510 (k) Summary – k081868

MAY 25 2011

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Date Summary Prepared	April 28, 2011
Trade/Proprietary Name	**SMART Leish**
Common or Usual Name	*Leishmania* Real-time Polymerase Chain Reaction Diagnostic Assay,
Classification Name	Trypanosoma spp. serological reagents
Regulation Number	866.3870
Product Code	OUZ
Review Panel	Microbiology 83
Classification	Class I
Predicate Device	Amizyme-Leishmania spp. Test Kit

Intended Use

The **SMART Leish** is a qualitative diagnostic real-time PCR test for the detection of *Leishmania* species and the identification of *L. major* in skin lesion scrapings and punch biopsies from individuals suspected of having cutaneous leishmaniasis. The test utilizes real-time polymerase chain reaction assay on the Cepheid SmartCycler® II Dx to detect *Leishmania* species and *L. major*.

WARNING

The **SMART Leish** is intended for use only by trained laboratory personnel in Department of Defense laboratories. Clinical performance has not been established with strains other than *L. major*.

Device Description

The **SMART Leish** consists of an assay reagent kit and assay definition files for the polymerase chain reaction (PCR) instrument platform. The kit contains sufficient reagents, in lyophilized bead form, to qualitatively assay 50 clinical samples for both *Leishmania* genus and *L. major* targets. Additional required accessories that are specified include the PCR instrument platform, a deoxyribonucleic acid (DNA) purification kit, and a positive extraction control. The device components and required accessories are listed as follows.

For the *Leishmania* assays, a tissue specimen (skin scraping or punch biopsy) from an individual suspected of being infected with *Leishmania* species *or L. major* is collected in 70% or 100% ethanol, and the DNA is extracted from the specimen using the Qiagen QIAamp DNA Mini Kit. An aliquot of this DNA is tested using the *Leishmania* genus assay, which will amplify a portion of DNA encoding the *Leishmania* species 16S ribosomal ribonucleic acid (rRNA) gene if present. Amplified targets are detected using a TaqMan® hybridization probe with 6-carboxy-fluorescein (FAM) reporter dye (517 nm) and a Black Hole Quencher® (BHQ). This assay also contains a positive internal control consisting of a nonsensical, non-naturally occurring DNA sequence, with Texas Red reporter dye (615 nm) and BHQ, used to detect evidence of PCR inhibition and confirm the integrity of assay reagents in negative specimens. If the sample tests positive for *Leishmania* species, another aliquot of DNA may be tested using the *L. major* assay, which will amplify a portion of DNA encoding the GPI gene that is specific to *L. major*. Amplified targets are detected using a TaqMan hybridization probe with FAM reporter dye and a BHQ. The DNA amplification, detection of fluorescence, and interpretation of signals are done automatically by the SmartCycler instrument for each assay. The thermocycling protocols take approximately 45–55 minutes.

Substantial Equivalence Information

1. Predicate device name:
   Amizyme-Leishmania spp. Test Kit

2. 510k number :
   K842526

Comparison with predicate:

Table 1. Similarities

Device Aspect	SMART Leish	Reference Device
Intended Use	Detection of *Leishmania* spp	Detection of *Leishmania* spp
Assay Type	Qualitative	Qualitative
Agent measured	*Leishmania* genus *and L. major* DNA	*Leishmania* spp
Technology	Qiagen QIAap DNA Mini Kit	None
Controls	External – positive and negative controls for both the extraction process and detection assay runs Internal – positive control for PCR inhibitors in sample	External positive and negative controls
Differences		
Instrument Platform	Cepheid SmartCycler	None
Automation	Automated DNA amplification cycling, probe detection, and interpretation of results	Manual method
Sample Collection	Skin punch biopsy or scrapings, stored in ethanol	Serum
Endpoint detection method	PCR amplification of DNA sequences unique to the organisms of interest with detection by hybridization probes incorporating reporter dyes	Indirect Immunofluoresence (IFA) Fluorescent conjugate secondary antibodies on a prepared slide

Clinical Performance Testing

The clinical performance of the **SMART** Leish assay was established in a multi-center, retrospective clinical study conducted at two military U.S. hospital sites.

