JCP Online First, published on June 16, 2011 as 10.1136/jcp.2011.090639


# Comparison between the Midi Parasep and Midi Parasep Solvent Free (SF) faecal parasite concentrators

Agatha C Saez,[1] Monika M Manser,[1] Nick Andrews,[2] Peter L Chiodini[1]

[1]Department of Clinical Parasitology, UKNEQAS Parasitology, Hospital for Tropical Diseases, London, UK
[2]Statistics Unit, Centre for Infections, HPA, UK

**Correspondence to**
Professor Peter L Chiodini, Department of Clinical Parasitology, Hospital for Tropical Diseases, Mortimer Market, Capper Street, London WC1E 6JB, UK; peter.chiodini@uclh.nhs.uk

Accepted 15 May 2011

## ABSTRACT

**Aim** To compare the recovery of parasites in faecal samples using the Midi Parasep with ethyl acetate and Midi Parasep Solvent Free (SF) faecal parasite concentrators.

**Methods** 23 preserved and 11 fresh faecal samples were microscopically examined for the presence of parasites using the Midi Parasep concentrator with ethyl acetate centrifuged for 1 and 3 min and the Midi Parasep SF concentrator.

**Results** The Midi Parasep SF faecal parasite system recovered significantly fewer ova and cysts and resulted in a notably larger deposit than the Midi Parasep concentrator with ethyl acetate.

**Conclusions** Parasites present in small numbers that would be detected using the Midi Parasep concentrator with ethyl acetate could be missed using the SF faecal parasite system.

## INTRODUCTION

The microscopic examination of faeces is essential for the recognition and identification of intestinal parasites. The use of a faecal concentration method increases the sensitivity and probability of finding ova, cysts and larvae in faecal specimens, particularly in those specimens where they are too few to be seen by direct microscopy.[1]

The faecal concentration method used in the Department of Clinical Parasitology, Hospital for Tropical Diseases, is the Midi Parasep, which is an enclosed, single-use disposable system based on the modified Ridley–Allen[2] concentration technique. This method uses ethyl acetate and Triton X as an extractor of fat and debris from faeces, and then filtration followed by centrifugation to leave the ova, cysts and larvae in the sediment at the bottom of the tube. The advantage of this method is that it will recover most ova, cysts and larvae while retaining their morphology, thus facilitating identification. The method can also be used on samples that have been preserved in formalin, sodium acetate–acetic acid–formalin and polyvinyl alcohol, but it has the disadvantage of destroying trophozoite stages and distorting cellular exudates. Liquid faeces do not concentrate well, so it is also necessary to examine the stool by direct microscopy in addition to the concentration technique.

The Midi Parasep Solvent Free (SF) product was introduced in 2006. This does not use ethyl acetate or ether-derived products, so it has health and safety benefits by eliminating the risk of solvent disposal and exposure. It employs the dual-filter technology to remove smaller faecal debris and solubilise the fat content so that it does not interfere with the examination of the resulting sediment.

As a result of the health and safety advantages of the Midi Parasep SF system, many clinical laboratories have employed this technique in their routine practice for examining faecal specimens for parasites. However, 14 participants using this product as part of the UKNEQAS Parasitology, Faecal Scheme, have expressed their concern at failing to observe parasite ova, namely those of hookworm species, *Taenia* species and *Trichuris trichiura*, present in UKNEQAS specimens and consequently losing points in their cumulative scores. Many users were also concerned that they were recovering lower numbers of ova and cysts than those seen by UKNEQAS in the predistribution examination of the specimens. This prompted UKNEQAS to conduct a study to compare the recovery of parasites using the original solvent-based Midi Parasep product and the Midi Parasep SF product. Both commercial kits are supplied by DiaSys Ltd, Sapphire Centre, Fishpond Road, Workingham, Berkshire, UK.

## MATERIALS AND METHODS

### Faecal samples

A total of 23 faecal samples preserved in 10% formalin in water (table 1) and 11 fresh, unpreserved samples (table 2) containing ova, cysts and larvae were concentrated and examined using both the Midi Parasep with ethyl acetate and the Midi Parasep SF in accordance with the manufacturer's instructions. In addition, eight serial doubling dilutions were made from two specimens, one fresh sample containing cysts (table 3) and one preserved sample containing ova (table 4), in order to establish the dilution ratio at which the parasites could no longer be detected.

Prior to the microscopic examination of the specimens, 75 µl (three drops) of saline was added to all the faecal deposits and mixed well in order to resuspend them. For all specimens, 50 µl of the diluted deposit was dispensed onto a microscope slide and a 22 mm by 22 mm coverslip was applied. The whole of the coverslip was examined and the number of ova, cysts and larvae was recorded. All specimens were processed in duplicate.

