

TOTAL FIX SENSIBILITY, STABILITY, SPECIFICITY AND REPRODUCIBILITY STUDIES



Margaret Clarke, Minoo Ghajar, Karen McLean and Richard Alexander

Orange County Public Health Laboratory, California

INTRODUCTION

- Microscopic examination of fecal material is standard laboratory practice for diagnosis of intestinal parasites.
- The ova and parasite (O&P) examination is traditionally performed using a collection kit that chemically fixes organisms in polyvinyl alcohol (PVA) and formalin. However, as a result of disposal problems related to the use of mercuric chloride in PVA stool preservatives, many laboratories have switched to other, non-mercury based preservatives.
- This study evaluated the performance of the Total-Fix single vial collection system compared to Para Pak LV-PVA/formalin system.

METHODS

- A total of 100 specimens were collected from 50 refugee patients in duplicate from the Refugee Preventative Health Services Clinic (RPHS). Each set of specimens included a Total-Fix collection bottle, a PVA and a formalin bottle.
- Of the 100 specimens, 97 were acceptable in both systems to be evaluated. Three specimens were excluded for reasons of either no stool in vial or duplicate specimens collected on the same date.
- Specimens were blinded by a third person before being processed or read by two technologists.
- Specimens were processed and stained according to each manufacturer's directions.
- Concentration methods and Trichrome stains were performed on all specimens from both type of preservatives.
- Specimens were randomly read by two technologists, with no knowledge of results from the second preservative to prevent reader bias.
- Discrepant results were re-read by both technologists to help eliminate reading errors by one person.

RESULTS

- 43 of 97 specimens (44%) evaluated were found to be positive without discrepancies; several had multiple parasites per specimen.
- 43 of 97 specimens (44%) were negative in both systems.
- 11 specimens (11%) had discrepant results between the two different preservatives (Table 1), 4 of which were the same type.

Durviz s.l.
Parque Tecnológico Valencia
Leonardo Da Vinci, 10
Tno. 96 136 61 07 - Fax 96 136 61 68
46980 Paterna - Valencia-España

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Durviz s.l. C/Leonardo da Vinci, 10 Parque Tecnológico de Valencia, Paterna - Valencia - Spain

Tel. +34 96 136 61 07, fax +34 96 136 61 68 Email : durviz@durviz.com

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Table 1. Summary of discrepancies

Method	Discrepancy	No.
PVA/Formalin	D. fragilis and E. coli not recovered	1
PVA/Formalin	E. nana not recovered	1
Total-Fix	B. hominis not recovered	4
Total-Fix	D. fragilis not recovered	1
Total-Fix	E. nana and B. hominis not recovered	1
Total-Fix	D. fragilis and E. nana not recovered	1
Total-Fix	Results between technologists did not agree	2

Table 2. Enumeration of parasites observed during the evaluation

Organism	No.	%
<i>E. coli</i>	3	6
<i>E. nana</i>	7	13
<i>D. fragilis</i>	1	2
<i>C. mesnili</i>	2	4
<i>B. hominis</i>	37	70
<i>E. histolytica/E. dispar</i>	3	6



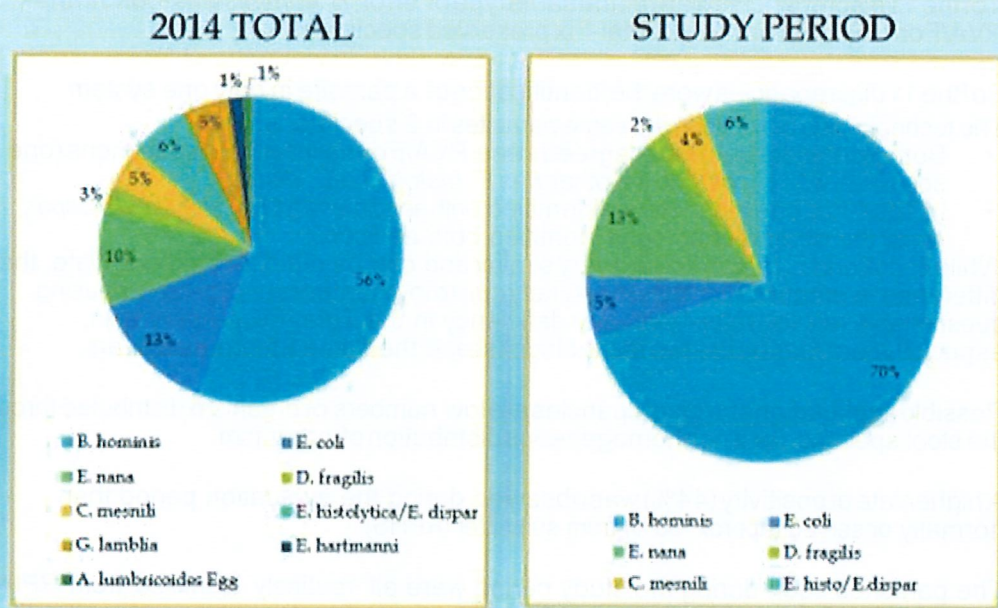
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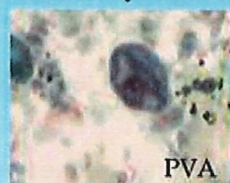
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Orange County Public Health Laboratory, California

