510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K121565

B. Purpose for Submission:

To obtain a substantial equivalence determination for a new device

C. Measurand:

Cryptosporidum parvum antigen

D. Type of Test:

Lateral flow immunoassay

E. Applicant:

Trinity Biotech

F. Proprietary and Established Names:

Uni-Gold™ Cryptosporidium

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3220 *Entamoeba histolytica* serological reagents

2. Classification:

   Class II

3. Product code:

   MHJ – *Cryptosporidium spp.*

4. Panel:

   83 Microbiology

H. Intended Use:

1. Intended use(s):

   Trinity Biotech Uni-GoldTM Cryptosporidium is a single use rapid immunoassay for the qualitative detection of *Cryptosporidium parvum* (*C. parvum*) antigens in human stool specimens. This test is intended for use with patients with gastrointestinal symptoms as an aid in the diagnosis of suspected *Cryptosporidium* gastrointestinal infections. As with other *Cryptosporidium* tests, results should be considered in conjunction with the clinical evaluation and medical history. For In Vitro Diagnostic use.

2. Indication(s) for use:
   Trinity Biotech Uni-GoldTM Cryptosporidium is a single use rapid immunoassay for the qualitative detection of *Cryptosporidium parvum* (*C. parvum*) antigens in human stool specimens. This test is intended for use with patients with gastrointestinal symptoms as an aid in the diagnosis of suspected *Cryptosporidium* gastrointestinal infections. As with other *Cryptosporidium* tests, results should be considered in conjunction with the clinical evaluation and medical history. For In Vitro Diagnostic use.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Not applicable

I. Device Description:

The Trinity Biotech Uni-Gold^{TM} Cryptosporidium test strip (5mm x 60mm) combines a nitrocellulose membrane with designated fiber pads (conjugate, sample and absorbent). The test strip is placed into a plastic housing and is sealed constituting the Test Device. The test strip consists of A) Mouse anti-*Cryptosporidium parvum* antibody coated onto the Test Line region, B) Goat anti-mouse IgG antibody coated onto the Control Line region, C) Mouse anti- *Cryptosporidium parvum* antibodies and mouse IgG antibodies conjugated to red latex particles and dried onto the inert glass fiber conjugate pad which is positioned on the test strip below the nitrocellulose zone. The housing contains a window where the diluted stool sample is added (Sample Well) and a window above where the results are read in 15 minutes.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Remel Xpect® Cryptosporidium Lateral Flow Assay

2. Predicate 510(k) number(s):

   K031965

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	Trinity Biotech Uni-Gold™ Cryptosporidium	Remel Xpect® Cryptosporidium Lateral Flow Assay
Intended use	Detection of *Cryptosporidium parvum* antigens in human stool specimens	Detection of *Cryptosporidium* antigens in preserved and unpreserved fecal specimens
Technology	Qualitative immunochromatographic assay	Qualitative immunochromatographic assay
Specimen types	Human stool samples, unpreserved: fresh/frozen, preserved: 10% formalin, SAF, Cary-Blair, or C&S Transport Medium	Human stool samples, unpreserved and preserved: 10% formalin, SAF, or Cary-Blair Transport Medium
Test time	15 minutes	15 minutes
Reading method	Visual	Visual

Differences		
Item	Device	Predicate
	Trinity Biotech Uni-Gold™ Cryptosporidium	Remel Xpect™ Cryptosporidium Lateral Flow Assay
Capture antibodies on membrane	Mouse anti-*Cryptosporidium parvum* "Test Line", Goat anti-mouse IgG "Control Line"	Rabbit anti-*Cryptosporidium* "Test Line", Goat anti-mouse IgG "Control Line"
Conjugate antibodies	Mouse anti-*Cryptosporidium parvum*, Mouse IgG	Mouse monoclonal anti-*Cryptosporidium*

