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

A. 510(k) Number:

K170728

B. Purpose for Submission:

To obtain a substantial equivalence determination for the detection of *Entamoeba histolytica* antigens in human stool

C. Measurand:

Entamoeba histolytica antigen

D. Type of Test:

Qualitative membrane enzyme immunoassay

E. Applicant:

TechLab Inc.

F. Proprietary and Established Names:

E. HISTOL YTICA QUIK CHEK

G. Regulatory Information:

1. Regulation section:

   21 CFR Part 866.3220 *Entamoeba histolytica* Serological Reagents

2. Classification:

   II

3. Product code:

   KHW

4. Panel:

83 Microbiology

H. Intended Use:

1. Intended use(s):

   The TECHLAB *E. HISTOLYTICA QUIK CHEK* test is a rapid membrane enzyme immunoassay for the qualitative detection of adhesin from *Entamoeba histolytica* in a single use cassette. It is intended for use with human fecal specimens from patients with diarrhea or dysentery as an aid in the diagnosis of *E. histolytica* gastrointestinal infection. Test results should be considered in conjunction with patient history.

   FOR IN VITRO DIAGNOSTIC USE

2. Indication(s) for use:

   The TECHLAB *E. HISTOLYTICA QUIK CHEK* test is a rapid membrane enzyme immunoassay for the qualitative detection of adhesin from *Entamoeba histolytica* in a single use cassette. It is intended for use with human fecal specimens from patients with diarrhea or dysentery as an aid in the diagnosis of *E. histolytica* gastrointestinal infection. Test results should be considered in conjunction with patient history.

   FOR IN VITRO DIAGNOSTIC USE

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   None

I. Device Description:

The *E. HISTOLYTICA QUIK CHEK* test uses antibodies to adhesin. The device contains a reaction window with two vertical lines of immobilized antibodies. The test line ("T") contains monoclonal antibodies specific for *E. histolytica* adhesin. The control line ("C") contains antibodies to horseradish peroxidase (HRP). The Conjugate consists of polyclonal antibodies to *E. histolytica* adhesin coupled to horseradish peroxidase. The "T" reaction is examined visually for the appearance of a vertical blue line on the "T" side of the Reaction Window. A blue line indicates a positive test. A positive "C" reaction, indicated by a vertical blue line on the "C" side of the reaction window, monitors/confirms that the sample and reagents were added correctly, the reagents were active at the time of performing the assay, and that the sample migrated properly through

the membrane device. It also confirms the reactivity of the other reagents associated with the assay.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   E. histolytica II

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

   K994101

3. Comparison with predicate:

Similarities		
Item	Device K170728	Predicate K994101
Indications for use	The TECHLAB *E. HISTOLYTICA QUIK CHEK*™ test is a rapid membrane enzyme immunoassay for the qualitative detection of adhesin from *Entamoeba histolytica* in a single use cassette. It is intended for use with human fecal specimens from patients with diarrhea or dysentery as an aid in the diagnosis of *E. histolytica* gastrointestinal infection. Test results should be considered in conjunction with patient history. FOR *IN VITRO* DIAGNOSTIC USE.	The *E. histolytica II* is an enzyme immunoassay for the rapid detection of the adhesin of *E. histolytica* in human fecal specimens. It is indicated for use with fecal specimens from patients with diarrhea or dysentery to determine the presence of *E. histolytica* gastrointestinal infection. The test can be used for fecal specimens submitted for routine clinical testing from adults or children. Conventional microscopy is not a prerequisite for use of the test. FOR *IN VITRO* DIAGNOSTIC USE.
Measured analyte	*E. histolytica* specific antigen	Same
Target Population	Persons suspected of having *E. histolytica* infection	Same
Antibody Format	Monoclonal/Polyclonal	Same
Type of Test	Qualitative	Same
Sample Matrix	Human Fecal Specimen	Same

Similarities		
Item	Device K170728	Predicate K994101
Controls	Positive and negative control included in the kit	Same
Storage	Refrigerated (2°C – 8°C)	Same

