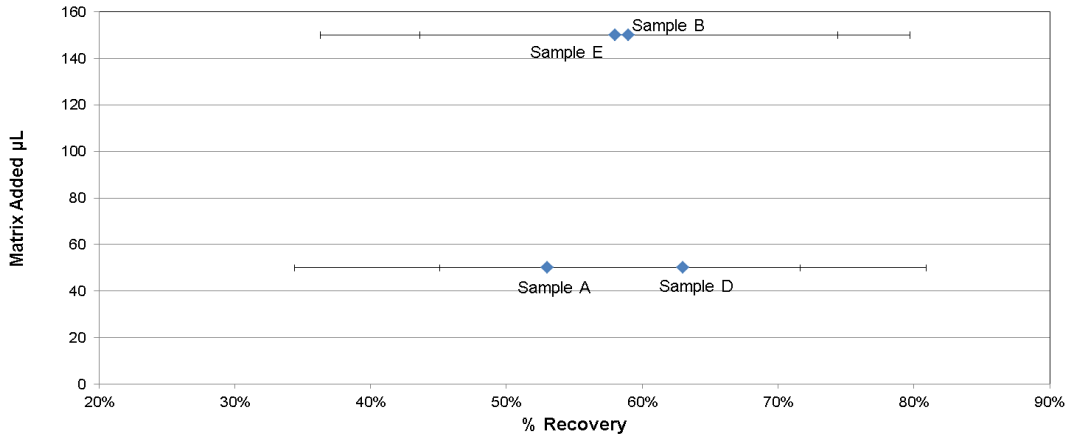
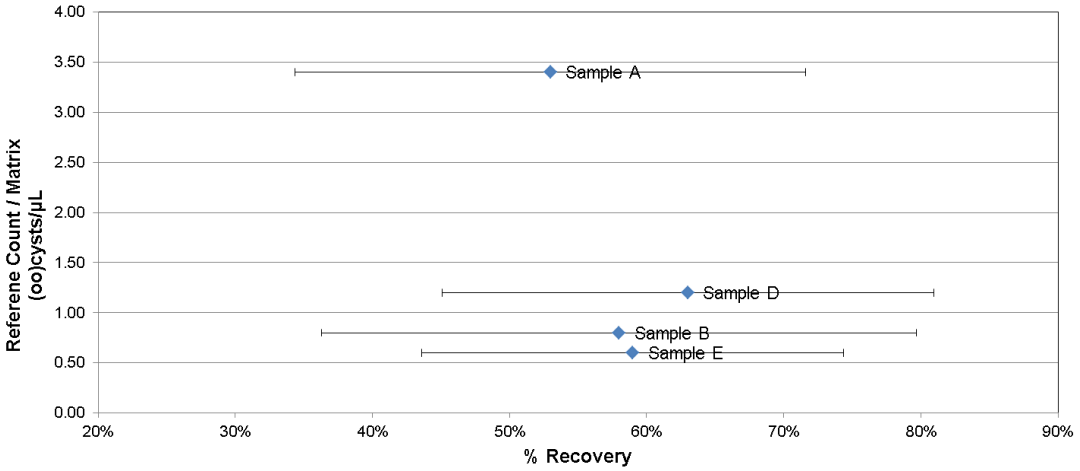


Figure 3C: Reference Count/Matrix vs % Recovery for *Giardia*



1. The blue diamonds represent the mean recoveries for each sample.
2. The horizontal bars (error bars) represent measurement uncertainties.

Measurement Uncertainty (MU) Estimation

Results including MU calculations are presented in two formats, including absolute oocyst numbers (so that participants can compare numeric recoveries against seed doses) and relative % recoveries, as seen in Tables B and C below. These tables and comments are provided for information purposes only, and do not affect the evaluation of participants' results.

TABLE B: *Cryptosporidium* and *Giardia* Round 39 (Oo)cyst Recovery - % Measurement Uncertainty

SAMPLE	ORGANISM	(OO)CYST MEDIAN RECOVERY	STANDARD DEVIATION	RELATIVE STANDARD DEVIATION (RSD - %)	✦MEASUREMENT UNCERTAINTY (± [OO]CYSTS)	REFERENCE COUNT
A	*Giardia*	83	41	49	98	170
	Cryptosporidium	27	19	70	140	60
B	*Giardia*					
	Cryptosporidium	66	17	26	52	90
C	*Giardia*	84	34	40	80	120
	Cryptosporidium					
D	*Giardia*	43	14	33	66	60
	Cryptosporidium	76	21	28	56	110
E	*Giardia*	58	18	31	62	90
	Cryptosporidium	36	9	25	50	50

Notes for Table B:
1. ✦ = All measurement uncertainty values are at the 95% level of confidence.
2. Sample C did not include *Cryptosporidium*.
3. Sample B did not contain *Giardia.*

TABLE C: *Cryptosporidium* and *Giardia* Round 39 Recovery - % Measurement Uncertainty

SAMPLE	ORGANISM	MEDIAN RECOVERY (%)	STANDARD DEVIATION	RELATIVE STANDARD DEVIATION (RSD - %)	✦MEASUREMENT UNCERTAINTY (RSD - %)	REFERENCE COUNT
A	*Giardia*	49	24	49	48	170
	Cryptosporidium	45	32	71	64	60
B	*Giardia*					
	Cryptosporidium	73	19	26	38	90
C	*Giardia*	70	28	40	56	120
	Cryptosporidium					
D	*Giardia*	72	23	32	46	60
	Cryptosporidium	69	19	28	38	110
E	*Giardia*	64	20	31	40	90
	Cryptosporidium	72	18	28	36	50

Notes for Table C:
1. ✦ = All measurement uncertainty values are at the 95% level of confidence.
2. Sample C did not include *Cryptosporidium*.
3. Sample B did not contain *Giardia.*

The table below shows *Cryptosporidium* oocyst levels for each round.