Differences		
Item	Device	Predicate
Conjugate material	Red latex conjugated antibodies dried on a conjugate pad	Red and blue polystyrene microparticles coated with antibodies and diluted in buffer
Sample volume	100 µL	2 drops ~ 40-60 µL
Membrane material	Nitrocellulose	Mylar-backed nitrocellulose

K. Standard/Guidance Document Referenced (if applicable):

CLSI M28-A2 Procedures for the Recovery and Identification of Parasites from the Intestinal Tract

CLSI EP12-A2 User Protocol for Evaluation of Qualitative Test Performance (In Vitro Diagnostics)

CLSI EP17-A Protocols for Determination of Limits of Detection and Limits of Quantitation

L. Test Principle:

A buffered solution is added to a dilution tube followed by the addition of two drops of the stool specimen via a disposable pipette. This mixture is then dispensed in total into the sample well of the lateral flow cartridge device with a dropper pipette. The mixture migrates through a pad containing red latex microspheres that have been coated with an antibody specific for the Cryptosporidium antigen.

When Cryptosporidium antigens are present in the sample they combine with the antibody/red latex conjugate. As this complex migrates it binds to the antibodies in the test region of the device forming a visible pink/red band.

Excess red latex conjugate forms a second pink/red band in the control region of the device. The control line should always appear as a visible pink/red band in the control region of the device to indicate that the test device is functioning correctly.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility was assessed at three sites using a blind coded sample panel with varying amounts of Cryptosporidium oocysts spiked into negative human stool matrix. The panel consisted of four low positive samples, four

moderately positive samples, and four negative samples. The panels were tested in two runs at each site by two operators each day for five days. Each site ran positive and negative controls for each day of testing. Reproducibility was 100%. Additionally, lot-to-lot repeatability and between-user repeatability were assessed internally with panels of positive and negative clinical samples. Repeatability was 100%. The Reproducibility and Repeatability study results are acceptable.

b. *Linearity/assay reportable range:*

Not applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Controls:
Good Laboratory Practice necessitates the use of control specimens to ensure proper device performance at least once daily. Uni-Gold™ Cryptosporidium Controls are available separately for use only with Uni-Gold™ Cryptosporidium. These controls are used to verify correct device performance, operator procedure and result interpretation. The positive control will produce a reactive test result and the negative control will produce a non-reactive test result as described in the package insert.

It is recommended that positive and negative controls are run
- By all new operators performing testing on patient specimens
- With each new kit lot and whenever a new shipment of test kits is received
- At periodic intervals as specified in the laboratory Quality Assurance program

Uni-Gold™ Cryptosporidium Controls must give the expected reactive or non-reactive results; otherwise the test results are not valid and must be repeated.

Sample stability:
Cryptosporidium oocysts were spiked into human stool samples in the following fixative and transport medium types: 10% formalin, SAF, Cary Blair, C&S, or fresh (unpreserved). Negative samples were also prepared in each medium type. The samples were stored under different temperature conditions and tested at various times during storage. All samples produced the expected results. The data supports the following sample storage parameters:

Fresh stool	48 hours at 2-8°C
Frozen Stool	2 months at -20°C
Cary Blair or C&S transport media	7 days at 2-8°C
Cary Blair or C&S transport media	2 months at -20°C

10% formalin or SAF fixed stool 2 months at 2-30°C

A multiple freeze/thaw study was also conducted with negative and positive spiked fresh, C&S, and Cary Blair stool samples. All samples produced the expected results in the study. Multiple freeze/thaw cycles should be avoided.