### Midi Parasep

The Parasep faecal parasite concentrator is an enclosed system that employs the principle of the Ridley–Allen formol-ether sedimentation technique. One gram of faeces is mixed with 6 ml of 10% formalin in the mixing chamber. Two

**Table 1** Comparison of recovery of parasites (preserved in formalin)

| Specimen content | Method 1: Midi Parasep with ethyl acetate, 3000 rpm for 3 min | Method 2*: Midi Parasep with ethyl acetate, 3000 rpm for 1 min | Method 3*: Midi Parasep Solvent Free (SF), 1400 rpm for 3 min |
|---|---|---|---|
| *Ascaris lumbricoides* | 807 | 490 | 161 |
| *Taenia* spp. | 942 | 374 | 204 |
| Hookworm spp. | 76 | 64 | 19 |
| *Diphyllobothrium latum* | 98 | 101 | 20 |
| *Trichuris trichiura* | 45 | 26 | 3 |
| *Toxocara* spp. | 95 | 114 | 40 |
| *Hymenolepis nana* | 72 | 43 | 33 |
| *Schistosoma mansoni* | 85 | 82 | 17 |
| *Trichostrongylus* spp. | 17 | 17 | 1 |
| *Fasciola* spp. | 8 | 8 | 2 |
| *Enterobius vermicularis* | 8 | 13 | 4 |
| Rhabditiform larvae of *Strongyloides stercoralis* | 3 | 2 | 1 |
| *Isospora belli* | 106 | 40 | 25 |
| *Entamoeba coli* | 466 | 521 | 355 |
| *Giardia intestinalis* | 299 | 189 | 110 |
| *Endolimax nana* | 609 | 494 | 364 |
| *Blastocystis hominis* | 118 | 90 | 80 |
| *Chilomastix mesnili* | 143 | 164 | 125 |
| | **Method 1 vs method 2** | **Method 1 vs method 3** | **Method 2 vs method 3** |
| Sign test p value | 0.06 | 0.0005 | 0.0005 |

*Indicates the method recommended by the manufacturer for each device.

millilitres of ethyl acetate are added (1 drop of Triton-X-100 is added to the mixture, as it helps to emulsify the faecal matter). Parasep is assembled and sealed by screwing the filter thimble and sedimentation cone onto the mixing chamber. The mixture is vortexed for 15 s and the system is then inverted to allow the mixture to be filtered through the filter thimble and centrifuged at 1000 g or 3000 rpm for 1 min.[2 3] The process was also tested with a centrifugation speed of 3 min to assess the effect of centrifugation on the deposition of ova and cysts. The mixing chamber and the filter thimble are unscrewed together and discarded. Like the conventional Ridley–Allen sedimentation method, there is an upper ethyl acetate layer, fatty plug, formalin supernatant and deposit. The fatty plug is loosened and the supernatant is safely discarded according to the Control of Substances Hazardous to Health regulations. The concentration procedure apart from the centrifugation stage is undertaken in a safety cabinet.

**Table 2** Comparison of unpreserved specimens

| Specimen content | Method 1: Midi Parasep with ethyl acetate, 3000 rpm for 3 min | Method 2*: Midi Parasep with ethyl acetate, 3000 rpm for 1 min | Method 3*: Midi Parasep Solvent Free (SF), 1400 rpm for 3 min |
|---|---|---|---|
| *Giardia intestinalis* | 4236 | 3604 | 2163 |
| *Iodamoeba butschlii* | 1013 | 1019 | 624 |
| *Entamoeba coli* | 112 | 91 | 45 |
| *Entamoeba histolytica/dispar* | 25 | 36 | 20 |
| *Endolimax nana* | 21 | 17 | 3 |
| *Hymenolepis nana* | 37 | 22 | 18 |
| *Enterobius vermicularis* | 3 | 3 | 8 |
| | **Method 1 vs method 2** | **Method 1 vs method 3** | **Method 2 vs method 3** |
| Sign test p value | 0.69 | 0.015 | 0.015 |

*Indicates the method recommended by the manufacturer for each device.