TABLE D: Comparison of *Cryptosporidium* Oocyst Levels for Each Round

Round	*Cryptosporidium* levels (Counts)	Round	*Cryptosporidium* levels (Counts)
1	50-200	21	100-200
2	50-200	22	50-300
3	50-300	23	50-250
4	110	24	50-200
5	50-200	25	50-250
6	25-75	26	50-200
7	50-100	27	50-200
8	65-140	28	50-250
9	125	29	50-140
10	110-235	30	80-135
11	50-200	31	70-140
12	110-235	32	50-130
13	90-205	33	50-170
14	55-135	34	50-130
15	55-135	35	50-120
16	55-120	36	50-130
17	90-310	37	50-120
18	50-300	38	50-110
19	50-300	39	50-110
20	50-200		

The table below shows *Giardia* cyst levels for each round.

TABLE E: Comparison of *Giardia* Cyst Levels for Each Round

Round	*Giardia* levels (Counts)	Round	*Giardia* levels (Counts)
1	50-200	21	90-200
2	50-200	22	50-250
3	50	23	50-300
4	40	24	50-200
5	50-200	25	50-250
6	75-120	26	50-250
7	50	27	50-200
8	65-140	28	50-200
9	55	29	50-150
10	70-85	30	85-150
11	50-200	31	50-140
12	110-125	32	50-170
13	90-145	33	50-130
14	55-200	34	50-170
15	55-200	35	50-120
16	120-255	36	50-170
17	135-310	37	60-120
18	150-300	38	60-170
19	150-300	39	60-170
20	50-120		

Method Commentary

Analysis of Results by Method Groups

In order for methods to be grouped for analysis, PTA requires at least 11 sets of results from the same method group. As there were less than 11 results submitted for each method, reliable conclusions cannot be drawn from analysing grouped methods on this occasion. Therefore, results from all method groups have been pooled for analysis.

Of the nine participating laboratories, four indicated the use of filtration for their bulk water concentration method, one used flocculation, one used centrifugation, one used filtration and centrifugation, and two did not list a method. With regards to each laboratory's preferred filtration method, six laboratories used a cartridge method, whilst one used flat-bed, and one used sponge filtration. All laboratories used IMS as their purification method and immunofluorescence microscopy as their presumptive ID and total count enumeration method. All nine laboratories reported use of DAPI staining for confirmation, of which three also indicated additional use of DIC microscopy. One laboratory used an undefined method.

Laboratory 2 *Cryptosporidium* mean recoveries were the lowest (20%) of all participating laboratories. This laboratory used sponge filtration for bulk water concentration. Laboratory 9 had the lowest mean *Giardia* recovery (22%); followed by laboratory 6 (30%). These laboratories used cartridge filtration and flocculation for bulk water concentration, respectively.

TABLE F: Recoveries and recovery variability by bulk water concentration method

Bulk Water Concentration Method	**Mean *Cryptosporidium* Recovery and Variability (RSD)**	**Mean *Giardia* Recovery and Variability (RSD)**	**Number of laboratories using method**
Cartridge Filtration	64-80 (7-31%)	22-87 (11-50%)	6
Flat Bed Filtration	55 (34%)	63 (31%)	1
Flocculation	46 (13%)	30 (25%)	1
Sponge Filtration	20 (77%)	46 (16%)	1

Laboratories 2 and 4 obtained substantially lower confirmation of *Cryptosporidium* oocysts and *Giardia* cysts than other laboratories (A1.7, A2.0). These laboratories used DAPI + DIC, and DAPI-only confirmation techniques, respectively. They also used sponge and cartridge filtration for bulk water concentration, respectively.

Overall Laboratory Performance

Overall, recoveries of both *Cryptosporidium* and *Giardia* oocysts were higher than the previous round (38).

With regards to the analysis of *Cryptosporidium*, performance was mostly satisfactory for all sample doses and matrix amounts with one laboratory reporting a false negative for sample A and all other results for all samples inside the acceptable recovery rate limit (10% - 110%). The analysis of *Giardia* resulted (on average) in a lower recovery result compared to *Cryptosporidium*.

Laboratories with relatively low recoveries (2, 6, 9) should investigate causal factors, including failure to concentrate from bulk water and/or disperse (oo)cysts from matrix materials prior to IMS; and/or failure to add, capture or dissociate *Cryptosporidium* IMS beads; and/or status of associated reagents are potential areas for investigation.

Laboratories 2 and 4 should review confirmation procedures including DAPI staining and/or DIC procedures (setup, use of prism, etc.) in light of relative performance and take corrective action(s) to investigate sources of failure to confirm during sample analysis. Some laboratories experienced issues with low percentages of DAPI stained cysts and oocysts. Several methods suggest use of heat and/or acid for dissociation of oocysts from IMS beads, as well as permeabilisation for subsequent DAPI staining. Laboratories 2 and 4 may find the following publication of interest if such techniques to optimise DAPI staining are not already employed: Ware, MW, Wymer, L, Lindquist, and Schaefer, FW. (2003)

Measurement Uncertainty (MU)

Estimated uncertainties of measurement between laboratories were not atypical for these measurands. Note that not all laboratories provided sufficient information for relative MU assessment (i.e. laboratory 2).

The highest reported MU (140% RSD) was associated with generally the most difficult measurand and matrix combination (*Cryptosporidium*, low matrix, low reference count).

Laboratory code 2 reported uncertainties that do not appear related to their total (or confirmed counts). Example: Sample A *Giardia* - total count 64 cysts; MU 40 ± 24. This laboratory also failed to report associated *n*'s. The facility is advised to clarify and/or report MU as either a relative standard uncertainty related to their total count, or as a range with respect to same. And to report associated *n*'s.

Laboratory 5 reported MU sample *n* as a decimal fraction, e.g. *Giardia* sample A, *n* = 2885.0. Samples are discreet rather than continuous data. They can therefore only be reported as whole numbers.

Laboratory code 8 reported different *n* values for *Cryptosporidium* (32) and *Giardia* (39). It is generally atypical for these to differ between measurands for this test type.

Laboratory code 9 reported MU as $\log_{10}$ value with $n = 5$. MU should be reported as non-$\log_{10}$-transformed whole numbers for the purposes of PE reporting. Note also that the minimum *n* generally considered acceptable for MU estimation is ≥ 10.

Laboratory 3 failed to report MU.

