**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION**
**DECISION SUMMARY**
**ASSAY ONLY TEMPLATE**

**A. 510(k) Number:**

K171078

**B. Purpose for Submission:**

To obtain a substantial equivalence determination for the simultaneous detection of *Giardia spp*., *Cryptosporidium spp*. and/or *Entamoeba histolytica* antigens in human stool

**C. Measurand:**

*Giardia spp*. antigen

*Cryptosporidium spp*. antigen

*Entamoeba histolytica* antigen

**D. Type of Test:**

Enzyme Linked Immunosorbant Assay

**E. Applicant:**

TechLab Inc.

**F. Proprietary and Established Names:**

Tri-Combo Parasite Screen

**G. Regulatory Information:**

1. Regulation section:

   21 CFR Part 866.3220 *Entamoeba histolytica* Serological Reagents

2. Classification:

   II

3. Product code:

   MHJ, MHI and KHW

4. Panel:

   83 Microbiology

## H. Intended Use:

1. Intended use(s):

   The TECHLAB TRl-COMBO PARASITE SCREEN test is an enzyme immunoassay for the simultaneous qualitative detection of *Giardia* spp., *Cryptosporidium* spp. and/or *E. histolytica* antigen in human fecal specimens. The test is indicated as an aid in the diagnosis of gastrointestinal infection when giardiasis, cryptosporidiosis and amebiasis is suspected. The test does not differentiate between the three parasites and follow-up testing is required for all positive results to confirm the specific diagnosis.

2. Indication(s) for use:

   The TECHLAB TRl-COMBO PARASITE SCREEN test is an enzyme immunoassay for the simultaneous qualitative detection of *Giardia* spp., *Cryptosporidium* spp. and/or *E. histolytica* antigen in human fecal specimens. The test is indicated as an aid in the diagnosis of gastrointestinal infection when giardiasis, cryptosporidiosis and amebiasis is suspected. The test does not differentiate between the three parasites and follow-up testing is required for all positive results to confirm the specific diagnosis.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   A spectrophotometric plate reader capable of reading the following wave lengths 450nm or 450/620nm.

## I. Device Description:

The *TRI-COMBO PARASITE SCREEN* test is an enzyme immunoassay for the simultaneous qualitative detection of *Giardia* spp., *Cryptosporidium* spp. and/or *E. histolytica* antigen in human fecal specimens. The test uses monoclonal and polyclonal antibodies to cell-surface antigens of *Giardia*, *Cryptosporidium* and *E. histolytica*. The microassay plate in the kit contains immobilized monoclonal antibodies against the antigens, and the *Conjugate* consists of polyclonal antibodies against the antigens. In the assay, an aliquot of a diluted fecal specimen is transferred to a microassay well. The immobilized monoclonal antibodies bind

the *Giardia*, *Cryptosporidium* and/or *E. histolytica* antigens if they are present. Upon addition, *Conjugate* then binds to the antigen/ antibody complex. Any unbound materials are removed during the washing steps. Following the addition of *Substrate*, a color is detected due to the enzyme-antibody-antigen complexes that formed in the presence of antigens and conjugate. The color if positive is a yellow color that may be read visually or at the following wave lengths 450nm or 450/620 Nm.

