Compatibility of Alcorfix for *Giardia* and *Cryptosporidium* Antigen Testing

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Abstract

Background: Our laboratory recently converted to a single-vial stool fixative (Alcorfix) for ova & parasite testing; eliminating formalin from our laboratory and simplifying specimen processing. A limitation with this change is requiring an additional frozen stool aliquot for parasitic antigen testing. Alcorfix compatibility with antigen detection ELISAs has not been evaluated and it is unknown whether the polyvinyl alcohol (PVA) is inhibitory. We assessed and validated Alcorfix for detection of *Giardia* and *Cryptosporidium* [Crypto] antigens and whether concentrated stool sediments were also compatible.

Methods: Unpreserved stools previously tested by antigen detection ELISA for Crypto or Giardia were collected. Aliquots of the stool were preserved in Alcorfix at a 1:3 ratio and spiked with various concentrations of Crypto oocysts (n=40) or *Giardia* cysts (n=40). Spiked specimens of each organism were also concentrated using a Parasep concentrator tube. The pellet and the supernatant were tested for the presence of Crypto or *Giardia* antigen by ELISA. The pellet was tested like a fresh stool specimen (1:4 in diluent). The supernatant was directly tested without dilution. Antigen stability for the Crypto and *Giardia* in Alcorfix was also assessed.

Results: The analytical sensitivity was 100% (40/40) for the detection of *Giardia* and 92.5% (37/40) for Crypto. The majority of *Giardia* antigen was reactive in the pellet rather than the supernatant. Crypto antigen was also concentrated in the pellet though there was still significant reactivity in the supernatant. The stability of the antigens in Alcorfix was limited to 7 days with Crypto (vs 14 days frozen unpreserved) and 14 days for *Giardia* (same as unpreserved).

Conclusions: Alcorfix, despite containing PVA, is compatible with *Giardia* and Crypto antigen testing by ELISA. A pellet from concentrated stool is suboptimal for detecting Crypto, as low parasite burden specimens may not be detected. Furthermore, the supernatant from the concentrated stool specimen is not acceptable for antigen testing. Using a Parasep tube with Alcorfix is a compatible combination for *Giardia* and Crypto antigen detection only when the entire sample is filtered, pelleted, and then resuspended before testing. The remaining suspension can then be pelleted again for further microscopic examination as needed.

Methods/Results

Table 1: ***Stability of spiked stool specimens fixed in Alcorfix by Giardia and Cryptosporidium antigen ELISAs***

Stability of *Giardia* and Crypto antigens were determined by spiking stool and preserving in Alcorfix. Aliquots were then stored at ambient, 4°C, and -20°C and tested at 4, 7 and 14 days using the TecLab *Giardia* II or *Cryptosporidium* II antigen detection ELISAs (Blacksburg, VA) per manufacturer's instructions. The optical density (OD) and qualitative interpretation were assessed. OD values >= 0.150 are positive for both ELISAs.

- Stability of the *Giardia* antigen is 14 days at ambient, 4°C, and -20°C.
- Stability of the Crypto antigen is 7 days at ambient, 4°C, and -20°C.

	Giardia		*Crypto*	
4°C	**OD**	**Result**	**OD**	**Result**
T=0	3.167	**Positive**	0.369	**Positive**
T= 4 days	1.199	**Positive**	0.354	**Positive**
T=7 days	3.251	**Positive**	0.376	**Positive**
T=14 days	3.078	**Positive**	0.087	Negative
Ambient				
T=0	3.167	**Positive**	0.369	**Positive**
T=4 days	2.929	**Positive**	0.332	**Positive**
T=7 days	3.623	**Positive**	0.473	**Positive**
T=14 days	3.814	**Positive**	0.287	**Positive**
-20°C				
T=0	3.167	**Positive**	0.369	**Positive**
T=14 days	3.096	**Positive**	0.116	Negative

Methods/Results

TABLE 2: ***Accuracy of spiked stool specimens fixed in Alcorfix by Giardia and Cryptosporidium antigen ELISAs***

Unpreserved stool, previously run on the Crypto and *Giardia* antigen ELISAs were collected as spiking matrices. Aliquots of stool were fixed with Alcorfix at a ratio of 1:3 and spiked with Crypto oocysts or *Giardia* cysts (Waterbourne Inc, New Orleans, LA) at low, medium, and high levels (defined below).