TABLE G: Overall Laboratory Performance

Round	Sample Type	Percentage false positive and false negative results reported	Number of laboratories reporting false results	Percentage low/high recovery results reported	Number of laboratories reporting low/high percentage recovery rates
1	10 Litres - tap water	11.0%	6	11.0%	7
2	10 Litres - tap water	6.7%	1	7.8%	3
3	10 Litres -Milli-Q water	3.8%	3	4.7%	3
4	10 Litres - RO water + QC mud + confounding organisms	10.3%	3	11.8%	4
5	10 Litres - RO water + QC mud*	7.0%	4	11.0%	5
6	10 Litres - RO water + QC mud	8.3%	4	8.3%	5
7	Concentrate samples - QC mud - Labs. add to 10 Litres distilled water	8.2%	4	6.4%	5
8	10 Litres - RO water	1.2%	1	1.2%	1
9	10 Litres - RO water + QC mud	2.7%	1	7.3%	4
10	Concentrate samples - QC mud - Labs. add to 10 Litres distilled water & 10 Litres - RO water + QC mud	2.3%	1	3.5%	2

Round	Sample Type	Percentage false positive and false negative results reported	Number of laboratories reporting false results	Percentage low/high recovery results reported	Number of laboratories reporting low/high percentage recovery rates
11	10 Litres - RO water + QC mud	0.0%	0	6.8%	4
12	10 Litres - RO water + QC mud	5.5%	2	17.5%	6
13	10 Litres - RO water + QC mud	0.0%	0	10.0%	4
14	10 Litres - RO water + QC mud*	2.6%	1	2.6%	1
15	Concentrate samples - QC mud* - Labs. add to 10 Litres distilled water	1.3%	1	5.0%	2
16	Concentrate samples - QC mud - Labs. add to 10 Litres distilled water	0.0%	0	3.3%	2
17	Concentrate samples - QC mud - Labs. add to 10 Litres distilled water	1.5%	1	2.9%	1
18	Concentrate samples - QC mud - Labs. add to 10 Litres water	0.0%	0	0.0%	0
19	Concentrate samples - QC mud - Labs. add to 10 Litres water	6.0%	1	11.4%	1
20	Concentrate samples - QC mud - Labs. add to 10 Litres water	10.0%	4	7.1%	3

Round	Sample Type	Percentage false positive and false negative results reported	Number of laboratories reporting false results	Percentage low/high recovery results reported	Number of laboratories reporting low/high percentage recovery rates
21	Concentrate samples - QC mud - Labs. add to 10 Litres water	5.4%	1	10.7%	2
22	Concentrate samples - QC mud - Labs. add to 10 Litres water	1.4%	1	1.4%	1
23	Concentrate samples - QC mud - Labs. add to 10 Litres water	0.0%	0	1.7%	1
24	Concentrate samples - QC mud - Labs. add to 10 Litres water	1.4%	1	0.0%	0
25	Concentrate samples - QC mud - Labs. add to 10 Litres water	0.0%	0	0.0%	0
26	Concentrate samples - QC mud - Labs. add to 10 Litres water	1.4%	1	4.3%	2
27	Concentrate samples - QC mud - Labs. add to 10 Litres water	0.0%	0	0.0%	0
28	Concentrate samples - QC mud - Labs. add to 10 Litres water	0.0%	0	3.3%	1

Round	Sample Type	Percentage false positive and false negative results reported	Number of laboratories reporting false results	Percentage low/high recovery results reported	Number of laboratories reporting low/high percentage recovery rates
29	Concentrate samples - QC mud - Labs. add to 10 Litres water	10.0%	2	18.8%	3
30	Concentrate samples - QC mud - Labs. add to 10 Litres water	2.5%	1	3.75%	3
31	Concentrate samples - QC mud - Labs. add to 10 Litres water	0.0%	0	1.4%	1
32	Concentrate samples - QC mud - Labs. add to 10 Litres water	0.0%	0	3.5%	1
33	Concentrate samples - QC mud - Labs. add to 10 Litres water	0.0%	0	0.0%	0
34	Concentrate samples - QC mud - Labs. add to 10 Litres water	0.0%	0	0.0%	0
35	Concentrate samples - QC mud - Labs. add to 10 Litres water	3.3%	1	0.0%	0
36	Concentrate samples - QC mud - Labs. add to 10 Litres water	0.0%	0	0.0%	0
37	Concentrate samples - QC mud - Labs. add to 10 Litres water	10%	2	9.38%	3

Round	Sample Type	Percentage false positive and false negative results reported	Number of laboratories reporting false results	Percentage low/high recovery results reported	Number of laboratories reporting low/high percentage recovery rates
38	Concentrate samples - QC mud - Labs. add to 10 Litres water	1.25%	1	7.81%	5
39	Concentrate samples - DWPFBW - Labs. add to 10 Litres water	1.11%	1	0.0%	0

Notes for Table F:
1. RO = reverse osmosis.
2. * = For Round 5, QC mud was only added to Sample types 1, 2, 3 and 5. For Round 14, QC mud was only added to Samples A, B, D and E. For Round 15, QC mud was only added to Samples A, B, C and E. For Round 34, QC mud was only added to Samples A, B, D and E.
3. For Round 35, a combination of QC mud or Drinking Water Plant Filter Backwash (DWPFBW) was added to samples.

Conclusions

This round saw the *Cryptosporidium* and *Giardia* recovery rates increase in comparison to the previous round, with only one laboratory reporting false results for *Cryptosporidium*.

Except for the one false negative, all laboratories reported recoveries in the acceptable range for each of the two respective measurands (*Giardia* and *Cryptosporidium*).

8. REFERENCES

[1] *Guide to Proficiency Testing Australia,* 2016 (this document can be found on the PTA website, www.pta.asn.au).

[2] *Evaluation of an alternative IMS dissociation procedure for use with Method 1622: detection of Cryptosporidium in water*. Ware, MW, Wymer, L, Lindquist, and Schaefer, FW (2003).

[3] USEPA (1996) *Microscopic particulate analysis (MPA) for filtration plant optimization.* EPA 910-R-96-001.

