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

A. 510(k) Number:

K141341

B. Purpose for Submission:

To obtain a substantial equivalence determination for a new device

C. Measurand:

Antigen from species of *Leishmania* that cause cutaneous leishmaniasis

D. Type of Test:

Qualitative immunochromatographic assay

E. Applicant:

InBios International, Inc.

F. Proprietary and Established Names:

CL *Detect*™ Rapid Test for Cutaneous Leishmaniasis

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3870 *Trypanosoma spp.* serological reagents

2. Classification:

   Class I

3. Product code:

   PIT– Reagent, *Leishmania spp.* antigen detection

4. Panel:

   83 Microbiology

H. Intended Use:

1. Intended use(s):

   The CL *Detect*™ Rapid Test is a qualitative, in vitro immunochromatographic assay for the rapid detection of *Leishmania* species antigen in ulcerative skin lesions. The test is intended for use with dental broach samples from less than four month old ulcerative skin lesions that are obtained from patients with suspected cutaneous leishmaniasis (CL). The test targets the peroxidoxin antigen of *Leishmania* species that may cause CL. The CL *Detect*™ Rapid Test is intended to aid in the diagnosis of CL, and must be interpreted within the context of all relevant clinical and laboratory findings.

2. Indication(s) for use:

   The CL *Detect*™ Rapid Test is a qualitative, in vitro immunochromatographic assay for the rapid detection of *Leishmania* species antigen in ulcerative skin lesions. The test is intended for use with dental broach samples from less than four month old ulcerative skin lesions that are obtained from patients with suspected cutaneous leishmaniasis (CL). The test targets the peroxidoxin antigen of *Leishmania* species that may cause CL. The CL *Detect*™ Rapid Test is intended to aid in the diagnosis of CL, and must be interpreted within the context of all relevant clinical and laboratory findings.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Not applicable

I. Device Description:

The CL *Detect*™ Rapid Test is a qualitative membrane-based immunoassay for the detection of antigens expressed by *Leishmania* amastigotes present in skin lesions of infected individuals. The test strip membrane is pre-coated with an affinity-purified polyclonal antibody to a *Leishmania* antigen (peroxidoxin) on the test line region and goat anti-mouse IgG on the control line region. The test strip also contains a control line in an area pre-coated with a dye-conjugated mouse monoclonal antibody to the amastigote peroxidoxin antigen. The control line serves as verification of sufficient sample volume, proper sample flow, and also as a control for the reagents.

Kit Components:

1. Twenty-five (25) individually pouched test strips or twenty-five (25) test strips in a vial.

2. One (1) vial of Lysis Buffer, 6 mL.
3. One (1) vial of Chase Buffer solution, 6 mL.
4. One (1) vial of Positive Control solution, 6 mL.
5. One (1) vial of Negative Control solution, 6 mL.
6. Twenty-five (25) sterile dental broaches for sample collection.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Kalazar *Detect*™ Rapid Test for Visceral Leishmaniasis

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

   K023483

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	CL *Detect*™ Rapid Test for Cutaneous Leishmaniasis	Kalazar *Detect*™ Rapid Test for Visceral Leishmaniasis
Intended Use	Aid in the diagnosis of *Leishmania spp.* infection	Same
Technology	Immunochromatographic assay	Same
Dye conjugate	Colloidal gold	Same
Interpretation	Qualitative	Same
Reading method	Visual, manual	Same

Differences		
Item	Device	Predicate
	CL *Detect*™ Rapid Test for Cutaneous Leishmaniasis	Kalazar *Detect*™ Rapid Test for Visceral Leishmaniasis
Indication	Aid in the diagnosis of cutaneous leishmaniasis	Aid in the presumptive diagnosis of visceral leishmaniasis
Specimen type	Dental broach samples from ulcerative skin lesions	Serum
Analyte	Antigen from *Leishmania spp.*	Antibodies to members of the *L. donovani* complex
Test line capture reagent	Anti-*Leishmania* peroxidoxin polyclonal antibody	Recombinant rK39 antigen
Conjugate reagent	Anti-*Leishmania* peroxidoxin monoclonal antibody conjugate	Protein A conjugate

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

Not applicable

L. Test Principle:

Sample collection:
Only ulcerative skin lesions less than four months old may be tested with this kit. A sample should be obtained from a skin lesion by a trained medical professional. After cleaning, debriding, and anesthetizing the lesion and surrounding skin if necessary, the sample is collected by twisting a sterile dental broach at the border of the lesion. The broach is then placed into a sample cup containing three drops of lysis buffer. Material should remain in lysis buffer for at least five to ten minutes but no longer than 30 minutes prior to testing.