High-dose hook effect:
High levels of Cryptosporidium oocysts were spiked into negative human stool matrix, serially diluted, and then tested. High oocyst concentrations produced the expected positive results in the Uni-Gold Cryptosporidium and did not produce a high-dose hook effect.

d. Detection limit:

The limit of detection was determined by spiking purified Cryptosporidium oocysts quantified by DFA microscopy into negative human stool samples. The samples were serially diluted and three replicates from each dilution were tested with the Uni-Gold Cryptosporidium to determine the concentration that produced a positive result 95% of the time. A limit of detection concentration of 9920 oocysts/mL was confirmed by testing an additional 20 replicates with the Uni-Gold Cryptosporidium.

e. Analytical specificity:

Cross reactivity:
No cross reactivity was observed with samples containing the following microorganisms:

Adenovirus serotype 3	Coronavirus OC43	*Iodamoeba butschlii*
Adenovirus serotype 5	Coxsackievirus	*Isospora sp.*
Adenovirus serotype 7	*Cyclspora cayetanensis*	*Klebsiella pneumoniae*
Adenovirus serotype 41	Cytomegalovirus (CMV)	*Microsporidia*
Adenovirus serotype 40	*Dientamoeba fragilis*	*Salmonella typhimurium*
Aeromonas hydrophila	*Diphyllobothrium latum*	*Shigella dysenteriae*
Ascaris lumbricoides	Echovirus 20	*Shigella flexneri*
Bacteroides fragilis	*Endolimax nana*	*Shigella sonnei*
Bacillus cereus	*Entamoeba coli*	*Staphylococcus aureus*
Bacillus subtilis	*Entamoeba hartmanni*	*S. aureus (Cowan's)*
Blastocystis hominis	*Entamoeba histolytica*	*Staphylococcus epidermidis*
Campylobacter coli	*Enterobius vermincularis*	*Strongyloides stercoralis*
Campylobacter fetus	*Enterococcus faecalis*	*Taenia sp.*
Campylobacter jejuni	*Escherichia coli*	*Trichurius trichiura*
Candida albicans	*Escherichia coli 0157H7*	*Vibrio parahaemolyticus*

Chilomastix mesnili	*Giardia lamblia*	*Yersinia enterocolitica*
Clostridium difficile	Hookworm	
C. biffermentans	*Hymenolepis nana*	

Cross-reactivity to *E. dispar* was not evaluated.

Cross-reactivity study results are acceptable.

Interference study:
The following substances did not interfere with positive or negative results when tested in spiked human stool samples at the following concentrations:

Human blood (20% v/v), Mucin (3.5% w/v), Stool fat (Triglycerides 0.14mg/ml or Stearic Acid 20% v/v), Pepto-Bismol (Bismuth) (20% v/v), Imodium A-D (Loperamide HCl) (20% v/v), Kaopectate (Attapugite) (20% v/v), Vancomycin (0.6mg/ml), K-Y jelly (0.289mg/ml), Vasoline (0.22mg/ml), Condom lubricant (1.716mg/ml), Maalox (magnesium hydroxide, calcium carbonate) (20% v/v), Tagamet (Cimetidine) ($2.0x10^{-2}$ mg/ml), Pepsid (Famotidine) ($6.0x10^{-4}$ mg/ml), Zantac (Ranitidine) ($6.0x10^{-3}$ mg/ml), Prilosec (Omeprazole) ($6.0x10^{-3}$ mg/ml), Nitrazoxanide ($6.96x10^{-3}$ mg/ml), Atovaquone (0.031mg/ml), Azithromycin ($1.2x10^{-2}$ mg/ml), Metronidazole (0.12mg/ml), Paromomycin (0.42mg/ml), Trimethoprim-sulfamethoxazole (TRM 0.04mg/ml & Sulf 0.4mg/ml).

Interference study results are acceptable.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

The Uni-Gold Cryptosporidium was compared to a commercially available lateral flow test on 299 retrospective stool samples in the following stool matrix types: unpreserved frozen (37), Cary Blair (4), SAF (159), and formalin (99). The percent agreement of Uni-Gold Cryptosporidium versus the comparator device was as follows:

Site 1		Comparator Device		
Uni-Gold™ Cryptosporidium		+	-	% Agreement
	+	24	1	100% Pos Agr
	-	0	52	98.1% Neg Agr

Site 2		Comparator Device		
Uni-Gold™ Cryptosporidium		+	-	% Agreement
	+	56	0	100% Pos Agr
	-	0	55	100% Neg Agr