APPENDIX A

Summary of Results

Results *Cryptosporidium* (total counts)

REFERENCE COUNTS	60	90	0	110	50	
DWPFBW per vial	**50µL**	**500µL**	**150 µL**	**50µL**	**150µL**	
Lab Code No.	**Sample A**	**Sample B**	**Sample C**	**Sample D**	**Sample E**	**Method Codes**
Code 1 - Total Count	59	65	0	64	36	3, 6, 7, 8, 9, 10, 11 (FITC - Confirmation method)
Confirmed Count	51	60	0	59	33	
MU	±18 *n* = 15	±20 *n* = 15	-	±19 *n* = 15	±11 *n* = 15	
Code 2 - Total Count	0	22	0	21	19	
Confirmed Count	0	12	0	11	14	1, 7, 8, 9, 10
MU	-	24 ± 24	-	21 ± 26	21 ± 25	
Code 3 - Total Count	55	66	0	85	38	
Confirmed Count	50	66	0	85	38	3, 7, 8, 9
MU	-	-	-	-	-	
Code 4 - Total Count	35	72	0	79	20	
Confirmed Count	18	54	0	44	9	3, 7, 8, 9
MU	35 - 117 *n* = 56	73 - 241 *n* = 56	-	80 - 264 *n* = 56	20 - 67 *n* = 56	
Code 5 - Total Count	24	77	0	76	43	
Confirmed Count	24	77	0	75	42	3, 7, 8, 9, 10
MU	70.2% *n* = 2885.0	70.2% *n* = 2885.0	70.2% *n* = 2885.0	70.2% *n* = 2885.0	70.2% *n* = 2885.0	
Code 6 - Total Count	27	49	0	50	20	
Confirmed Count	27	49	0	50	20	5, 7, 8, 9, 10
MU	25.1% *n* = 353	25.1% *n* = 353	25.1% *n* = 353	25.1% *n* = 353	25.1% *n* = 353	
Code 7 - Total Count	18	66	0	59	31	
Confirmed Count	18	64	0	57	29	2, 7, 8, 9, 10
MU	18 ± 12 (95% CI, *n* = 800)	66 ± 43 (95% CI, *n* = 800)	-	59 ± 38 (95% CI, *n* = 800)	31 ± 20 (95% CI, *n* = 800)	

Results *Cryptosporidium* (total counts) - continued

Code 8 - Total Count	50	69	-	78	39	
Confirmed Count	50	69	-	78	39	3, 7, 8, 9
MU	46 - 54 / 32	64 - 74 / 32	-	73 -84 / 32	36 - 42 / 32	
Code 9 - Total Count	26	58	0	86	36	
Confirmed Count	26	56	-	84	34	3, 6, 7, 8, 9, 10
MU	0.10 log (*n* = 5)	0.10 log (*n* = 5)	0.10 log (*n* = 5)	0.10 log (*n* = 5)	0.10 log (*n* = 5)	

Note:1. A "-" indicates that no result was returned for this sample/test.

Summary Statistics for *Cryptosporidium* (total counts)

Reference Counts	**60**	**90**	**0**	**110**	**50**
DWPFBW	**50 µL**	**500 µL**	**150 µL**	**50 µL**	**150 µL**

	Sample A	**Sample B**	**Sample C**	**Sample D**	**Sample E**
No. of Results	9	9	9	9	9
Minimum	0	22	0	21	19
Maximum	59	77	0	86	43
Average	33	60	0	66	31
Median	27.0	66.0	0.0	76.0	36.0
SD	19.1	16.5	0.0	20.9	9.3
Median Absolute Deviation (%)	71	26	-	28	25

Method Codes

Analysis	**Method used to obtain results**	**Code**
Concentration	Filtration (Sponge)	1
	Filtration (Flat Bed)	2
	Filtration (Cartridge)	3
	Filtration (Tangential Flow)	4
	Flocculation	5
	Centrifugation	6
Purification	IMS	7
Enumeration	Immunofluorescence Microscopy	8
Confirmation	DAPI Staining	9
	DIC Microscopy	10
Methods not defined	-	11

Results *Giardia* (total counts)

REFERENCE COUNTS	170	0	120	60	90	
DWPFBW	**50 µL**	**500 µL**	**150 µL**	**50 µL**	**150 µL**	
Lab Code No.	**Sample A**	**Sample B**	**Sample C**	**Sample D**	**Sample E**	**Method Codes**
Code 1 - Total Count	140	0	91	49	58	3, 6, 7, 8, 9, 10, 11 (FITC - Confirmation method)
Confirmed Count	122	0	80	43	50	
MU	±42 *n* = 15	-	±28 *n* = 15	±15 *n* = 15	±17 *n* = 15	
Code 2 - Total Count	64	0	52	33	45	
Confirmed Count	42	0	33	24	31	1, 7, 8, 9, 10
MU	40 ± 24	-	36 ± 37	36 ± 34	37 ± 33	
Code 3 - Total Count	157	0	124	44	72	
Confirmed Count	145	0	120	44	72	3, 7, 8, 9
MU	-	-	-	-	-	
Code 4 - Total Count	83	0	84	50	45	
Confirmed Count	39	0	20	35	17	3, 7, 8, 9
MU	112 - 396 *n* = 56	-	113 - 401 *n* = 56	67 - 239 *n* = 56	61 - 215 *n* = 56	
Code 5 - Total Count	97	0	62	43	63	
Confirmed Count	97	0	61	39	61	3, 7, 8, 9,11
MU	49.1% *n* = 2885.0	49.1% *n* = 2885.0	49.1% *n* = 2885.0	49.1% *n* = 2885.0	49.1% *n* = 2885.0	
Code 6 - Total Count	64	0	28	21	21	
Confirmed Count	58	0	25	19	19	5, 7, 8, 9
MU	23.2% *n* = 353	23.2% *n* = 353	23.2% *n* = 353	23.2% *n* = 353	23.2% *n* = 353	
Code 7 - Total Count	60	0	85	39	73	
Confirmed Count	49	0	64	24	52	2, 7, 8, 9
MU	60 ± 33 (95% CI, *n* = 800)	-	85 ± 46 (95% CI, *n* = 800)	39 ± 22 (95% CI, *n* = 800)	73 ± 40 (95% CI, *n* = 800)	

A1.4

Results *Giardia* (total counts) - continued

Code 8 - Total Count	115	-	88	52	67	
Confirmed Count	85	-	79	27	49	3, 7, 8, 9
MU	112 - 118 / 39	-	86 - 90 / 39	51 - 53 / 39	65 - 69 / 39	
Code 9 - Total Count	30	0	16	11	34	
Confirmed Count	28	-	14	7	32	3, 6, 7, 8, 9,10
MU	0.11 log (*n* =5)	0.11 log (*n* = 5)	0.11 log (*n* = 5)	0.11 log (*n* = 5)	0.11 log (*n* = 5)	

Note:
1. A "-" indicates that no result was returned for this sample/test.