## J. Substantial Equivalence Information:

1. Predicate device name(s):

   GIARDIA/CRYPTOSPORIDIUM CHEK

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

   K051929

3. Comparison with predicate:

| **Similarities** | | |
|---|---|---|
| Item | Device (K171028) | Predicate (K051929) |
| Intended Use | The TECHLAB TRl-COMBO PARASITE SCREEN test is an enzyme immunoassay for the simultaneous qualitative detection of *Giardia* spp., *Cryptosporidium* spp. and/or *E. histolytica* antigen in human fecal specimens. The test is indicated as an aid in the diagnosis of gastrointestinal infection when giardiasis, cryptosporidiosis and amebiasis is suspected. The test does not differentiate between the three parasites and follow-up testing is required for all positive results to confirm the specific diagnosis. | The *GIARDIA/ CRYPTOSPORIDIUM CHEK* test is an enzyme immunoassay for the qualitative detection of *Giardia* cyst and *Cryptosporidium* oocyst antigen in human fecal specimens. It is indicated for use as an aid in the diagnosis of patients with diarrhea suspected of *Giardia* and/or *Cryptosporidium* gastrointestinal infections |
| Technology | Enzyme Linked Immunoassay (ELISA) | Same |
| Antibody Format | Monoclonal capture Ab Polyclonal secondary Ab | Same |
| Type of Test | Qualitative | Same |
| Format/Tests | Microassay Well Plate (96 tests) | Same |
| Controls | Positive and negative control are included in the kit | Same |
| Interpretation | Spectrophotometrically and visually | Same |

| Differences | | |
|---|---|---|
| Item | Device | Predicate |
| Analyte Detected | *Giardia* spp., *Cryptosporidium* spp., and *E. histolytica* specific antigens | *Giardia* spp., and *Cryptosporidium* spp., specific antigens |
| Acceptable Specimen Type | Fecal specimens in Cary-Blair and C&S Transport Media | Specimens in preservation media of 10% buffered formalin or Sodium Acetate Formalin (SAF) |

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

CLSI EP07-A2 Interference Testing In Clinical Chemistry; Approved Guideline - Second Edition

CLSI EP15-A3 User Verification of Precision And Estimation Of Bias; Approved Guideline - Third Edition

CLSI EP17-A2 Evaluation Of Detection Capability For Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition

**L. Test Principle:**

Enzyme Linked Immunosorbant Assay

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

*a. Precision/Reproducibility:*
The precision of the *TRI-COMBO PARASITE SCREEN* test was determined using a masked fecal specimen panel comprised of 24 specimens spiked with different levels of whole organisms. The samples were prepared using a negative fecal pool and spiked with the following organisms individually *Giardia* cysts, *Cryptosporidium* oocysts or *E. histolytica* whole organisms. The panel consisted of two negative specimens, two high negative specimens (just below C5), two low positive specimens (just above LoD), and two moderate positive (2-3x higher than the C95) specimens for each of the three organisms (eight specimens for each organism). Each fecal specimen was spiked using whole organism to achieve the desired level. The specimens were tested twice a day over a 12 day period by multiple technicians using two different kit lots. Positive and negative controls were run with each sample panel of masked specimens. The positive specimens tested positive 100% of the time and the negative specimens tested negative 100% of the time (Only 2 of 1152 test results for the masked specimens were misclassified).

The reproducibility of the *TRI-COMBO PARASITE SCREEN* test was determined using masked fecal specimen panel comprised of 20 specimens spiked with different levels of whole organisms that were prepared the same way as described for the precision study except that the same two negatives were used for all three sample

types. Testing was performed at two independent external laboratories and one internal site at TECHLAB, Inc. The specimens were tested twice a day over a five day period by multiple technicians at each site using two different kit lots. Positive and negative controls were run with each sample panel of masked specimens. The result from each laboratory were submitted to TECHLAB Inc. and compared. The results were consistent among all three locations.

b. *Linearity/assay reportable range:*

Not applicable

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

Storage stability study
The effect of specimen storage on antigen stability was evaluated for both fresh samples and samples in transport media. The following transport media were used for the study: Thermo Scientific *Protocol* Cary Blair media and Meridian Bioscience Inc., *Para-Pak* C&S. For the analysis, a total of 39 fecal specimens were tested with the *TRI-COMBO PARASITE SCREEN* test. The samples were prepared using a negative fecal pool and spiked with the following organisms individually *Giardia* cysts, *Cryptosporidium* oocysts or *E. histolytica* whole organisms. The panel consisted of three negative specimens used for all three anayltes, three high negative (just below C5), three low positive (just above LoD), three moderate positive (2-3x higher than the C95) and three high positive (4-5x higher than the C95) specimens for each of the three organisms (12 for each and three negatives used for all three organisms). Each fecal specimen was spiked using whole organism to achieve the desired level.