Test procedure:
During testing, 20 microliters of the sample collected in lysis buffer is pipetted onto the sample pad of the test strip. The strip is then placed into a cup containing two to three drops of chase buffer to facilitate capillary action. The mixture reacts with the dye-conjugate monoclonal antibody on the test strip and migrates upward on the membrane chromatographically. When *Leishmania* amastigote antigens are present, the mixture reacts with the affinity-purified polyclonal antibody to generate a red line at the test line region of the membrane. Regardless of the presence of amastigote antigens, the mixture continues to migrate across the membrane to the immobilized goat anti-mouse IgG region where a red line at the control line region is expected to appear. The presence of the control line serves as verification of sufficient sample volume, proper sample flow, and also as a control for the reagents. The presence of the control line in the presence of the test line indicates a positive result. The presence of the control line in the absence of the test line indicates a negative result. The absence of the control line indicates an invalid result.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   A reproducibility study of the CL *Detect*™ Rapid Test kit was performed at three sites by two operators at each site for five days. Each site was provided sufficient material to run the assay in triplicate, for a total of 90 tests per panel sample. Each site was given a blinded coded panel of samples containing varying amounts of microscopy-quantified *L. major* promastigotes spiked into lysis buffer (six negative, six low positive, and six medium positive panel samples). Reproducibility was 98.9% for two of the negative panel samples and 100% for each of the other positive and negative panel samples. An additional study was performed in-house with six operators over five days using a blinded coded panel of lysed *L. major* promastigotes spiked at levels close to the estimated assay detection limit for that strain (negative, 300 parasite equivalents/test strip, and 450 parasite equivalents/test strip). A total of

90 tests were run per panel sample. Reproducibility was 97.8% for the negative sample, 80% for the 300 parasite equivalents/test strip sample, and 92.2% for 450 parasite equivalents/test strip sample.

b. Linearity/assay reportable range:

Not applicable

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

Controls:
One vial each of positive and negative control is provided with each test kit. Positive and negative controls should be used to test each new kit of 25 test strips to ensure kit integrity. If either the positive or negative control fails, retest with new test strips. If kit controls fail a second time and tips of bottles have not been contaminated, then open a new kit box for testing.

Sample stability:
The test should be performed as soon as possible after sample collection. Do not leave samples at room temperature for prolonged periods. Material should remain in lysis buffer for at least five to ten minutes but no longer than 30 minutes prior to testing.

High-dose hook effect:
Purified *Leishmania* peroxidoxin antigen produced reduced color intensity test line but otherwise positive results with the CL *Detect*™ when tested at 100 µg/mL, a concentration projected to exceed the concentrations expected in clinical samples. No false negative results were observed and all positive test lines remained positive up to purified antigen concentrations of 100 µg/mL.

d. Detection limit:

The limit of detection was estimated by an expert operator using promastigotes from culture isolates of various *Leishmania* species that may cause cutaneous leishmaniasis. Quantified parasites were serially diluted in lysis buffer and simulated sample matrix, and three replicates of each dilution were tested with the CL *Detect*. The estimated limit of detection for each culture isolate (detected at least 95% of the time after testing an additional 20 replicates by an expert operator) is reported in Table 1.

Table 1. Estimated limit of detection

Species	**Parasite equivalents / test**
*L. tropica (*WR-2995)	187
L. major (9/22/09 YS)	200
L. donovani (WR-378)	380

Species	Parasite equivalents / test
L. panamensis (WR-2307)	1080
L. mexicana (WR-2798)	1440
*L. brazi*liensis (WR-2353)	1440

e. Analytical specificity:

Cross reactivity:
A cross-reactivity study was performed to determine the effects of potentially cross-reactive species with the CL *Detect*™ Rapid Test kit. The potentially cross-reactive species are listed in Table 2 below and included organisms that cause the majority of secondary infections in CL. Bacteria were diluted to 10^4, 10^5 and 10^6 CFU equivalents per mL. Viruses were diluted to 10^3, 10^4 and 10^5 pfu per mL. Mammalian cells were diluted to 10^4, 10^5 and 10^6 cells per mL. Fungi were diluted to 10^4, 10^5 and 10^6 spores/mL and parasites to 10^4, 10^5 and 10^6 parasites/mL. Species were tested in duplicate. Four mammalian cell lines [HeLa (human cervical cancer), MCF-7 (human breast cancer), WI-38 (human fibroblast) and WM-115 (human melanoma)] resulted in slight cross-reactivity with CL *Detect*™ at the highest concentration tested (10^6 cells/mL). With all four cell lines, testing was negative once cells were diluted to 10^5 cells/mL. None of the bacterial, viral and fungal species commonly known to cause secondary infections in CL patients, and none of the closely related parasite species cross-reacted with the CL *Detect*™ Rapid Test kit at any concentration tested.