A total of 312 *Leishmania* genus and 187 *Leishmania major* prospective specimens were collected from adults 18 years or older and evaluated in the **SMART** Leish assay and compared to culture and/or microscopy. Evaluated specimens were skin lesion scrapings and punch biopsies from individuals suspected of having cutaneous leishmaniasis. The patient population consisted entirely of military personnel who were at the time, or had previously been, deployed to Southwest Asia—primarily Afghanistan and Iraq. **SMART** Leish performance versus culture and microcopy, including 95% confidence intervals, is detailed below.

Table 2. Summary of SMART Leish Combined Studies for the *Leishmania* genus Assay

		Clinical Truth		
		Positive	Negative	Total
SMART Leish – *Leishmania* Genus Assay	Positive	223	29	252
	Negative	2	58	60
	Total	225	87	312
*Sensitivity = 99.1% (223/225) 95%CI (96.5 – 99.8) *Specificity = 67% (58/87) 95%CI (55 - 76)				

*Diagnosis of leishmaniasis by culture and slide techniques results in lower clinical sensitivity than PCR assays. In this clinical data set there were 29 false positives generated for the *Leishmania* Genus assay. Twenty seven of these Genus false positives were determined to be Genus positive using sequencing methods.

Table 3. Summary of SMART Leish Combined Studies for the *Leishmania major* Assay

		Clinical Truth		
		Positive	Negative	Total
SMART Leish – *L. major* Assay	Positive	92	8	100
	Negative	4	83	87
	Total	96	91	187
Sensitivity =95.8 (92/96) 95%CI (89.1– 98.7)* Specificity = 91.2% (83/91) 95%CI (82.9 – 95.9)				

*Diagnosis of leishmaniasis by culture and slide techniques results in lower clinical sensitivity than PCR assays. In this clinical data set there were eight false positives generated for the *L. major* assay, of which three of were determined to be *L. major* using sequencing methods.

ANALYTICAL PERFORMANCE

Analytical Reactivity Study

Two separate studies were done with Leishmania genus and Leishmania major assays using 36 Leishmania strains. The goal was to determine whether the assays cross-reacts with the nucleic acids from non-target organisms. In the Leishmania genus study, DNA from 36 *Leishmania* strains was tested and gave robust positive results with the Leishmania genus PCR assay. In the Leishmania major study, 11 of 36 strains were Leishmania major species. All 11 tested gave robust positive results with the *Leishmania major* PCR assay. None of the 25 non-*Leishmania major* samples tested positive. See **Table 4.**

Table 4. List of Leishmania Organisms Tested in Reactivity Study

Leishmania major (11)	*Leishmania mexicana*
Leishmania tropica (9)	*Leishmania amazonensis*
Leishmania infantum (7)	*Leishmania peruviana*
Leishmania donovani (2)	*Leishmania guyanensis (2)*
Leishmania braziliensis	*Leishmania tarentolae*

Analytical Specificity

A total of 121 DNA samples were evaluated in the specificity study. The DNA from 11 *Leishmania major* strains tested in this study gave robust positive results with the *Leishmania major* PCR assay. Twenty-five non-*Leishmania major* samples tested negative; hence the analytical specificity was 100%. The percentage of false positives was 0%, and the percentage of false negatives is 0%. In the *Leishmania genus* study, 85 of the 121 samples were non-target DNA samples. Eighty-five were negative, 2 false positives and 36 were positive. The analytical specificity of the *Leishmania genus* PCR assay was 98.3%, false positive rate was 2.4% and the false negative rate was 0%. See **Table 5** for list of organisms used in the study.