**Table 3** Comparison of methods using doubling dilutions of a stool containing *Iodamoeba butschlii* cysts (unpreserved specimen)

| Dilution | Method 1: Midi Parasep with ethyl acetate, 3000 rpm for 3 min | Method 2*: Midi Parasep with ethyl acetate, 3000 rpm for 1 min | Method 3*: Midi Parasep Solvent Free (SF), 1400 rpm for 3 min |
|---|---|---|---|
| 1 (Neat) | 775 | 709 | 235 |
| 2 (1:2) | 445 | 387 | 116 |
| 3 (1:4) | 183 | 184 | 55 |
| 4 (1:16) | 87 | 63 | 35 |
| 5 (1:32) | 33 | 55 | 15 |
| 6 (1:64) | 17 | 17 | 8 |
| 7 (1:128) | 6 | 5 | 0 |
| 8 (1:256) | 2 | 5 | 0 |
| | **Method 1 vs method 2** | **Method 1 vs method 3** | **Method 2 vs method 3** |
| Sign test p value | ≤1 | 0.008 | 0.008 |

*Indicates the method recommended by the manufacturer for each device.

## Midi Parasep Solvent Free (SF)

The Midi Parasep SF was developed specifically to eliminate the need for a solvent in faecal concentration; it uses a two-stage filtration process, first filtering out unwanted debris and then detaching fat content from the smaller debris so that it can be efficiently removed from the resulting sediment without the use of solvent.

In the mixing tube, 0.5 g of faeces is mixed with 6 ml of 10% formalin and one drop of Triton-X-100. The system is sealed by assembling the filter thimble and conical tube. The mixture is vortexed for 15 s, then centrifuged at 1400 rpm or 200 g for 3 min. The filter thimble plus the mixing tube is unscrewed and discarded. The supernatant is discarded to leave only the sediment for microscopic examination. Similar to the Midi Parasep, the concentration procedure apart from the centrifugation stage is undertaken in a safety cabinet to minimise the smell of formalin and specimen odours.

## RESULTS

A comparison of the Midi Parasep with ethyl acetate centrifuged at 3000 rpm for 3 min, Midi Parasep with ethyl acetate centrifuged at 3000 rpm for 1 min and Midi Parasep SF centrifuged at

**Table 4** Comparison of methods using doubling dilutions of a stool containing *Diphyllobothrium latum* ova (preserved in formalin)

| Dilution | Method 1: Midi Parasep with ethyl acetate, 3000 rpm for 3 min | Method 2*: Midi Parasep with ethyl acetate, 3000 rpm for 1 min | Method 3*: Midi Parasep Solvent Free (SF), 1400 rpm for 3 min |
|---|---|---|---|
| 1 (Neat) | 234 | 221 | 99 |
| 2 (1:2) | 96 | 90 | 38 |
| 3 (1:4) | 61 | 57 | 19 |
| 4 (1:16) | 40 | 35 | 9 |
| 5 (1:32) | 34 | 13 | 0 |
| 6 (1:64) | 7 | 9 | 0 |
| 7 (1:128) | 5 | 7 | 0 |
| 8 (1:256) | 5 | 4 | 0 |
| | **Method 1 vs method 2** | **Method 1 vs method 3** | **Method 2 vs method 3** |
| Sign test p value | 0.3 | 0.008 | 0.008 |

*Indicates the method recommended by the manufacturer for each device.

**Table 5** Summary statistics of the volume of deposit present after concentration (preserved specimens)

| | Method 1: Midi Parasep with ethyl acetate, 3000 rpm for 3 min | Method 2*: Midi Parasep with ethyl acetate, 3000 rpm for 1 min | Method 3*: Midi Parasep Solvent Free (SF), 1400 rpm for 3 min |
|---|---|---|---|
| Mean | 80.52174 | 60.91304 | 123.2174 |
| SE | 5.585791 | 5.759993 | 4.513903 |
| Median | 67 | 67 | 125 |
| Mode | 67 | 67 | 125 |
| SD | 26.78851 | 27.62396 | 21.64792 |
| Sample variance | 717.6245 | 763.083 | 468.6324 |
| Kurtosis | −0.21184 | 3.906991 | 5.025034 |
| Skewness | 0.787442 | 1.371964 | −1.89303 |
| Range | 100 | 125 | 100 |
| Minimum | 37 | 25 | 50 |
| Maximum | 137 | 150 | 150 |
| Sum | 1852 | 1401 | 2834 |
| Count | 23 | 23 | 23 |

| | Method 1 vs method 2 | Method 1 vs method 3 | Method 2 vs method 3 |
|---|---|---|---|
| Sign test p value | 0.002 | <0.001 | <0.001 |

*Indicates the method recommended by the manufacturer for each device.

1400 rpm for 3 min was done to determine differences in the number of parasites present in the deposit. The size of the deposit was also compared.