Fresh samples were stored at refrigerated temperatures (between 2°C and 8°C) and were tested at day 0, 3, 4, 5, 6, 7 and 10. For refrigerated conditions, the positive and negative specimens gave the expected results 100% of the time.

Fecal specimens added to Cary Blair were transported as recommended in the Cary Blair package insert; Cary Blair samples were stored between 20°C and 25°C. *Para-Pak* C&S samples were stored at both refrigerated (2°C to 8°C) and room temperature (15°C to 30°C) conditions as indicated in the package insert. Samples stored in transport media were tested at 24 hour intervals form 0 to 96 hours. Positive and negative controls were tested daily.

Transport media stability:
Storage in transport media affected the stability of the samples. Based on the results from stability studies, the recommended storage times/temperatures are as follows:

For Cary Blair transport media up to 96 hours between 20°C to 25°C,

For C&S transport media up to 96 hours between 2 to 8°C and 48 hours between 15°C to 30°C based on the study results.

Frozen sample stability:
Stability of frozen raw stool samples was established using 39 masked fecal specimens panel prepared the same way as was described for the storage stability study. Samples were stores at ≤ -10°C for 8 weeks. Specimens were tested at 0, 1, 4 and 8 weeks. Positive samples remained positive and negative samples remained negative throughout the study.

Freeze/Thaw
A study was conducted to determine stability after one freeze/thaw cycle for Cary Blair and C&S media compared to raw specimens for each analyte and to support and facilitate testing of samples in analytical and clinical studies. Eighty masked samples were tested [10 true negative, 30 high negative (just belowC5 (whole organisms)), 30 low positives (1-2 times C95 (whole organisms)), and 10 high positives (4-5 x LoD (whole organisms)] were tested for each of the three organisms in each transport media and raw stool. Based on the data submitted, the freeze-thaw cycle enhances the performance for *E. histolytica* in raw stool from 0% detected before freezing to 100% reactivity for the high negative samples tested after a single freeze thaw cycle. *Giardia* and *Cryptosporidium* raw samples demonstrated a slight decrease from 100% to 86.7% reactivity for the high negative samples.

*Giardia* and *Cryptosporidium* samples preserved in Cary Blair demonstrated a very slight improvement from 93.3%-100% reactivity for the high negative samples only. All other results at different concentrations did not change during the study. Testing for all samples with the *TRI-COMBO PARASITE SCREEN* was done before and after a single freeze thaw cycle.

The information suggests that there is no influence on sample reactivity after a single freeze-thaw cycle, except for a slight increase the reactivity of the high negative sample.

*d. Detection limit:*

The cutoff point (limit of detection, LoD) for the *TRI-COMBO PARASITE SCREEN* test was determined using whole organism spiked into unpreserved (raw stool) and preserved (Cary Blair and C&S media). The cutoff is the concentrations of *Giardia, Cryptosporidium* or *E. histolytica* that yields a positive result 95% of the time and a negative result 5% of the time. The cutoff point was determined as the concentration that provided the correct result 95% of the time based on testing dilutions of whole organism in a negative fecal sample matrix, in replicates of 20. LoD testing was read at both single and dual wave length reads. The LoD between single wave lengths reads and duel wave length reads was not significantly different; however single wavelength reads trended slightly higher for some but not all analytes and sample types. Single wavelength reads are as follows:

Test results determined the LoD for whole organism in raw stool to be 8450 cysts/mL for *Giardia* (equivalent to 169) cysts detected per test), 47962 oocysts/mL for

*Cryptosporidium* (equivalent to 959) oocysts detected per test), and 1676 whole organisms/mL for *E. histolytica* (equivalent to 34 whole organisms per test).