Summary Statistics for *Giardia* (total counts)

Reference Counts	**170**	**0**	**120**	**60**	**90**
DWPFBW	**50 µL**	**500 µL**	**150 µL**	**50 µL**	**150 µL**

	Sample A	**Sample B**	**Sample C**	**Sample D**	**Sample E**
No. of Results	9	9	9	9	9
Minimum	30	0	16	11	21
Maximum	157	0	124	52	73
Average	90	0	70	38	53
Median	83.0	0.0	84.0	43.0	58.0
SD	41.2	0.0	33.9	14.0	18.0
Median Absolute Deviation (%)	49	-	40	32	31

Method Codes

Analysis	**Method used to obtain results**	**Code**
Concentration	Filtration (Sponge)	1
	Filtration (Flat Bed)	2
	Filtration (Cartridge)	3
	Filtration (Tangential Flow)	4
	Flocculation	5
	Centrifugation	6
Purification	IMS	7
Enumeration	Immunofluorescence Microscopy	8
Confirmation	DAPI Staining	9
	DIC Microscopy	10
Methods not defined	-	11

Summary of Percentage Recovery Rates and Charts

A1.5

Recovery Results for *Cryptosporidium* (%)

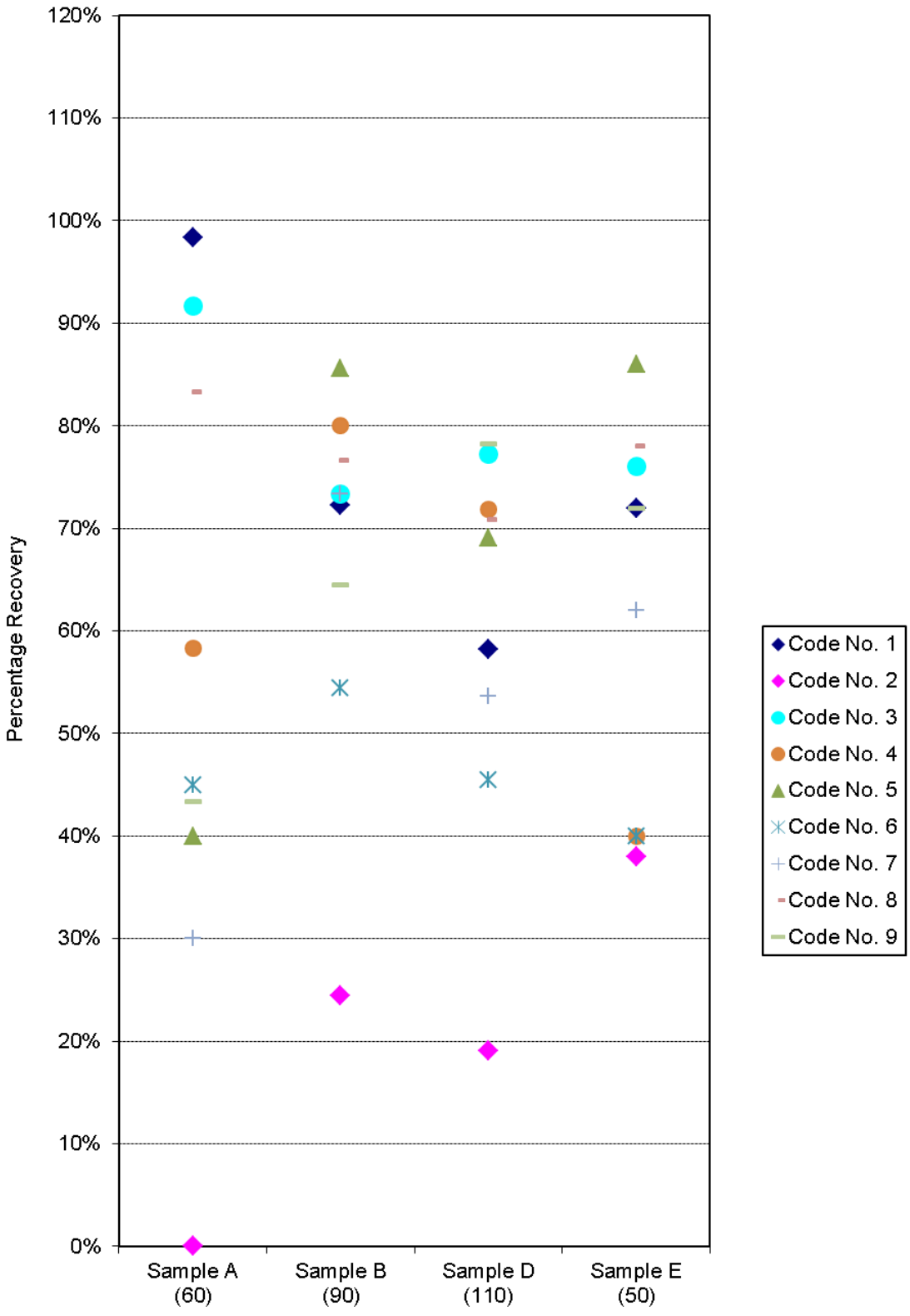
Calculated as a % of TOTAL Counts / Reference Counts