Site 3		Comparator Device		
Uni-Gold™ Cryptosporidium		+	-	% Agreement
	+	27	51*	96.4% Pos Agr
	-	1**	32	38.6% Neg Agr

*At Site 3, out of 51 samples that tested positive on Uni-Gold Cryptosporidium and negative on the comparator device, 30 samples were positive by Modified Kinyoun Stain light microscopy and three samples were positive for Cryptosporidium by DFA microscopy in agreement with the Uni-Gold Cryptoporidium result.
**The one sample that tested negative on Uni-Gold Cryptosporidium and positive on the comparator device was negative by Modified Kinyoun Stain microscopy in agreement with the Uni-Gold Cryptoporidium result.

b. Matrix comparison:

An in-house study was conducted to evaluate test performance with negative human stool samples and whole *Cryptosporidium* oocysts spiked into stool samples in the following sample matrices: 10% formalin, SAF, Cary Blair, C&S, and unpreserved (fresh). All samples produced the expected result with the test device.

3. Clinical studies:

The clinical performance of the Uni-Gold Cryptosporidium was evaluated on 564 retrospective stool samples at three external laboratories and on 378 prospective stool samples at a fourth external laboratory.

a. Clinical Sensitivity:

Retrospective study:
The sensitivity and specificity of the test was compared against DFA microscopy with retrospective samples at sites 1 and 2 as shown in the

following tables.

Site 1

		DFA Microscopy	
		+	-
Uni-Gold™ Cryptosporidium	+	28	0
	-	0	103

Site 2

		DFA Microscopy	
		+	-
Uni-Gold™ Cryptosporidium	+	49	0
	-	0	54

Total

		DFA Microscopy	
		+	-
Uni-Gold™ Cryptosporidium	+	77	0
	-	0	157

Sensitivity: 100% (77/77), 95% CI 94 - 100%
Specificity: 100% (157/157), 95% CI 97 - 100%

The positive samples were tested in the following stool matrix types: formalin (47), SAF (11), unpreserved frozen (14), Cary Blair (2), and C&S (3). The negative samples were tested in the following stool matrix types: formalin (71), SAF (49), unpreserved frozen (23), Cary Blair (2), C&S (12).

Additional retrospective studies:
Performance of the test was compared to non-fluorescent microscopy (staining) at two external laboratories. At site 2, 47 retrospective samples were evaluated and demonstrated a Positive Percent Agreement (PPA) of 100% (26/26) and a Negative Percent Agreement (NPA) of 100% (21/21) versus Modified Acid-Fast Stain. At site 3, 281 retrospective SAF samples were evaluated and demonstrated a PPA of 92% (55/60) and a NPA of 90% (198/221) versus Modified Kinyoun Stain. Of the 23 negative samples (by Modified Kinyoun Stain) that tested positive on the Uni-Gold™ Cryptosporidium test, three of these samples subsequently tested positive for Cryptosporidium by DFA microscopy in agreement with the Uni-Gold™ Cryptosporidium result.

Prospective study
Test performance was compared against DFA microscopy at site 4 with 378 prospective samples in the following stool sample types: fresh (153), frozen (45), 10% formalin (45), SAF (45), C&S (45), and Cary Blair (45). Due to infection prevalence, no positive samples were encountered during this study.

			DFA Microscopy	
			+	-
Site 4	Uni-Gold™ Cryptosporidium	+	0	0
		-	0	378

Specificity: 100% (378/378) 95% CI 99 – 100%

b. Clinical specificity:

See section M3a.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

The performance of the Uni-Gold™ Cryptosporidium Test Kit was evaluated at four external laboratories. The 940 (prospective and retrospective) samples were collected from Hospitals throughout the US and Canada and consisted of both male and female patients of all ages from pediatric to adult. The retrospective study included 163 positive samples and 399 negative samples confirmed by microscopy. The prospective study included 378 samples which were subsequently confirmed negative by microscopy. There were no differences observed in clinical performance between males or females, or between pediatric or adult populations.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.