Table 2. Cross reactivity

Bacteria	**Mammalian**	**Mycobacteria**
Acinetobacter baumannii	WI-38 (human fibroblast)	*Mycobacteria abscessus*
Bacillus cereus	MCF7 (human breast cancer)	*Mycobacteria fortuitum*
Bacillus subtilis	HeLa (human cervical cancer)	*Mycobacteria marinum*
Bacillus thuringiensis	293T/17 (HEK293 human embryonic kidney)	*Mycobacteria tuberculosis* (attenuated) H37Ra-1
Clostridium perfringens	WM-115 (human melanoma)	*Mycobacteria ulcerans*
Clostridium sordellii	U-937 (human lymphoma)	
Enterobacter aerogenes		**Viruses**
Enterococcus durans	**Fungi**	HSV-1
Enterococcus faecalis	*Cladophialophora carrionii*	HSV-2
Escherichia coli (Clinical Isolate)	*Fonsecaea pedrosoi*	VZV (Ellen)
Haemophilus influenzae	*Microsporum canis*	VZV (Isolate D)
Klebsiella pneumoniae	*Phialophora verrucosa*	
Moraxella catarrhalis	*Rhinocladiella compacta*	**Trypanosoma**
Neisseria lactamica	*Sporothrix schenckii*	*Crithidia fasciculate*
Pasteurella multocida	*Arthroderma benhamiae*	*Trypanosoma cruzi*
Proteus mirabilis	*Trichophyton soudanense*	*Trypanosoma lewisi*
Proteus vulgaris	*Trichophyton tonsurans*	*Trypanosoma rhodesiense*

Providencia stuartii	*Microsporum gypseum*	*Trypanosoma rangeli*
Pseudomonas aeruginosa		*Trypanosoma gambiense*
Staphylococcus aureus		
Staphylococcus hominis		
Stenotrophomonas maltophilia		
Streptococcus pyogenes		
Streptococcus agalactiae		
Streptococcus sp. ATCC 12392		
Klebsiella oxytoca		

Interference study:
Blood components and topical treatments were evaluated for potential interference with the CL *Detect*™ Rapid Test kit. Potentially interfering substances were tested at concentrations expected to exceed those encountered in clinical samples as a "worst case scenario." Lysed *L.* major promastigotes were diluted to generate a sample panel that included a negative sample and three positive samples (500, 1000, and 10,000 parasite equivalents per test strip). Final concentration of interfering substances in each sample was 5% in lysis buffer. Results are summarized in Table 3 below. Betadine and mercurochrome should not be used on lesions, as even minute traces can detrimentally impact performance of CL *Detect*™. This information is included in the limitations section of the package insert.

Table 3. Interfering substances

Substance	**Results**
Whole Blood	Interference observed [1]
Buffy Coat	No interference
Plasma	No interference
Betadine (10% povidone-iodine)	Interference observed [2]
Hydrogen peroxide (3%)	No interference
70% isopropyl alcohol	No interference
Saline	No interference
Mercurochrome (2%)	Interference observed [3]
EMLA cream (2.5% lidocaine, 2.5% prilocaine)	No interference
Xylocaine (lidocaine)	No interference
Antibacterial hand sanitizer (70% ethanol)	No interference
Neosporin (0.8% bacitracin, 0.05% polymyxin B, 0.5% neomycin)	No interference
Hydrocortisone (2.5%)	No interference
Boil-Ease (20% benzocaine)	No interference

[1] Whole blood at ≥5% final concentration showed some interference with the reading of low positive samples. No interference was observed with whole blood tested at 2.5% final concentration.

[2] False positives observed with betadine at >0.0008% final concentration.
[3] False negatives observed with mercurochrome at >0.025% final concentration.

Strain reactivity:
The analytical reactivity of the CL *Detect*™ Rapid Test was evaluated with promastigotes from various *Leishmania* culture isolates. Reactivity was reported for the lowest concentration of lysed parasite equivalents per test strip that was detected by the CL *Detect*™ in three out of three replicates. Results are summarized in Table 4 below.

Table 4. Analytical reactivity

Species	**Parasite Equivalents / test strip**
L. tropica (WR-3064)	312
L. tropica (WR-1091)	312
L. amazoniensis (WR-404)	156
L. amazoniensis (WR-2837)	626
L. amazoniensis (WR-2767)	1500
L. donovani (WR-2712)	626
L. donovani (WR-2801)	626
L. infantum (WR-2719)	626
L. infantum (WR-2455)	1250
L. infantum (WR-2317)	1500
L. mexicana (WR-2800)	312
L. mexicana (WR-3006)	2500
L. guyanensis (WR-3017)	750
L. guyanensis (WR-2334)	1250
L. guyanensis (WR-2949)	2500
L. major (WR-3087)	376
L. major (WR-779)	1250
L. major (WR-1088)	2500
L. braziliensis (WR-2356)	1250
L. braziliensis (WR-3088)	5000
L. panamensis (WR-2306)	2500
L. panamensis (WR-3098)	5000
L. peruviana (WR-2334)	1500
L. peruviana (WR-2771)	2500
L. peruviana (WR-2851)	5000

f. Assay cut-off:

Not applicable

2. Comparison studies:

   a. Method comparison with predicate device:

   Not applicable

   b. Matrix comparison:

   Not applicable

3. Clinical studies:

   a. Clinical Sensitivity:

   Clinical performance - Endemic population

   A prospective clinical study was conducted in two ex-US sites endemic for cutaneous leishmaniasis (Sidi Bouzid and Gafsa, Tunisia). These regions are known to be endemic for *L. major*. One hundred sixty eight (168) patients with suspected CL lesions were tested with the CL *Detect*™ Rapid Test and with the reference method, Giemsa microscopy. For each patient, a sample was collected with a dental broach and tested with the CL *Detect*™ Rapid Test. An additional sample from the same site was collected by scraping for microscopic identification of amastigotes. All 149 microscopy-positive samples also tested positive with CL *Detect*™. The sensitivity of the CL *Detect*™ was 100%, 95% C.I. [97.6%, 100%]. Out of 19 microscopy-negative samples, three samples tested positive with CL *Detect*™, so that the specificity in this endemic population was 84.2%, 95% C.I. [62.4%, 94.5%]. One microscopy-negative and CL *Detect*™-positive sample was positive by subsequent culture analysis. Results are summarized in Tables 5, 6, and 7 below.

   It is likely that only patients infected with *L. major* were enrolled in the clinical study. Clinical performance has not been established for other *Leishmania* species that cause cutaneous leishmaniasis.

   Table 5. Clinical performance endemic site 1:

		Microscopy		
		Positive	**Negative**	**Total**
CL *Detect*™ Rapid Test	**Positive**	92	1	93
	Negative	0	12	12
	Total	92	13	105
Sensitivity:		100% (92/92, 95% C.I.: 96.0% - 100%)		
Specificity:		92.3% (12/13, 95% C.I.: 66.7% - 98.6%)		

Table 6. Clinical performance endemic site 2:

		Microscopy		
		Positive	**Negative**	**Total**
CL *Detect*™ Rapid Test	**Positive**	57	2	59
	Negative	0	4	4
	Total	57	6	63
Sensitivity:		100% (57/57, 95% C.I.: 93.7% - 100%)		
Specificity:		66.7% (4/6, 95% C.I.: 30% - 90.3%)		

Table 7. Combined endemic population performance

		Microscopy		
		Positive	**Negative**	**Total**
CL *Detect*™ Rapid Test	**Positive**	149	3*	152
	Negative	0	16**	16
	Total	149	19	168
Sensitivity:		100% (149/149, 95% C.I.: 97.1% - 100%)		
Specificity:		84.2% (16/19, 95% C.I.: 62.4%-94.5%)		

* One sample was positive with subsequent culture analysis.
** Two samples were positive with subsequent culture analysis.

Clinical performance – Non-endemic population

A prospective clinical study was conducted in one non-endemic site in the US. Although this site observes occasional patients with microscopically confirmed CL, the probability of CL in this non-endemic population is very low. One hundred fifty (150) samples from patients with skin lesions that had a clinical presentation similar to cutaneous leishmaniasis were tested with the CL *Detect*™ Rapid Test and by Giemsa microscopy. All 150 samples were negative for CL by microscopy. One hundred forty-four (144) samples tested negative with the CL *Detect*™, and six samples were false positive. Therefore, the specificity of the CL *Detect*™ in this non-endemic population was 96.0%, 95% C.I. [91.6%, 98.2%]. Results are summarized in Table 8 below.

Table 8. Clinical performance non-endemic population

		Microscopy		
		Positive	**Negative**	**Total**
CL *Detect*™ Rapid Test	**Positive**	0	6	6
	Negative	0	144	144
	Total	0	150	150
Sensitivity:		n/a*		
Specificity:		96.0% (144/150, 95% C.I.: 91.6% - 98.2%)		

***** No microscopy-positive CL lesions were observed in this population.

*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:

In an endemic population in Tunisia, CL *Detect*™ Rapid Test demonstrated positive results in 90.5% (152/168) of human ulcerative skin lesion samples. The endemic study population was 55.4% female and 44.6% male with an age range of 18 to 79 years old.

In a non-endemic population in the United States, CL *Detect*™ Rapid Test demonstrated positive results in 4.0% (6/150) of human ulcerative skin lesion samples. The non-endemic study population was 57.3% female and 42.7% male with an age range of 18 to 92 years old.

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.