Nucleic acids from non-target organisms were tested for both the *Leishmania* genus and *Leishmania major* assays. DNA samples with concentrations used in this study included purified DNA from bacteria (52; conc =50 - 46,250 pg/uL), fungi (11; conc= 50,000 – 97,000 pg/uL), viruses (7; conc= 5,950 – 30,990 pg/uL), mammals (3; conc = 20 pg/uL (human, bovine, and murine)), human melanoma cell lines (3; conc = 10,200 – 25,200 pg/uL), *Leishmania major* (11; conc = 21,540 – 83,000 pg/uL), 25 additional *Leishmania* species (not-*L. major*) (conc = 16,680 – 592,00 pg/uL), and 9 trypanosomes representing 6 species (conc = 12,760 – 95,000 pg/uL).

Table 5. List of Organisms Used in Specificity Study

Bacteria		
Acinetobacter baumanni	*Staphylococcus hominis*	*Rhinocladiella compacta*
Bacillus anthracis (3)	*Stenotrophomonas maltophilia*	*Phialophora verrucosa*
Bacillus cereus (2)	*Streptococcus pyogenes (2)*	*Trichophyton tonsurans*
Bacillus subtilis var. niger	*Streptococcus sp. (B)*	*Trichophyton mentagrophytes*
Bacillus thuringiensis	*Streptococcus (F2)*	*Trichophyton soudanense*
Brucella abortus	*Yesinia pestis*	*Arthroderma benhamiae*
Brucella melitiensis	*Yersinia*	*Sporothrix schenckii*
Brucella suis		*Microsporum canis*
Clostridium botulinum type A	***Mycobacteria***	
Clostridium botulinum type B	*Mycobacteria abscessus*	**Mammalian**
Clostridium perfringens (2)	*Mycobacteria fortuitum*	Bovine
Clostridium sordelli	*Mycobacteria marinum*	Human
Enterobacter aerogenes	*Mycobacteria spp*	Murine
Enterococcus durans	*Mycobacteria tuberculosis*	Human melanoma cell line (3)
Enterococcus faecalis	*Mycobacteria ulcerans*	
Escherichia coli		***Trypanosoma***
Francisella tularensis (2)	**Viruses**	*Trypanosoma cruzi (2)*
Haemophilus influenzae	Human Papilloma Virus	*Trypanosoma rhodesiense*
Klebsiella oxytoca	Herpes Simplex Virus Type I	*Trypanosoma rangeli (2)*
Klebsiella pneumoniae	Herpes Simplex Virus Type II (2)	*Trypanosomal lewisi lincicome*
Moraxella cattaharalis	Varicella Zoster Virus (2)	*Trypanosoma brucei gambiense*
Neisseria lactamica		*Crithidia fasciculata (2)*
Pasteurella multocida	**Fungi**	
Proteus mirabilis	*Microsporium gypseum*	
Proteus vulgaris	*Cladophialophora carrionii*	
Providencia stuartii	*Fonsecaea pedrosoi*	
Pseudomonas aeruginosa		
Staphylococcus aureus (4)		

Two strains of *C. fasciculata* were determined to be low-level cross-reactors based on false-positive results observed. The degree of cross-reactivity of the *Leishmania* genus assay for *C. fasciculata* can be considered low level or weak. No cross-reactors were determined for the *L. major* assay through the study.

Supplementary *in silico* analysis was done comparing the Smart Leish primer and probe sequences to sequences from the following organisms that could potentially result in a clinical presentation similar to cutaneous leishmaniasis: Corynebacterium diphtheria (veldt sores), Mycobacteria (Tropical Ulcer), Mycobacterium tuberculosis (Lupus vulgaris), Treponema pallidum (Tertiary Syphilis), Treponema pertenue (Yaws), and Blastomyces (Blastomycosis). Each Smart Leish primer and probe contained at least four mismatches to those sequences. Based on these alignments, no cross-reactivity was predicted with the Smart Leish primers and probes.

Analytical Sensitivity

The assay linearity of SMART Leish for *Leishmainia* genus and *Leishmania major* was determined using eight different concentrations of purified *L. major* DNA tested in a 10-fold dilution series from 14.26 ng to 0.001426 pg and 21.39 ng to 0.002139 pg, respectively, DNA per reaction. Based on these results, additional studies were done to determine the analytical sensitivity (limit of detection or LOD) which was defined as the lowest amount of purified *L. major* DNA that consistently provided positive test results 95% of the time.