The numbers of ova and cysts for each parasite species were added together and the results are shown in table 1 for the formalin-preserved specimens and in table 2 for the fresh, unpreserved samples. The results of the serial dilutions are shown in tables 3 and 4. The comparison of deposit size for the formalin-preserved and fresh, unpreserved samples are shown in tables 5 and 6.

In order to compare the recovery of parasites and deposit size for the three methods, the non-parametric sign test was used. This simple test is based on whether the number of parasites recorded is lower or higher in each observation, and not on their numerical magnitude.

**Table 6** Summary statistics of the volume of deposit present after concentration (unpreserved specimens)

| | Method 1: Midi Parasep with ethyl acetate, 3000 rpm for 3 min | Method 2*: Midi Parasep with ethyl acetate, 3000 rpm for 1 min | Method 3*: Midi Parasep Solvent Free (SF), 1400 rpm for 3 min |
|---|---|---|---|
| Mean | 87.90909 | 71.18182 | 150 |
| SE | 11.62322 | 10.22725 | 5.291503 |
| Median | 67 | 67 | 150 |
| Mode | 67 | 75 | 150 |
| SD | 38.54985 | 33.91996 | 17.54993 |
| Sample Variance | 1486.091 | 1150.564 | 308 |
| Kurtosis | −1.35133 | 0.469069 | −1.30728 |
| Skewness | 0.0009 | 0.888115 | -0.11939 |
| Range | 112 | 112 | 50 |
| Minimum | 25 | 25 | 125 |
| Maximum | 137 | 137 | 175 |
| Sum | 967 | 783 | 1650 |
| Count | 11 | 11 | 11 |

| | Method 1 vs method 2 | Method 1 vs method 3 | Method 2 vs method 3 |
|---|---|---|---|
| Sign test p value | 0.06 | <0.001 | <0.001 |

*Indicates the method recommended by the manufacturer for each device.

### Comparison of recovery of parasites (preserved in formalin)

A comparison between the Midi Parasep with ethyl acetate centrifuged for 1 and 3 min and the Midi Parasep SF showed a significant difference in the number of ova recovered (sign test p=0.0005), the Midi Parasep with ethyl acetate recovering considerably more ova than the SF product. Assessment of the effect of centrifugation time with the Midi Parasep with ethyl acetate method did not show a significant overall difference in parasite recovery between centrifugation times of 3 min and 1 min although there was an apparent difference in the number of *Ascaris lumbricoides, Taenia* species, *T trichiura* and *Hymenolepis nana* ova recovered (table 1).

### Comparison of unpreserved specimens

A comparison of ovum and cyst recovery in the unpreserved specimens showed a significant overall difference (sign test p=0.015), with the Midi Parasep with ethyl acetate recovering more parasites than the SF product. A comparison between the centrifugal times of Midi Parasep with ethyl acetate did not show a significant difference in the recovery of cysts or ova (table 2).

### Comparison of ovum and cyst recovery from serial doubling dilutions

Comparison of ovum and cyst recovery from serial doubling dilutions of stools containing cysts *of Iodamoeba butschlii* or ova of *Diphyllobothrium latum* showed a significant difference (sign test p=0.008), with the Midi Parasep with ethyl acetate recovering considerably more parasites than the SF product. Comparison of the effect of centrifugation time using Midi Parasep with ethyl acetate did not show a significant difference in ovum or cyst recovery. The endpoint dilution for the detection of cysts of *I butschlii* was 1:64 and for ova of *D latum* was 1:16 when using the Midi Parasep SF whereas the ova and cysts were recovered in dilutions of 1:256 using Midi Parasep with ethyl acetate (tables 3 and 4).

### Summary statistics of the volume of deposit present after concentration

Sediment size after centrifugation was significantly larger with the SF product than with the product with ethyl acetate for both preserved and unpreserved specimens (sign test p=0.008). This was also confirmed by the summary statistics (tables 5 and 6).

## DISCUSSION

The use of a concentration method for the examination of faecal samples is essential to maximise the number of organisms detected particularly in those specimens where they are too few to be seen by direct microscopy alone. Most commercial kits are based on the modified Ridley–Allen[2] concentration technique, which involves emulsifying the faeces in formalin, sieving the suspension, the addition of ether or ethyl acetate, centrifugation and examining the sediment. The method routinely used in the Department of Clinical Parasitology, Hospital for Tropical Diseases, is the Midi Parasep manufactured by DiaSys, which uses ethyl acetate as a solvent. The SF kit, Parasep SF, which is also manufactured by DiaSys, decreases the risk of solvent exposure and disposal.

