

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 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).

<i>Giardia</i>				Crypto			
Study ID	Spiking level	OD	Study Result	Study ID	Spiking level	OD	Study Result
RD-04	N/A	0.066	Negative	RD47	N/A	0.073	Negative
RD-12	N/A	0.068	Negative	RD49	N/A	0.102	Negative
RD-48	N/A	0.069	Negative	RD53	N/A	0.072	Negative
RD-28	N/A	0.070	Negative	RD29	N/A	0.101	Negative
RD-08	N/A	0.073	Negative	RD45	N/A	0.072	Negative
RD-20	N/A	0.074	Negative	RD28	N/A	0.098	Negative
RD-36	N/A	0.074	Negative	RD23	N/A	0.098	Negative
RD-24	N/A	0.076	Negative	RD12	N/A	0.083	Negative
RD-16	N/A	0.077	Negative	RD26	N/A	0.094	Negative
RD-32	N/A	0.077	Negative	RD25	N/A	0.075	Negative
RD-44	N/A	0.095	Negative	RD65	Medium	0.511	Positive
RD-40	N/A	0.106	Negative	RD56	Medium	0.077	Negative
RD-63	Low	0.210	Positive	RD32	Medium	0.663	Positive
RD-55	Low	0.231	Positive	RD33	Medium	0.616	Positive
RD-38	Low	0.255	Positive	RD67	Medium	0.440	Positive
RD-61	Low	0.269	Positive	RD29-P	Medium	0.405	Positive
RD-14	Low	0.275	Positive	RD47-P	Medium	0.496	Positive
RD-51	Low	0.300	Positive	RD53-P	Medium	0.702	Positive
RD-30	Low	0.323	Positive	RD15	Medium	0.253	Positive
RD-53	Low	0.360	Positive	RD48	Medium	0.701	Positive
RD-65	Low	0.368	Positive	RD51	Medium	0.230	Positive
RD-02	Low	0.388	Positive	RD50	Medium	0.544	Positive
RD-10	Medium	0.402	Positive	RD38	Medium	0.278	Positive
RD-46	Medium	0.418	Positive	RD40	Medium	0.201	Positive
RD-49	Medium	0.432	Positive	RD68	Medium	0.761	Positive
RD-42	Medium	0.452	Positive	RD35	Medium	0.316	Positive
RD-22	Medium	0.507	Positive	RD70	Medium	0.588	Positive
RD-58	Medium	0.508	Positive	RD52	Medium	0.650	Positive
RD-18	Medium	0.514	Positive	RD30	Low	0.242	Positive
RD-05	Medium	0.559	Positive	RD9	Low	0.359	Positive
RD-59	Medium	0.603	Positive	RD61	Low	0.077	Negative
RD-67	Medium	0.606	Positive	RD49-P	Low	0.639	Positive
RD-34	Medium	0.647	Positive	RD11	Low	0.081	Negative
RD-26	Medium	0.709	Positive	RD16	Low	0.257	Positive
RD-73	High	2.427	Positive	RD46	High	0.986	Positive
RD-79	High	2.930	Positive	RD36	High	0.270	Positive
RD-80	High	3.322	Positive	RD69	High	0.562	Positive
RD-75	High	3.446	Positive	RD70	High	0.955	Positive
RD-83	High	3.623	Positive	RD45-P	High	0.204	Positive
RD-69	High	3.654	Positive	RD10	High	0.660	Positive
RD-71	High	3.727	Positive				
RD-81	High	4.070	Positive				

Low spike = ~6.25x10⁵ cysts
 Medium spike = ~1.25 x10⁶ cysts
 High spike = 1.87x10⁶ cysts

- All samples detected as expected

Low spike = ~5.0x10⁵ oocysts
 Medium spike = ~1.0x10⁶ oocysts
 High spike = ~1.5x10⁶ oocysts

- Three discrepant specimens
- All detected upon re-spiking

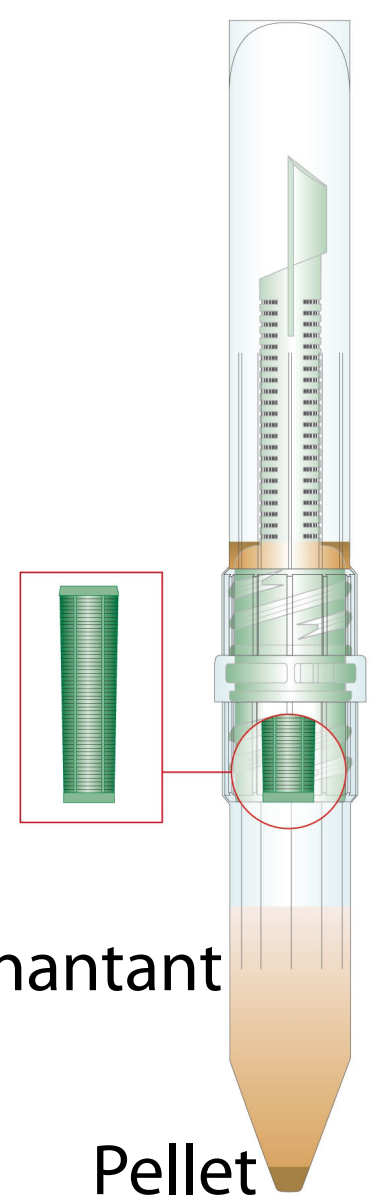
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Methods/Results

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 oocysts 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	<i>Giardia</i>	OD	Study Result	Study ID	Crypto	OD	Study Result
R&D1	Supernatant	0.078	Negative	R&D1	Supernatant	0.100	Negative
R&D1	Pellet	1.941	Positive	R&D1	Pellet	0.173	Positive
R&D2	Supernatant	0.729	Positive	R&D2	Supernatant	0.247	Positive
R&D2	Pellet	1.812	Positive	R&D2	Pellet	0.174	Positive
R&D3	Supernatant	0.269	Positive	R&D3	Supernatant	0.075	Negative
R&D3	Pellet	4.167	Positive	R&D3	Pellet	0.199	Positive
R&D4	Supernatant	0.206	Positive	R&D4	Supernatant	0.120	Negative
R&D4	Pellet	9.999	Positive	R&D4	Pellet	0.381	Positive
R&D5	Supernatant	0.441	Positive	R&D5	Supernatant	0.088	Negative
R&D5	Pellet	3.728	Positive	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.

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.

4°C	<i>Giardia</i>		Crypto	
	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

- 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.

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