Test results determined the LoD for whole organism in stool and Cary Blair to be 34155 cysts/mL for *Giardia* (equivalent to 427 cysts detected per test), 99456 oocysts/mL for *Cryptosporidium* (equivalent to 1243 oocysts detected per test), and 4655 whole organisms/mL for *E. histolytica* (equivalent to 49 whole organisms per test).

Test results determined the LoD for whole organism in stool and C&S to be 37095 cysts/mL for *Giardia* (equivalent to 464 cysts detected per test), 12299 oocysts/mL for *Cryptosporidium* (equivalent to 1529 oocysts detected per test), and 3948 whole organisms/mL for *E. histolytica* (equivalent to 49 whole organisms per test).

There was no significant difference observed for samples stored in transport media compared to fresh fecal samples when accounting for the difference in dilution for a fresh sample (1:5 dilution) versus a sample stored in transport media (1:2 dilution) for single or dual wavelength results. Because the *TRI-COMBO PARASITE SCREEN* test detects soluble antigen in fecal specimens in addition to cysts, oocysts, and trophozoites, this LoD study represents an estimate of analytical sensitivity based on purified cysts, oocysts, and whole organisms.

e. *Analytical specificity:*

Cross reactivity

The TRI-COMBO PARASITE SCREEN test was evaluated for cross-reactivity with the bacteria and viruses listed below. None of the strains were shown to interfere with the performance of the TRI-COMBO PARASITE SCREEN test. Bacteria were spiked at concentration of $>10^8$ CFU/mL and viruses at a range from $10^{3.3}$ to $10^{8.75}$ $TCID_{50}$ units per 0.2 mL.

*Aeromonas hydrophila*
*Bacillus cereus*
*Bacillus subtilis*
*Bacteroides fragilis*
*Campylobacter coli*
*Campylobacter fetus*
*Campylobacter jejuni*
*Candida albicans*
*Clostridium bifermentans*
*Clostridium difficile*
*Enterococcus faecalis*
*Escherichia coli*
*Escherichia coli O157:H7*
*Escherichia coli EIEC*
*Escherichia coli EPEC*
*Escherichia coli ETEC*
*Klebsiella pneumoniae*
*Salmonella typhimurium*
*Shigella dysenteriae*
*Shigella flexneri*
*Shigella sonnei*
*Staphylococcus aureus*
*Staphylococcus aureus (Cowan's)*
*Staphylococcus epidermidis*
*Vibrio parahaemolyticus*
*Yersinia enterocolitica*

*Calicivirus*
*Cytomegalovirus*

| | |
|---|---|
| *Echovirus 11, 18, 33* | *Human Rotavirus* |
| *Human Adenovirus 1 2,3, 5, 40, 41* | *Human Coronavirus* |
| *Human Coxsackievirus B2, B3, B4, B5* | *Human Echovirus 9* |
| *Human Enterovirus 68, 69, 70, 71* | *Human parechovirus 1 [Echovirus 22]* |

Cross reactivity with Norovirus is unknown because it was not tested in analytical studies. However, Norovirus GI/GII was identified in 35 clinical specimens and and Enterotoxigenic E. Coli - ETEC LT/ST was identified in 107 clinical specimens,using an FDA cleared multiplex NAAT assay during clinical testing and no cross reactivity was found using the TRI-COMBO PARASITE SCREEN in those samples

The following parasites were detected by microscopy in fecal specimens tested during clinical studies. The number in parenthesis is the number of individual clinical samples that were microscopy positive for the parasites listed below. No cross-reactivity was seen with the fecal specimens that were positive for: .