This study compared Midi Parasep SF against the original solvent-based product Midi Parasep. When comparing the techniques, the criteria considered were recovery of parasites, the density of the deposit, ease of handling and health and safety aspects. Although both kits are totally enclosed/sealed process and single use, disposable systems, their protocol differs in the

size of the faecal sample used (1 g vs 0.5 g), the centrifugal speed (3000 rpm vs 1400 rpm) and centrifugal time (1 min vs 3 min).

Although all parasites were seen in the three different techniques when concentrating neat preserved and unpreserved specimens, an analysis of parasite recovery showed a significant reduction in the number of ova and cysts reported in the SF system compared with those reported in the system using ethyl acetate. This observation was supported when comparing the end point of parasite detection in serial doubling dilutions of two parasite species (tables 3 and 4). The lower number of cysts and ova recovered could be due to the smaller amount of faeces used, the lower centrifugation speed and the lack of a solvent or a combination of all three. The recommended sample size is 1 g of faeces, so using 0.5 g as in the SF system very likely reduces the recovery of ova and cysts. Centrifugation speed is also an essential component of the concentration process. Our results suggest that centrifuging at a speed of 1400 rpm instead of 3000 rpm also contributes to reduced sedimentation of parasite stages.

This is further supported by the fact that some specimens centrifuged for 3 min using the Midi Parasep with ethyl acetate recovered more parasites than those that were centrifuged for 1 min using the same concentration device.

A significant difference was observed between the size of the final deposit from the Parasep ethyl acetate-based concentration technique and that of the Parasep SF technique. Samples prepared by the Parasep SF technique showed a larger volume of faecal debris although the size of faecal sample concentrated was smaller than that used in the product with ethyl acetate. The denser deposit is more difficult to examine and may obscure ova and cysts. The deposit of Parasep with ethyl acetate was less dense due to the removal of more faecal material by ethyl acetate, so the sediment obtained was clearer, thus facilitating the identification of ova and cysts. The deposit was denser when centrifuged at 3000 rpm for 3 min with the product containing ethyl acetate than that when centrifuged for 1 min using the same device. This was to be expected since more faecal matter was deposited, but it had the added benefit of more ova and cysts being recovered in the deposit for some parasite species.

When considering the health and safety aspects of the products, Midi Parasep, although still requiring the use of formalin and ethyl acetate, is an enclosed process. The product has an air/liquid seal and safety lock; the seal prevents the release of hazardous material and the lock ensures that the mixing chamber and filter thimble are removed together for safe disposal after centrifugation. The risk of solvent exposure is further minimised by performing the procedure in a safety cabinet. Disposal of the solvent is undertaken in accordance with local guidelines. Although the SF product further reduces the risk of solvent exposure, the risk associated with the product containing ethyl acetate is minimal when following the correct procedures.

### Take-home messages

- A concentration method increases the sensitivity and probability of finding ova, cysts and larvae in faecal specimens.
- The omission of a solvent in the concentration method reduces the recovery of ova, cysts and larvae in faecal specimens.
- The centrifugal force and time affects the recovery of ova, cysts and larvae in faecal specimens.

## CONCLUSIONS

In a concentration technique for the examination of faecal samples for parasites, the number of ova, cysts and larvae deposited is affected by the size of the sample used for concentration, the centrifugal force, the centrifugal time and the presence of a solvent. The Midi Parasep SF faecal parasite system recovered significantly fewer ova and cysts and resulted in a notably larger deposit than the Midi Parasep concentrator with ethyl acetate. This observation has implications for the examination of clinical samples for faecal parasites as our results suggest that parasites present in small numbers that would be detected with the Midi Parasep concentrator with ethyl acetate could be missed using the SF faecal parasite system.

**Funding** PLC is supported by the UCL Hospitals Comprehensive Biomedical Research Centre Infection Theme.

**Competing interests** None.

**Provenance and peer review** Not commissioned; externally peer reviewed.

## REFERENCES

1. **Ridley DS,** Hawgood BC. The value of formol-ether concentration of faecal cysts and ova. *J Clin Pathol* 1956;**9**:74—6.
2. **Allen AV,** Ridley DS. Further observations on the formol-ether concentration technique for faecal parasites. *J Clin Pathol* 1970;**23**:545—6.
3. Health Protection Agency (2008). Investigation of specimens other than blood for parasites. National Standard Method BSOP 31 Issue 3. http://www.hpa.org.uk/srmd/div_esl_su/pdf_bacteriology.htm.

# Comparison between the Midi Parasep and Midi Parasep Solvent Free (SF) faecal parasite concentrators

Agatha C Saez, Monika M Manser, Nick Andrews, et al.

*J Clin Pathol* published online June 16, 2011
doi: 10.1136/jcp.2011.090639