Reference Counts	60	90	110	50			
DWPFBW	50 µL	500 µL	50 µL	150 µL	Lab Average	Lab SD	Lab %RSD
Code No.	Sample A	Sample B	Sample D	Sample E			
1	98%	72%	58%	72%	75%	17%	22%
2	0%‡	24%	19%	38%	20%	16%	77%
3	92%	73%	77%	76%	80%	8%	10%
4	58%	80%	72%	40%	63%	17%	28%
5	40%	86%	69%	86%	70%	22%	31%
6	45%	54%	45%	40%	46%	6%	13%
7	30%	73%	54%	62%	55%	18%	34%
8	83%	77%	71%	78%	77%	5%	7%
9	43%	64%	78%	72%	64%	15%	24%
Minimum	0%	24%	19%	38%			
Maximum	98%	86%	78%	86%			
Average	54%	67%	60%	63%			
Median	45%	73%	69%	72%			

Notes:

1. The acceptable percentage recovery rate range is 10-110%.
2. A ‡ indicates a result outside of the acceptable recovery rate of 10-110%.
3. The median is provided for information only. It is the middle result. It is a measure of the centre of the data and is similar to the mean (or average), however, is less subject to outlier results.

Results *Cryptosporidium* (% Recovery Rate)



Note:

1. *Cryptosporidium* reference count included in brackets alongside corresponding sample name.

Confirmed Results for *Cryptosporidium* (%)

Calculated as a % of Confirmed Counts / Total Counts

Reference Counts	60	90	110	50	
DWPFBW	50 µL	500 µL	50 µL	150 µL	Lab Average
Code No.	Sample A	Sample B	Sample D	Sample E	
1	86%	92%	92%	92%	91%
2	N/A	55%	52%	74%	60%
3	91%	100%	100%	100%	98%
4	51%	75%	56%	45%	57%
5	100%	100%	99%	98%	99%
6	100%	100%	99%	98%	99%
7	100%	100%	100%	100%	100%
8	100%	97%	97%	94%	97%
9	100%	100%	100%	100%	100%
	8	9	9	9	
Minimum	51%	55%	52%	45%	
Maximum	100%	100%	100%	100%	
Average	91%	91%	88%	89%	
Median	100%	100%	99%	98%	

Recovery Results for *Giardia* (%)

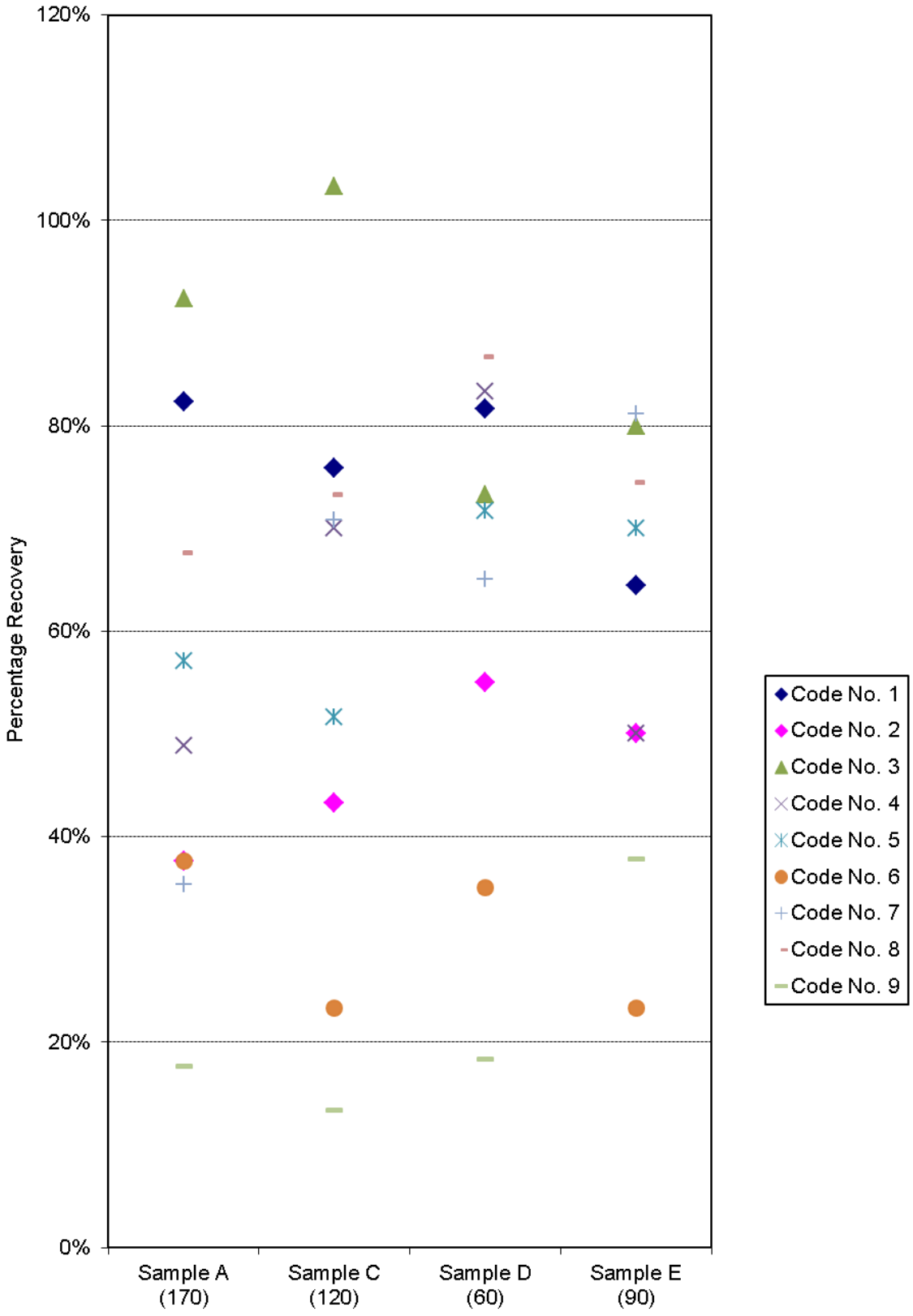
Calculated as a % of TOTAL Counts / Reference Counts