In addition to the LOD determination, six different concentrations of purified *L. major* parasites were tested in a 10-fold dilution of 5 X 10^2 parasites/mL (equivalent to 10^7 to 10^2 parasites/extraction) to determine the minimum number of *L. major* parasites that can be taken through the extraction and testing procedure and still give a positive result for the SMART Leish assays (extraction LOD).

Table 6 summarizes the results of the assay LOD for the *L. genus* and *L. major* assays, and Table 7 summarizes the results of the extraction LOD (eLOD) study.

Table 6. Summary of the Measuring Range and LOD for the *Leishmania* genus and *L. major* Assays (Values are per assay reaction)

Assay	Linear Range[1]	Assay LOD[1,2]	LOD Equates to
Leishmania Genus Assay	0.014–14,260 pg	0.14 pg	4 genome copies with ~200 copies/genome of target gene
L. major Assay	0.2139–21,390 pg	2.1 pg	62 genome copies

[1]Amount of *L. major* DNA in the PCR reaction. LOD refers to the minimum amount of L. major DNA that can be put into a SMART Leish PCR reaction and still result in a positive SMART Leish test greater than or equal to 95% of the time.

[2]95% accuracy across different operators and instruments and from multiple *L. major* strains

Table 7. Summary of Assay eLOD for the *Leishmania* genus and *L. major* Assays Using Parasite Samples. Values are per extraction.

Assay	eLOD[1]	LOD Equates to
Leishmania Genus Assay	250 parasites	~5 genome copies at assay reaction stage
L. major Assay	1,000 parasites	~30 genome copies at assay reaction stage

[1]95% accuracy across different operators and days and from multiple *L. major* strains

2. Extraction LOD refers to the minimum number of L. *major* parasites that can be taken through the Qiagen extraction procedure and still consistently result in a positive SMART Leish test greater than or equal to 95% of the time. Extraction LOD was determined across different operators, different days, and using multiple L. *major* strains.

Reproducibility

Precision was evaluated internally at the study sites. The following tables summarize the between laboratory reproducibility results for the *Leishmania* genus and *L. major* assays, respectively. These tables include the results of the studies done with purified DNA, cultured parasites, and mock human samples. The negative mock human samples were not further tested using the *L. major* assay if the initial *Leishmania* genus assay result was negative.

Table 8. Summary of Between Laboratory Reproducibility Results for the *Leishmania* Genus Assay

Sample Type	Sample ID	Concentration	Number of Replicates (over 5 days)	Laboratory A (WRAIR)	Laboratory B (BAMC)	Laboratory C (MAMC)	Total Agreement	% Total Agreement
		genome equivalents/µL						
Purified DNA	Low	2.0	20	20/20	20/20	20/20	60/60	100%
	Medium	20	20	20/20	20/20	20/20	60/60	100%
	High	200	20	20/20	20/20	20/20	60/60	100%
Positive Agreement for Purified DNA							180/180	100%
		(parasites/ extraction)						
Cultured Parasites	Low	1,000	20	20/20	20/20	20/20	60/60	100%
	Medium	10,000	20	20/20	20/20	20/20	60/60	100%
	High	25,000	20	20/20	20/20	20/20	60/60	100%
Positive Agreement for Cultured Parasites							180/180	100%
		(parasites/ extraction)						
Mock Human Samples	Negative	0	10	10/10	10/10	10/10	30/30	100%
	Positive	1.1x10^6	14	14/14	13/14	14/14	41/42	97.6%
Overall Agreement for Mock Human Samples							71/72	98.6%

Table 9. Summary of between Laboratory Reproducibility Results for the *Leishmania major* Assay

