

INTRODUCTION

UK NEQAS Parasitology has been providing External Quality Assessment (EQA) schemes for Faecal Parasite microscopy since 1986. For this scheme, specimens preserved in 10% formalin are distributed and participants are requested to use their routine concentration methods for parasite recovery and examination. A concentration method is required to increase the chances of recovering ova, cysts and larvae which may otherwise be too scanty to be detected by direct microscopy alone.

To ensure that all our specimens are fit-for-purpose; rigorous pre- and post- distribution quality controls (QC) checks are undertaken. Currently, unfilled faecal concentrators are used for parasite recovery which requires manual handling and adding of bulk 10% formalin, triton X 100 and ethyl acetate.

Due to health and safety implications of these manual steps which involves handling of hazardous chemicals, it is important to move to pre-filled concentration systems whilst maintaining the highest quality of EQA provision.

Data from the QC checks using pre-filled system are shown below, together with a validation study of 10 randomised specimens.

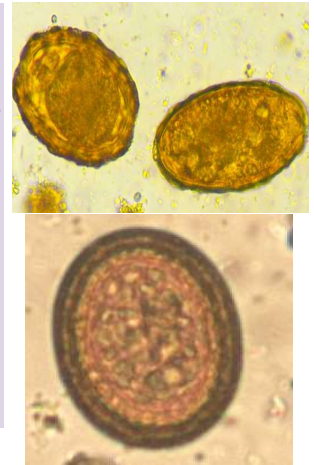
METHODS

10 randomised known positive specimens and 2 known negatives were chosen as controls.

All specimens were concentrated for parasite recovery in duplicate using an unfilled and a pre-filled concentrator.

Microscopy was performed to establish the species, stage and number of parasites recovered from each specimen, noting the number of parasites observed per coverslip.

A quantitative analysis was performed on the results to establish whether there was a significant difference between the 2 concentrators.



RESULTS

- Each specimen that was analysed contained between 1 and 4 different parasite species.
- The same species of parasites were recovered using both methods.
- No unexpected parasites were recovered in either method.
- No parasites were observed in the negative specimens using either method.
- In total, 12 different species of parasites were recovered and observed in this study.

Specimen	Cysts of <i>E. coli</i>	Cysts of <i>E. histolytica/dispar</i>	Cysts of <i>Iodamoeba butschlii</i>
1	25	26	46
2	22	26	39
3	23	20	44
4	25	27	48
5	19	23	40
6	14	17	50
7	20	25	46
8	22	27	49
9	19	23	41
10	25	27	39
Range	14-25 per coverslip	17-27 per coverslip	39-50 per coverslip
Average	21 per coverslip	24 per coverslip	44 per coverslip

Table 1 (above): Pre-QC of Pilot Distribution using Pre-filled Concentrator

Returns Lab ID	Cysts of <i>E. coli</i>	Cysts of <i>E. histolytica/dispar</i>	Cysts of <i>Iodamoeba butschlii</i>
9	27	20	45
16	30	21	49
68	24	16	49
93	20	27	39
Range	20-30 per coverslip	16-27 per coverslip	39-49 per coverslip

Table 2 (above): Post-QC of Pilot Distribution using Pre-filled Concentrator

Sample storage no/specimen no	Sample no assigned	List of parasites observed in each sample	No of parasites recovered in unfilled concentrator	No of parasites recovered in pre-filled concentrator
P334	1	Ova of <i>Ascaris lumbricoides</i>	19	23
	1	Ova of Hookworm species	1	1
	1	Ova of <i>Schistosoma mansoni</i>	6	8
P359	2	Cysts of <i>Entamoeba coli</i>	12	9
	2	Cysts of <i>Endolimax nana</i>	12	11
	2	Cysts of <i>Blastocystis hominis</i>	8	5
	2	Cysts of <i>Entamoeba histolytica/dispar</i>	6	7
P381	3	Ova of <i>Taenia</i> species	10	12
	3	Ova of <i>Diphyllobothrium latum</i>	7	14
P379	4	Ova of <i>Trichostrongylus</i> species	3	2
P380	5	Ova of <i>Schistosoma mansoni</i>	3	4
P319	6	Ova of <i>Diphyllobothrium latum</i>	10	18
	6	Ova of Hookworm species	24	32
P333	6	Ova of <i>Ascaris lumbricoides</i>	30	50
	7	Ova of <i>Trichuris trichiura</i>	6	4
	7	Ova of <i>Ascaris lumbricoides</i>	497	520
specimen 6239	8	Cysts of <i>Giardia duodenalis</i>	41	48
specimen 6340	9	Ova of <i>Schistosoma mansoni</i>	69	76
specimen 6141	10	Ova of <i>Ascaris lumbricoides</i>	422	471

Table 3 (above): Data showing parasite recovery and counts using the 2 types of concentrators

t-Test: Two-Sample Assuming Unequal Variances		
	Unfilled concentrator	Pre-filled concentrator
Mean	62.42105263	69.21053
P(T<=t) two-tail	0.887342815	

DISCUSSION

- A T test was performed to establish whether there was a significant difference between the 2 methods. The null hypothesis for this study was that there was no significant difference between the 2 methods and in the number of parasites recovered.
- The P value was 0.887, and therefore there was no significant difference between the 2 methods.
- Quality of the samples, i.e., recovery and morphology were not impacted by using pre-filled system.
- The QC data from the pilot distribution (Table 1 and 2) shows that recovery of the parasites was not affected in any way and the parasite numbers were similar in the pre- and post-QC of the pilot distribution.

CONCLUSIONS

- Both concentration systems work very well for recovery of parasites from faecal suspensions.
- This study reinforces the idea of a safer system of work by reducing health risks associated with the use of unfilled concentrators that require manual dispensing of hazardous chemicals.
- As per the pre- and post- QC results of the pilot distribution backed up by the validation studies using the pre-filled concentration system, we have demonstrated that our QC remained unaffected. The specimens remain fit-for-purpose. UK NEQAS will therefore start using a pre-filled concentration system for the QC of all their future faecal parasitology scheme distribution.

REFERENCES

Manser, M.M., Saez, A.C. and Chiodini, P.L. (2016) "Faecal Parasitology: Concentration Methodology Needs to be Better Standardised," *PLOS Neglected Tropical Diseases*, 10(4), p. e0004579. Available at: <https://doi.org/10.1371/journal.pntd.0004579>