Differences		
Item	Device K170728	Predicate K994101
Format	Single use membrane cassette 25 tests	96 well Mircoassay Plate
Specimen type	Fecal specimens in Cary-Blair and C&S Transport Media	Cannot use Transport Media
Time to result	30 minutes	2.5 hours
Controls	Internal "C" Control line	No internal control
Interpretation of result	Visual	Spectrophotometric and Visual

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:

Lateral flow immunochromatographic assay.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The precision of the *E. HISTOLYTICA QUIK CHEK* test was determined using an eight member masked fecal specimen panel. The panel consisted of 2 negative, 2 high negative (just below C5), 2 low positive (just above LoD), and 2 moderate positive (2-3x higher than the C95) specimens. 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. The samples produced the expected result 100% of the time.

The reproducibility of the *E. HISTOLYTICA QUIK CHEK* test was determined using eight masked fecal specimen panel prepared the same way as described for the precision study. Testing was performed at 2 independent laboratories and on-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 and exhibited a correlation of 100%. The samples produced the expected result 100% of the time.

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. For the analysis, a total of 15 fecal specimens were tested with the *E. HISTOLYTICA QUIK CHEK* test. The fecal specimens consisted 3 negative fecal samples, 3 *E. histolytica* low positive fecal samples (1-2 times C95 for whole organisms), 3 *E. histolytica* moderate positive fecal samples (2-3 times C95 for whole organism), and 3 *E. histolytica* high positive fecal samples (4-5 times C95 for whole organism). Both fresh samples and samples stored in transport media were tested.

For fresh samples, samples were stored between 2°C and 8°C and tested at 24 hour intervals for 120 hours. At the same time positive and negative controls were tested. The following transport media were used for the study: Thermo Scientific *Protocol* Cary Blair media and Meridian Bioscience Inc., *Para-Pak* C&S. As recommended in the Cary Blair package insert, Cary Blair samples were stored between 20°C and 25°C. C&S samples were stored at both refrigerated (2°C to 8°C) and prevailing 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 for 120 hours. At the same time positive and negative controls were tested.

Positive samples remained positive and negative samples remained negative throughout the study. Storage in transport media did not affect the stability of the samples. Based on these results, the recommended storage time for fresh samples is

up to 120 hours between 2°C and 8°C, Cary Blair transport media is up to 96 hours between 20°C and 25°C and C&S transport media is up to 120 hours between 2°C and 30°C based on the data and the package insert recommendation.

Frozen stability:

The frozen stability tests were determined for raw stool specimens using 15 masked fecal specimens panel prepared the same way as was described for the storage stability study. Samples were stores at $\leq$ -10°C for 28 weeks. Specimens were tested at 0, 1, 4, 6, 12, 16, 20, 24 and 28 weeks. Positive samples remained positive and negative samples remained negative throughout the study.

Freeze/Thaw:

Freeze/Thaw stability for Cary Blair and C&S media compared to raw specimens was demonstrated for one Freeze/Thaw cycle. 60 masked samples were tested, ten true negative, ten high negative (just belowC5 (whole organisms)), thirty low positives (1-2 times C95 (whole organisms)), and ten high positives (4-5 x LoD (whole organisms)) were tested for each transport media and raw stool. Based on the data submitted, the freeze-thaw cycle neither enhances nor impairs the performance of the *E. HISTOLYTICA QUIK CHEK* test.

d. Detection limit:

The cutoff point (limit of detection, LOD) for the *E. HISTOLYTICA QUIK CHEK* test was determined using whole organism spiked into unpreserved (raw stool) and preserved (Cary Blair and C&S media). Additional testing of spiked purified native antigen into raw stool was also performed. The cutoff is the concentrations of organisms or antigen 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 and purified antigen in a negative fecal sample matrix, in replicates of 20.

The LoD for the *E. HISTOLYTICA QUIK CHEK* test was determined to be 320 organisms/mL (equivalent to 15 organisms detected per test) or 0.2 ng/mL of adhesin in raw stool. For specimens in Cary Blair media, the LoD was established at 275 organisms/mL (equivalent to 14 organisms detected per test). For specimens in C&S media, the LoD was established at 245 organisms/mL (equivalent to 12 organism detected per test). No testing with adhesin was conducted in Cary Blair media or C&S.

e. *Analytical specificity:*

Cross reactivity.