Giardia

Study ID	Spiking level	OD	Study Result
RD-04	N/A	0.066	Negative
RD-12	N/A	0.068	Negative
RD-48	N/A	0.069	Negative
RD-28	N/A	0.070	Negative
RD-08	N/A	0.073	Negative
RD-20	N/A	0.074	Negative
RD-36	N/A	0.074	Negative
RD-24	N/A	0.076	Negative
RD-16	N/A	0.077	Negative
RD-32	N/A	0.077	Negative
RD-44	N/A	0.095	Negative
RD-40	N/A	0.106	Negative
RD-63	Low	0.210	**Positive**
RD-55	Low	0.231	**Positive**
RD-38	Low	0.255	**Positive**
RD-61	Low	0.269	**Positive**
RD-14	Low	0.275	**Positive**
RD-51	Low	0.300	**Positive**
RD-30	Low	0.323	**Positive**
RD-53	Low	0.360	**Positive**
RD-65	Low	0.368	**Positive**
RD-02	Low	0.388	**Positive**
RD-10	Medium	0.402	**Positive**
RD-46	Medium	0.418	**Positive**
RD-49	Medium	0.432	**Positive**
RD-42	Medium	0.452	**Positive**
RD-22	Medium	0.507	**Positive**
RD-58	Medium	0.508	**Positive**
RD-18	Medium	0.514	**Positive**
RD-05	Medium	0.559	**Positive**
RD-59	Medium	0.603	**Positive**
RD-67	Medium	0.606	**Positive**
RD-34	Medium	0.647	**Positive**
RD-26	Medium	0.709	**Positive**
RD-73	High	2.427	**Positive**
RD-79	High	2.930	**Positive**
RD-80	High	3.322	**Positive**
RD-75	High	3.446	**Positive**
RD-83	High	3.623	**Positive**
RD-69	High	3.654	**Positive**
RD-71	High	3.727	**Positive**
RD-81	High	4.070	**Positive**

Crypto

Study ID	Spiking level	OD	Study Result
RD47	N/A	0.073	Negative
RD49	N/A	0.102	Negative
RD53	N/A	0.072	Negative
RD29	N/A	0.101	Negative
RD45	N/A	0.072	Negative
RD28	N/A	0.098	Negative
RD23	N/A	0.098	Negative
RD12	N/A	0.083	Negative
RD26	N/A	0.094	Negative
RD25	N/A	0.075	Negative
RD65	Medium	0.511	**Positive**
RD56	Medium	0.077	Negative
RD32	Medium	0.663	**Positive**
RD33	Medium	0.616	**Positive**
RD67	Medium	0.440	**Positive**
RD29-P	Medium	0.405	**Positive**
RD47-P	Medium	0.496	**Positive**
RD53-P	Medium	0.702	**Positive**
RD15	Medium	0.253	**Positive**
RD48	Medium	0.701	**Positive**
RD51	Medium	0.230	**Positive**
RD50	Medium	0.544	**Positive**
RD38	Medium	0.278	**Positive**
RD40	Medium	0.201	**Positive**
RD68	Medium	0.761	**Positive**
RD35	Medium	0.316	**Positive**
RD70	Medium	0.588	**Positive**
RD52	Medium	0.650	**Positive**
RD30	Low	0.242	**Positive**
RD9	Low	0.359	**Positive**
RD61	Low	0.077	Negative
RD49-P	Low	0.639	**Positive**
RD11	Low	0.081	Negative
RD16	Low	0.257	**Positive**
RD46	High	0.986	**Positive**
RD36	High	0.270	**Positive**
RD69	High	0.562	**Positive**
RD70	High	0.955	**Positive**
RD45-P	High	0.204	**Positive**
RD10	High	0.660	**Positive**