*Ascaris lumbricoides* and with eggs (22)
*Entamoeba bangladeshi* (3)
*Blastocystis hominis* (11)
*Entamoeba coli* (16)
*Iodamoeba bütschlii* (10)
*Entamoeba moshkovskii* (3)
*Trichuris trichiura* eggs (12)

*f. Assay cut-off:*

Not applicable

*g. Prozone*

The purpose of this study was to ensure that a high concentration of analyte does not interfere with the performance of the *TRICOMBO PARASITE SCREEN* test. Five different dilutions of each analyte were prepared, starting with 2X the clinically observed high concentration. For *Giardia* cysts this was $6 \times 10^5$ cysts/gram stool, for *Cryptosporidium* oocysts this was $2 \times 10^5$ oocysts/gram stool, and for *E. histolytica* this was $4 \times 10^4$ whole organisms/mL. Serial dilutions were made at a 1:2 dilution into a negative fecal pool. Testing was performed in triplicate according to the Package Insert instructions. The results below demonstrated that there was no overall hook affect, that elevated levels of analyte did not affect the detection of the analyte.

2. Comparison studies:

*a. Method comparison with predicate device:*

Not applicable

*b. Matrix comparison:*

Not applicable

3. Clinical studies:

*a. Clinical Sensitivity:*

Prospective Study
The performance of the *TRI-COMBO PARASITE SCREEN* test was evaluated at 3 independent sites. The performance of the *TRI-COMBO PARASITE SCREEN* test was compared to light microscopy, and molecular testing by polymerase chain reaction (PCR) for sub-speciation of *E. histolytica*. Molecular testing consisted of a commercial FDA cleared device and PCR with sequencing if applicable for the identification of *Giardia* spp., *Cryptosporidium* spp., and *E. histolytica.*

Prospective testing consisted of 754 specimens from two geographically distinct areas in the US and one *E. histolytica* endemic area in Bangladesh. The three sites yield a total of 14 microscopy positive samples (13 *Giardia* and one *E. histolytica.* The remaining 740 samples were negative. Of the 740 negative samples, four were *E. histolytica* positive by molecular comparison only but were negative by *TRI-COMBO PARASITE SCREEN*, negative by microcopy and negative by FDA cleared antigen test for *E. histolytica.* Table 1 below summarizes the performance observed, which is primarily as study to evaluate specificity due to the low number of positive specimens (See retrospective study for evaluation of sensitivity). Prospective testing was also read visually and performance was not significantly different from spectrophotometric readings.

**Table 1. Summary of prospective clinical performance comparing the *TRI-COMBO PARASITE SCREEN* test to microscopy for *Giardia, Cryptosporidium* and *E. histolytica*.**

| ***TRI-COMBO PARASITE SCREEN* (N = 754)** | **Microscopy** | |
|---|---|---|
| | **Positive** | **Negative** |
| **Positive** | **13** | **14*** |
| **Negative** | **1** | **726** |
| | | **95% Confidence Limits** |
| **Sensitivity** | 92.9% | 68.5% - 98.7% |
| **Specificity** | 98.1% | 96.9% - 98.9% |

| | |
|---|---|
| Specimens Positive for *Giardia* by microscopy/<br>Specimens Detected by the *TRI-COMBO PARASITE SCREEN* | 12/13 |
| Specimens Positive for *E. histolytica* by microscopy/<br>Specimens Detected by the *TRI-COMBO PARASITE SCREEN* | 1/1 |

*The fourteen *TRI-COMBO PARASITE SCREEN* positives that were microscopy negative were confirmed to be positive for *Giardia* with an alternate FDA cleared antigen test or by PCR with sequencing.

Retrospective Study

Testing consisted of 96 archived specimens previously collected and frozen from the Bangladesh clinical site. The Frozen samples are included in the bank based on being characterized as microscopy and PCR positive. The specimens were collected form an *E. histolytica* endemic area and contained specimens also positives for *Giardia* and *Cryptosporidium.* Table 2 below summarizes the performance observed. Retrospective testing was also read visually and performance was not different from spectrophotometric readings.