Sample Type	Sample ID	Concentration	Number of Replicates (over 5 days)	Laboratory A (WRAIR)	Laboratory B (BAMC)	Laboratory C (MAMC)	Total Agreement	% Total Agreement
		genome equivalents/µL						
Purified DNA	Low	2.0	20	15/20	7/20	15/20	**37/60**	**61%**
	Medium	20	20	20/20	20/20	20/20	**60/60**	**100%**
	High	200	20	20/20	20/20	20/20	**60/60**	**100%**
Positive Agreement for Purified DNA							**157/180**	**87.2%**
		(parasites/ extraction)						
Cultured Parasites	Low	1,000	20	19/20	20/20	16/20	**55/60**	**91.6%**
	Medium	10,000	20	20/20	20/20	20/20	**60/60**	**100%**
	High	25,000	20	20/20	20/20	20/20	**60/60**	**100%**
Positive Agreement for Cultured Parasites							**175/180**	**97.2% CI**
		(parasites/ extraction)						
Mock Human Samples	Negative	0	0/0	0/0	0/0	0/0	**0/0**	**100%**
	Positive	1.1x10^6	14	14/14	13/13	14/14	**41/41**	**100%**
Overall Agreement for Mock Human Samples							**41/41**	**100%**

INTERFERING SUBSTANCES

The *Leishmania* genus assay and the multiplexed positive IC assay were evaluated with known PCR inhibitors. The inhibitors tested were: hemoglobin, hemin, ferric ammonium citrate (FAC), and EDTA. Presence of inhibition was defined as a false negative result (negative result for a true positive sample). The inhibitor concentrations tested and the maximum inhibitor concentration that did not induce inhibition in the *Leishmania* genus and positive IC assays are listed in the table below.

Table 10. Results of Testing the Effects of Known Inhibitors on the *Leishmania* Genus and IC Assays

PCR Inhibitor	Concentrations Tested (final concentration in PCR reaction tube)	Maximum Concentration without Inhibition Detected	
		Leishmania Genus Assay	IC Assay
Hemoglobin	0.03, 0.06, 0.12, 0.24, 0.48, 0.96, and 1.92 µg/µl	1.92 µg/µl	0.96 µg/µl
Hemin	0.03, 0.06, 0.12, 0.24, 0.48, 0.96, and 1.92 µg/µl	0.96 µg/µl	0.96 µg/µl
FAC	0.03125, 0.0625, 0.125, 0.25, 0.50, and 1.00%	0.0625%	0.0625%
EDTA	0.3125, 0.625, 1.25, 2.5, 5, and 10 mM	1.25 mM	1.25 mM

Conclusion from Nonclinical and Clinical Performance Testing

The conclusions drawn from the nonclinical and clinical testing, and comparative methodology review, demonstrate the effectiveness of the **SMART Leish** for the intended use.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

Department of the Army
U.S Army Medical Material Development Activity
c/o Robert E. Miller, Ph.D., RAC
Director, Division of Regulated Activities and Compliance
1430 Veterans Drive
Fort Detrick, MD 21702-5009

Re: k081868
Trade/Device Name: SMART Leish PCR Assay
Regulation Number: 21 CFR 866.3870
Regulation Name: Trypanosoma spp. Serological Reagents
Regulatory Class: Class I
Product Code: OUZ
Dated: May 23, 2011
Received: May 24, 2011

MAY 25 2011

Dear Dr. Miller:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket

notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Sally A. Hojvat, Ph.D., MSc.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health

INDICATIONS FOR USE STATEMENT

510(k) Number (if known): k081868

Device Name: SMART Leish

Indications for Use:

The **SMART Leish** is a qualitative diagnostic real-time PCR test for the rapid detection of *Leishmania* species and the identification of *L. major* in skin lesion scrapings and punch biopsies from individuals suspected of having cutaneous leishmaniasis. The test utilizes real-time polymerase chain reaction assay on the Cepheid SmartCycler® II Dx to detect *Leishmania* species and *L. major*. The **SMART Leish** is indicated for use in patients with clinical presentations and travel history.

WARNING

The **SMART Leish** is intended for use only by trained laboratory personnel in Department of Defense laboratories. Clinical performance has not been established with strains other than *L. major*.

Prescription Use √ (Part 21 CFR 801 Subpart D) AND/OR Over-The-Counter Use _____ (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE OF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Division Sign-Off

Office of In Vitro Diagnostic Device Evaluation and Safety

510(k) K081868