Reference Counts	60	90	120	170			
DWPFBW	50 µL	150 µL	50 µL	150 µL	Lab Average	Lab SD	Lab %RSD
Code No.	Sample A	Sample C	Sample D	Sample E			
1	82%	76%	82%	64%	76%	8%	11%
2	38%	43%	55%	50%	46%	8%	16%
3	92%	103%	73%	80%	87%	13%	15%
4	49%	70%	83%	50%	63%	17%	26%
5	57%	52%	72%	70%	63%	10%	16%
6	38%	23%	35%	23%	30%	8%	25%
7	35%	71%	65%	81%	63%	20%	31%
8	68%	73%	87%	74%	76%	8%	11%
9	18%	13%	18%	38%	22%	11%	50%
Minimum	18%	13%	18%	23%			
Maximum	92%	103%	87%	81%			
Average	53%	58%	63%	59%			
Median	49%	70%	72%	64%			

Notes:

1. The acceptable percentage recovery rate range is 10-110%.
2. The median is provided for information only. It is the middle result. It is a measure of the centre of the data and is similar to the mean (or average), however, is less subject to outlier results.

Results *Giardia* (% Recovery Rate)



Note:
1. *Giardia* reference count included in brackets alongside corresponding sample name.

A2.0

Confirmed Results for *Giardia* (%)

Calculated as a % of Confirmed Counts / Total Counts

Reference Counts	170	120	60	90	
DWPFBW	50 µL	150 µL	50 µL	150 µL	Lab Average
Code No.	Sample A	Sample C	Sample D	Sample E	
1	87%	88%	88%	86%	87%
2	66%	63%	73%	69%	68%
3	92%	97%	100%	100%	97%
4	47%	24%	70%	38%	45%
5	100%	98%	91%	97%	96%
6	100%	98%	91%	97%	96%
7	91%	89%	90%	90%	90%
8	82%	75%	62%	71%	72%
9	74%	90%	52%	73%	72%
	9	9	9	9	
Minimum	47%	24%	52%	38%	
Maximum	100%	98%	100%	100%	
Average	82%	80%	80%	80%	
Median	87%	89%	88%	86%	

APPENDIX B

Homogeneity Testing
and
Trip Control

Homogeneity Testing and Trip Control

The samples for Round 39 were produced in line with EasySeed batch number 595, which are certified reference samples. The preparation of these certified reference samples is considered to have satisfied the homogeneity testing requirements.

An estimate of uncertainty, expressed as Relative Standard Deviation (RSD), for each organism for the *Cryptosporidium* and *Giardia* proficiency testing program was calculated for each dose within the sample set. These are presented in the table below:

TABLE H: Relative Standard Deviation for Various Sample Doses (Round 39)

ORGANISM	DOSE	RSD (%)	MU as RSD (Absolute)	Resultant dose with absolute uncertainty
Cryptosporidium	50	2.8	3	50 ± 3
Cryptosporidium	60	2.8	3	60 ± 3
Cryptosporidium	90	1.5	3	90 ± 3
Cryptosporidium	110	1.5	3	110 ± 3
Giardia	60	2.4	3	60 ± 3
Giardia	90	1.5	3	90 ± 3
Giardia	120	1.4	3	120 ± 3
Giardia	170	1.4	5	170 ± 5

Notes for Table G:

1. Historical QC data and homogeneity testing data have been used to calculate the information in the above table.
2. All measurement uncertainty estimates are at the 95% level of confidence.
3. All numbers have been rounded to whole numbers. Although it may appear that the “MU as RSD is always 2 x RSD%” rule has been ignored, the rule itself ignores the impact of the continuous data used to calculate each value (the impact of rounding up/down).

Trip Control

Water concentrate sample F, spiked with 110 *Cryptosporidium* oocysts and 170 *Giardia* cysts was used as the trip control.

BTF Pty Ltd retained a 3.5 mL water concentrate samples F (F_{NoT}), on their premises after preparation. Sample F (F_{NoT}) was added to 10 L of distilled water, concentrated and analysed on 15 June 2017.

One nominated laboratory (Code 9) was provided with a 3.5 mL water concentrate samples F (F_T) and was requested to return the sample immediately upon receipt. Sample F (F_T) was subsequently added to 10 L of distilled water and analysed by BTF Pty Ltd on 15 June 2017. Trip control samples were concentrated using membrane filtration, and then analysed using the Dynal IMS system and epifluorescence microscopy.

Results for Control Samples F_{NoT}, F_T

Date Analysed	*Crypto.* Counts	No. DAPI positive	*Giardia* Counts	No. DAPI positive
15 June 2017 (F_{NoT}; Sample kept on premises)	73	100%	116	96%
15 June 2017 (F_T; Sample sent to laboratory and returned)	69	100%	95	94%
Actual counts	**110**		**170**	
F_{NoT} % Recovery Rate	66%		68%	
F_T % Recovery Rate	63%		56%	

The trip controls sent to the laboratory indicated sample stability during transport. Percentage recovery rates for trip control samples lie within the acceptable range of 10% - 110%.

APPENDIX C

Instructions to Participants

and

Results Sheet

Proficiency Testing Australia

Proficiency Testing Program

Cryptosporidium and *Giardia* Round 39

INSTRUCTIONS TO PARTICIPANTS

To ensure results from this program can be properly analysed, participants are asked to carefully adhere to the following instructions.

1. For this round each participant will be supplied with a sample set consisting of five, 3.5 mL bulk-water-concentrate samples. Each sample contains reverse osmosis water that may contain matrix material(s) sourced from surface water (added to simulate an environmental water sample). Samples *may* have been spiked with *Cryptosporidium* oocysts and/or *Giardia* cysts at various concentrations.

Your laboratory may receive an additional 3.5 mL bulk-water-concentrate sample that will be utilised as the proficiency testing program *Trip Control*. If you receive this sample (labelled "PTA Sample F"), please refer to the included associated covering letter for further instructions.