Low spike = ~6.25x10^5 cysts
Medium spike = ~1.25 x10^6 cysts
High spike = 1.87x10^6 cysts

- All samples detected as expected

Low spike = ~5.0x10^5 oocysts
Medium spike = ~1.0x10^6 oocysts
High spike = ~1.5x106 oocysts

- Three discrepant specimens
- All detected upon re-spiking

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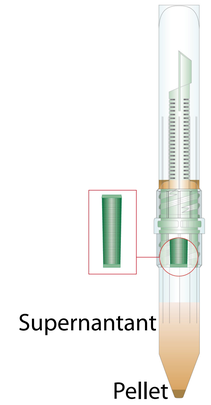
Methods/Results

TABLE 3: ***Pellet from concentrated stool in Alcorfix is compatible with Giardia and Cryptosporidium antigen detection***

Specimens fixed in Alcorfix using Parasep Concentration Tube

- Stools were spiked with *Giardia* cysts or Crypto oocyts and scooped into a MIDI Parasep Concentrator tube per manufacturer's recommendations.
- Samples were concentrated per manufacture's recommendations
- A sample of the supernatant (normally discarded after concentration) and the pellet were both tested for the presence of antigen.
- The supernatant was treated as a fixed sample per ELISA protocol according to manufacturer's recommendation.
- The pellet was tested as a raw specimen and diluted 1:4 in sample diluent.



Study ID	Giardia	OD	Study Result
R&D1	Supernatant	0.078	Negative
R&D1	Pellet	1.941	**Positive**
R&D2	Supernatant	0.729	**Positive**
R&D2	Pellet	1.812	**Positive**
R&D3	Supernatant	0.269	**Positive**
R&D3	Pellet	4.167	**Positive**
R&D4	Supernatant	0.206	**Positive**
R&D4	Pellet	9.999	**Positive**
R&D5	Supernatant	0.441	**Positive**
R&D5	Pellet	3.728	**Positive**

Study ID	Crypto	OD	Study Result
R&D1	Supernatant	0.100	Negative
R&D1	Pellet	0.173	**Positive**
R&D2	Supernatant	0.247	**Positive**
R&D2	Pellet	0.174	**Positive**
R&D3	Supernatant	0.075	Negative
R&D3	Pellet	0.199	**Positive**
R&D4	Supernatant	0.120	Negative
R&D4	Pellet	0.381	**Positive**
R&D5	Supernatant	0.088	Negative
R&D5	Pellet	0.137	Negative

***Giardia*:** Antigen was detected in both supernatant and pellet. Higher concentrations of antigen were observed in the pellet. Overall, antigen testing from the concentrated stool specimen or supernatant are compatible.

Crypto: Antigen was detected in both supernatant and pellet. R&D5 may have been spiked below the LoD. R&D2 was a watery stool and did not produce a discernible pellet which would account for the increased amount of antigen seen in the supernatant. Overall, the antigen concentrates in the pellet and it is compatible (but not optimal) for detecting Crypto antigen.

Conclusions

- **The presence of PVA in Alcorfix does not significantly interfere with *Giardia* or Crypto antigen ELISAs from TechLab. Compatibility with other products must be investigated by individual laboratories.**
- **Concentrated stool specimens can be tested, but must be diluted and treated as an unpreserved specimen prior to testing.**
- **Frozen unpreserved stool is preferred for antigen detection, however if that is not available on submission, stool fixed in Alcorfix is also compatible*. Importantly, testing should be performed as soon as possible to ensure antigen stability, as Crypto antigen became undetectable at 7 days.**

Parasep tubes kindly supplied by Apacor
***Apacor does not make claims of compatibility for Alcorfix with antigen detection assays**