**Table 2. Summary of retrospective clinical performance comparing the *TRI-COMBO PARASITE SCREEN* test to Microscopy and PCR**

| ***TRI-COMBO PARASITE SCREEN*** **(N = 96)** | **Microscopy and PCR** | |
|---|---|---|
| | **Positive** | **Negative** |
| **Positive** | **85** | **0** |
| **Negative** | **5** | **6** |
| | | **95% Confidence Limits** |
| **Sensitivity** | 94.4% | 87.7% - 97.6% |
| **Specificity** | 100% | 61.0% - 100% |

| | |
|---|---|
| Specimens Positive for *Giardia*/ Specimens Detected by the *TRI-COMBO PARASITE SCREEN* | 41/41 |
| Specimens Positive for *Cryptosporidium*/ Specimens Detected by the *TRI-COMBO PARASITE SCREEN* | 27/30 |
| Specimens Positive for *E. histolytica*/ Specimens Detected by the *TRI-COMBO PARASITE SCREEN* | 28/30 |

Note: Eight specimens were dual positive for *Giardia* and *E. histolytica* by the Microscopy and PCR and tested positive in the *TRI-COMBO PARASITE SCREEN* test. Three specimens were dual positive for *Giardia* and *Cryptosporidium* by Microscopy and PCR and tested positive in the *TRI-COMBO PARASITE SCREEN* test.

The prospective study results were analyzed by considering a composite result from multiple tests that consisted of light microscopy, molecular testing consisting of a commercial FDA cleared device and PCR with sequencing for the identification of *Giardia* spp., *Cryptosporidium* spp., in addition to identification and subspeciation of *E. histolytica.* This testing was mainly done because identification of *E. histolytica* organisms cannot be determined solely by microscopy because it is morphologically indistinguishable from the non-pathogenic *E. dispar*. Use of an alternate molecular testing is needed to confirm *Entamoeba* speciation. The molecular testing algorithm used provides a comparator method that is highly sensitive at the detection of *Giardia* spp., *Cryptosporidium* spp. and *E. histolytica*. The performance is summarized in Table 3 below and is presented as positive percent agreement and negative percent agreement. The algorithm used is presented in Table 4 below.

**Table 3. Summary of prospective clinical performance comparing the *TRI-COMBO PARASITE SCREEN* test to microscopy and molecular testing**

| | **Microscopy and Molecular testing** | |
|---|---|---|
| ***TRI-COMBO PARASITE SCREEN* (N = 754)** | **Positive** | **Negative** |
| **Positive** | **18** | **9*** |
| **Negative** | **11**** | **716** |
| | | **95% Confidence Limits** |
| **Positive Percent Agreement** | 62.1% | 44.0% - 77.3% |
| **Negative Percent Agreement** | 98.8% | 97.7% - 99.4% |

| | |
|---|---|
| Specimens Positive for *Giardia*/ Specimens Detected by the *TRI-COMBO PARASITE SCREEN* | 17/24 |
| Specimens Positive for *E. histolytica*/ Specimens Detected by the *TRI-COMBO PARASITE SCREEN* | 1/5 |
| Specimens Positive for *Cryptosporidium*/ Specimens Detected by the *TRI-COMBO PARASITE SCREEN* | 0/0 |

*These nine speciemns were tested with an alternate FDA cleared antigen test resulting in 9/9 Giardia determined to be antigen paositive.

**These eleven specimens were tested with an alternate FDA cleared antigen test resulting in 6/7 *Giardia* and 4/4 *E. histolytica* determined to be antigen negative.

**Table 4. Microscopy and molecular testing algorithm**

| Microscopy | Luminex xTag GPP | Alternate PCR with sequencing | Algorithm Result |
|---|---|---|---|
| Pos | Pos | Pos | Pos |
| Pos | Pos | Neg | Neg |
| Pos | Neg | Pos | Pos |
| Pos | Neg | Neg | Neg |
| Neg | Pos | Pos | Pos |
| Neg | Pos | Neg | Neg |
| Neg | Neg | N/A | Neg |

*b. Clinical specificity**:***

See section M3a. above.

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:

   Not applicable

**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.