The *E. HISTOLYTICA QUIK CHEK* 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 *E. HISTOLYTICA QUIK CHEK* test. Bacteria was 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

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

Cross reactivity with Norovirus is unknown because it was not tested in analytical studies. However, Norovirus GI/GII was identified in 50 clinical specimens using an FDA cleared multiplex NAAT assay during clinical testing and no cross reactivity was found using the E. HISTOLYTICA QUIK CHEK in those samples

The following parasites were found during clinical testing and found not to be cross reactant. The fecal specimens were documented to be positive for parasites by microscopy. 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 any of the following organisms.

Ascaris lumbricoides and with eggs (21)
Entamoeba bangladeshi (3)
Giardia spp. (45)
Blastocystis hominis (12)

Entamoeba coli (13)
Iodamoeba bütschlii (10)
Cryptosporidium spp. (30)
Entamoeba moshkovskii (3)
Trichuris trichiura eggs (11)
Cryptosporidium spp. (30)
Entamoeba moshkovskii (3)
Trichuris trichiura eggs (11)

f. Assay cut-off:

Not applicable

g. *Prozone:*

The purpose of this study was to demonstrate that a high concentration of *E. histolytica* adhesion (antigen) does not interfere with a positive reaction in the *E. HISTOLYTICA QUIK CHEK* test. High samples were prepared by spiking a negative fecal pool with 20,000 x the LoD described in section M.1.c above down to a concentration possibly observed in clinical specimens. A total of 5 different dilutions of the analyte, including the clinically observed high concentration, were prepared and tested. 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 *E. HISTOLYTICA QUIK CHEK* test was evaluated at three independent sites. The sensitivity and specificity for the *E. HISTOLYTICA QUIK CHEK* device was determined using the following composite reference method (CRM) table 1 at all three sites:

Table 1. CRM algorithm

Microscopy	FDA cleared multiplex NAAT test	Alternate PCR with sequencing	CRM 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

Prospective testing consisted of 755 specimens from two non-endemic areas in the US and one endemic area in Bangladesh. The endemic area had 5 CRM positive specimens. The remaining 750 samples were all negative. Table 2 below summarizes the performance observed, which mainly focused on evaluation of specificity due to the low number of positive specimens (See retrospective study).

Table 2. Performance….

N = 755	**Composite Reference Method Positive**	**Composite Reference Method Negative**
***E. HISTOLYTICA QUIK CHEK*™ Positive**	**2**	**0**
***E. HISTOLYTICA QUIK CHEK*™ Negative**	**3**	**750**

		95% Confidence Limits
Sensitivity	40.0%	7.3% - 83.0%
Specificity	100%	99.4% - 100%
Predictive Positive Value	100%	19.8% - 100%
Predictive Negative Value	99.6%	98.7% - 99.9%
Correlation	99.6%	99.6% - 99.6%

It should be noted additional discrepant analysis was done on the three false positive results because all three were microscopy negative, FDA cleared multiplex NAAT test positive and alternate PCR positive. Additional testing was performed and all three samples were determined to be negative by an alternative antigen test.

Retrospective Study

Retrospective testing consisted of 96 archived specimens previously collected and frozen. The Frozen samples are characterized as microscopy and NAAT positive for inclusion into the specimen bank. The specimens were collected form an *E. histolytica* endemic area. Table 3 below summarizes the performance observed

Table 3……

N = 96	**Composite Reference Method Positive**	**Composite Reference Method Negative**
***E. HISTOLYTICA QUIK CHEK*™ Positive**	**30**	**0**
***E. HISTOLYTICA QUIK CHEK*™ Negative**	**0**	**66**

		95% Confidence Limits
Sensitivity	100%	85.9% - 100%
Specificity	100%	93.1% - 100%

The age range for the 851 samples is one year to 100 years old. 161 of the sample tested were from children less than 21 years old. 57.3 percent of the specimens were from males and 42.7% of the specimens were from females.

*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.