2. On receipt at your facility, samples must be stored at 1-8ºC. The date and time of sample receipt must be recorded on the *Results Sheet.*

3. Mix the 3.5 mL tube containing the bulk-water-concentrate sample by inversion, then immediately place the bottom of the tube on a vortex mixer and mix such that the vortex extends to the bottom of the tube. Add each of the 3.5 mL bulk-water-concentrates to individual, respective 10 L bulk water samples of your choice, taking care not to mix-up the order of the sample vials in relation to their respective 10 L water samples. Ensure the bulk water used for dilution does not contain any *Giardia* cysts or *Cryptosporidium* oocysts. For example, use reverse osmosis or membrane-filtered (suggested pore size ≤ 45 µm) water. Ensure the bulk-water-concentrate sample vial is effectively rinsed and the concentrate thoroughly dispersed throughout the 10 L bulk water. The following rinse procedure is recommended to ensure optimal sample transfer:

   i) Carefully add the contents of the proficiency testing sample (bulk-water-concentrate) tube to respective 10 L water samples.

   ii) Add 3 mL 0.05% (v/v) Tween® 20* to the empty sample tube, recap and vortex for 20 sec. Empty contents into the 10 L water sample.

   iii) Add 3 mL reagent grade water to the empty sample vial, recap and vortex for 20 sec. Empty contents into 10 L water sample.

   iv) Repeat steps ii-iii.

   *Laureth-12 Envirocheck® elution buffer or other Tween®-containing solutions for rinsing filters may alternatively be used to rinse bulk-water-concentrate sample tubes.

4. A Senior QA/QC Officer (or similar) must sign the *Results Sheet* declaration to confirm your facility has diluted the bulk-water-concentrate samples to 10 L.

5. Laboratories must then proceed to analyse the 10 L samples using their **routine test method** (that most frequently employed). Samples are to be tested in the respective order on the *Results Sheet*. One hundred percent (100%) of each sample supplied

6. must be analysed. Participants are advised that analytical methods used will be noted in the *Final Report*. To allow for confidential treatment of results in the *Final Report*, your facility has been allocated a laboratory code number, which appears on your *Results Sheet.*
7. PTA is aware of the internal positive control reference material ColorSeed™. Although PTA understands the advantage of this material as an internal positive control, laboratories should note that it is not acceptable for laboratories to adjust results obtained with the PTA proficiency testing samples on the basis of recoveries obtained using ColorSeed™ unless the respective laboratory routine practice/standard operating procedure uses ColorSeed™ as a true internal standard, i.e. addition to *every* sample, *and* correction of observed count using internal standard recovery during routine sample reporting.

8. Record the results for each sample on the *Results Sheet* provided. Participants must report both *Total* and *Confirmed* Counts on the PTA *Results Sheet* and specify the method(s) used for confirmation. **Please be advised** that PTA uses *Total Counts* (rather than *Confirmed Counts*) in data analysis. Participants must not report non-numerical values (i.e. less than/greater than values, presence/absence, detected/not detected, etc.) on the PTA *Results Sheet*. Actual counts observed under the microscope must be reported. Participants must not use conversion (recovery) factors derived from quality control to adjust raw data unless a true internal standard is employed for every routine sample as described above. If such internal standard correction is used, this must be indicated.

9. Participants are requested to calculate and report an estimate of measurement uncertainty (MU) for each reported *Total Count* result. All MU must be reported as a 95% confidence interval (coverage factor k ≈ 2). Estimates must be reported as either relative (% RSD – e.g. 100 +/- 10% [oo/cysts] at 95% CI) or absolute (e.g. 100 +/- 10 [oo/cysts] at 95% CI) and include the number (*n*) of determinations used to generate the respective MU estimate.

10. Commence testing as soon as possible after samples are received. IMPORTANT: All participants must return completed *Results Sheets* no later than

Friday 2 June 2017 to:

Kathy Weller
Proficiency Testing Australia
PO Box 1122
ARCHERFIELD QLD 4108

phone: +61 7 3721 7373
fax: +61 7 3217 1844
email: kathy.weller@pta.asn.au

PTA would like to thank you for participating in this *Cryptosporidium* and *Giardia* proficiency testing program.

Proficiency Testing Australia

Cryptosporidium and *Giardia* Round 39 - Proficiency Testing Program

Results Sheet

Laboratory Code:

Date / Time of Sample Receipt: _____

Condition of Samples Upon Receipt: _____

Sample	*Cryptosporidium* Counts			*Giardia* Counts			Date & time of testing
	Total Count	MU and **n*	*Confirmed Count*	*Total Count*	MU and **n*	*Confirmed Count*	
A							
B							
C							
D							
E							

n – number of determinations used to generate MU estimate.

Methods used:

Bulk Water Concentration (e.g. Flocculation) _____

Filtration Type (please tick): Sponge ☐ Flat Bed ☐ Cartridge ☐ Tangential Flow ☐ Other ☐

Describe_____

Purification (e.g. IMS) _____

Enumeration (e.g. Microscopy) _____

Confirmation method(s) (e.g. DAPI, DIC) _____

Please be advised that methods used to obtain results will be noted in the final report.

Print Name: _____ **Date:** _____

Signed: _____ _____**(Analyst/s)**

I confirm that the concentrate was added to 10 L of water prior to analysis.

Print Name: _____ **Date:** _____

Signed: _____ **(Senior QA/QC Officer or similar)**

Return no later than **Friday 2 June 2017** to:

Kathy Weller
Proficiency Testing Australia, PO Box 1122, Archerfield QLD 4108.
Email: kathy.weller@pta.asn.au Phone: +61 7 3721 7373 Fax: +61 7 3217 1844

GLOSSARY

Trip Control	A sample used to monitor the effect(s) of sample set transport. Sent to a nominated laboratory and returned.
Seed Sample	Sample containing *Cryptosporidium* oocysts and/or *Giardia* cysts in various doses, prior to dispensing into the PTA sample container.
Water Concentrate Sample	Final proficiency testing sample, containing *Cryptosporidium* oocysts and/or *Giardia* cysts, DWPFBW and Milli-Q^{TM} water.
IMS	Immunomagnetic separation
DAPI	4',6-diamidino-2-phenylindole
DIC	Differential Interference Contrast (Microscopy)
IFA	Immunofluorescent Antibody
FITC	Fluorescein isothiocyanate

---------- End of report ---------