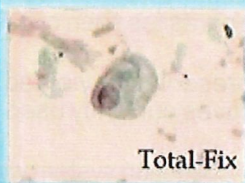
Figure 1. Parasites observed from RPHS during 2014 compared to study period



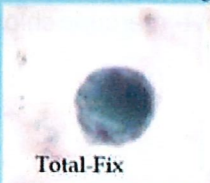
***Chilomastix mesnili* Cysts**



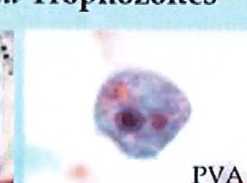
***Chilomastix mesnili* Trophozoites**



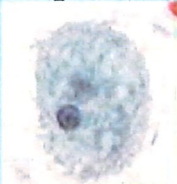
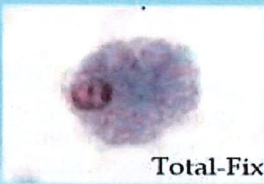
Blastocystis hominis



***Endolimax nana* Trophozoites**



***Entamoeba coli* Trophozoites**



***Entamoeba coli* Cysts**



Durviz s.l.u.
Parque Tecnológico Valencia
Leonardo Da Vinci, 10 3
Tno. 96 136 61 07 - Fax 96 136 61 68
Valencia, Spain
Tno. 96 136 61 07 - Fax 96 136 61 68



Durviz s.l. C/Leonardo da Vinci, 10 Parque Tecnológico de Valencia, Paterna 46100, Spain
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DISCUSSION

- 4 of the 11 discrepancies were all the same type of error; *B. hominis* was found in the PVA/Formalin, but not in the Total-Fix preserved specimens.
- 5 of the 11 discrepancies were the identification of a parasite in only one system.
- The technologists did not see the same parasites in 2 specimens:
 - Both technologists' results agreed in the PVA/Formalin for both specimens (one specimen was *E. coli*, and the other was *E. histolytica/E. dispar*).
 - In Total-Fix, one technologist identified both specimens as *E. histolytica/E. dispar*, while the other technologist identified both as *E. coli*.
- While *E. coli* and *E. histolytica* are very similar and can be difficult to differentiate, the difference in identification was most likely due to low numbers of organism causing questionable results, rather than any deficiency in the Total-Fix preservative, especially considering the two technologists read the same trichrome smears.
- Possible explanations for discrepancies are low numbers of organism distributed through the stool specimen or a nonhomogeneous distribution of organism.
- A higher rate of positivity (44%) was observed during the evaluation period than normally observed (approx. 35%) from submitter (RPHS).
- The parasites seen during the study period were all routinely identified from RPHS in approximately the same percentages as observed throughout the year.
- One exception was that *Giardia* was not seen during the evaluation period even though it accounted for 5% of the parasites seen over the course of the year.
- Overall, the Total-Fix preservative yielded comparable results to the traditional PVA/Formalin preservatives currently used by OCPHL.
- Use of the Total-Fix stool collection system would eliminate the need for costly hazardous waste disposal of the PVA mercuric chloride.
- Staining times between the two preservatives differ by about 15 minutes. This is due to the elimination of the first 3 staining steps that are required to remove mercuric chloride.

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Tno. 96 136 61 07 - Fax 96 136 61 61
46980 Paterna - Valencia-España

