510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:

K081868

B. Purpose for Submission:

To obtain a 510(k) clearance for a new device.

C. Measurand:

Leishmania species DNA and *L. major* DNA

D. Type of Test:

Real-time polymerase chain reaction assay

E. Applicant:
Department of the Army
U.S. Army Medical Research and Matériel Command
1430Veterans Drive
Fort Detrick, MD 21702-5012

F. Proprietary and Established Names:

SMART Leish

G. Regulatory Information:

1. Regulation section:

   866.3870 Trypanosoma spp. serological reagents

2. Classification:

   Class I

3. Product code:

   OUZ

4. Panel:

   Microbiology 83

H. Intended Use:

1. Intended 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 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*.

2. 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 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*.

3. Special conditions for use statement(s):

   The device is for prescription use only.

4. Special instrument requirements:

   Cepheid SmartCycler® II Dx

I. 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.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Amizyme-Leishmania spp. Test Kit

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

   K842526

3. Comparison with predicate:

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 specific* DNA	*Leishmania* spp.
Technology	Qiagen QIAamp 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 Immunofluorescence (IFA) Fluorescent conjugate secondary antibodies on a prepared slide

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

N/A

L. Test Principle:

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 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 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Studies were conducted to evaluate the reproducibility of the SMART Leish assay. The precision of the Leishmania genus and L. major assays between laboratories was evaluated using purified L. major DNA and cultured L. major parasites. Additionally, between-laboratory precision of the assays was evaluated using mock human samples (punch biopsies from porcine tissue injected with L. major parasites).

Reproducibility Results for the Leishmania Genus Assay

Sample Type	Sample ID	Concentration	Number of Replicates (over 5 days)	(WRAIR)	(BAMC)	(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%

b. Linearity/assay reportable range:

The assay linearity of SMART Leish for Leishmania 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 21.39ng to 0.002139 pg, respectively, DNA per reaction.

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

Not applicable

d. *Detection limit*:

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). The following tables summarize the results of the assay LOD for the L. genus and L. major assays, and summarizes the results of the extraction LOD (eLOD) study.

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	0.014–14,000 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.

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[2]
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.

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.

e. 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 the following table for a 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).

List of Organisms Used in Specificity Study

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

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.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Clinical performance of the SMART Leish was compared to culture and microscopy.

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

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 303 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. For the BAMC study, DNA was extracted from specimens and *Leishmania* genus and *L. major* assays were run. For the WRAIR study, *Leishmania* genus and *L. major* assays were run on archived DNA samples that were previously extracted from clinical specimens. For both studies, the results were compared to "clinical truth" that had been established for each specimen or sample.

Summary of Smart Leish WRAIR Studies for the

***Leishmania* genus Assay**

		Clinical Truth		
		Positive	Negative	Total
Smart Leish – Leishmania Genus Assay	Positive	160	29	189
	Negative	1	56	57

	Total	161	85	246
Sensitivity = 99.4% (96.1% - 99.9%) Specificity = 65.9% (54.7% - 75.6%)				

Summary of Smart Leish WRAIR Studies for the *Leishmania* major Assay

		Clinical Truth		
		Positive	Negative	Total
Smart Leish – L. major	Positive	70	8	78
	Negative	4	77	81
	Total	74	85	159
Sensitivity = 94.6% (86.0% - 98.3%) Specificity = 90.6% (81.8% - 95.6%)				

Summary of Smart Leish BAMC Studies for the *Leishmania* genus Assay

		Clinical Truth		
		Positive	Negative	Total
Smart Leish – Leishmania Genus	Positive	63	0	63
	Negative	1	2	3
	Total	64	2	66
Sensitivity = 98.4% (90.5% - 99.9%) Specificity = 100% (19.8% - 100%)				

Summary of Smart Leish BAMC Studies for the *Leishmania* major Assay

		Clinical Truth		
		Positive	Negative	Total
Smart Leish – L. major	Positive	22	0	22
	Negative	0	6	6
	Total	22	6	28
Sensitivity = 100.0% (81.5% - 100.0%) Specificity = 100.0% (51.7% - 100.0%)				

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% (96.5 – 99.8) Specificity = (67% (55% - 76%)				

Diagnosis of leishmaniasis by culture and slide techniques results in lower clinical sensitivity than PCR assays (46%-84% and 33%-74% respectively). In this clinical data set there were 29 false positives generated for the *Leishmania* Genus assay. Twenty seven of these Genus false positives were confirmed to be Genus positive using sequencing methods. Clinical and specificity values were recalculated counting the 27 Genus false positives that were confirmed by sequencing to be *Leishmania* as true positives. These "adjusted" calculation results are as follows: Sensitivity Genus: 99.2% (96.9 - 99.9); Specificity Genus: 96.7% (87.5 – 99.9). *Diagnosis of leishmaniasis by culture and slide techniques results in lower clinical sensitivity than PCR assays (46%-84% and 33%-74% respectively)

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% (89.1 – 98.7) *Specificity = = 91.2% (82.9 - 95.9)				

In this clinical data set there were eight false positives generated for the *L. major* assay, of which three of were confirmed to be *L. major* using sequencing methods. Clinical and specificity values were recalculated counting the 27 three *L. major* false positives that were confirmed by sequencing to be *L. major* as true positives. These "adjusted" calculation results are as follows: Sensitivity *L. major:* 96.0% (89.4 - 98.7); Specificity *L. major:* 94.3% (86.6 - 97.9).

*b. Clinical specificity**:***

Not applicable

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

None

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

In the clinical studies conducted, for this study, on U.S. military personnel that served in the Middle East and Afghanistan, 57% of 2,783 samples tested using an in-house developed PCR assay in an accredited clinical laboratory were positive for *Leishmania major*. In the investigational study of the Smart Leish assay, 72% of 312 skin scrapings and punch biopsy specimens from U.S. service members that served in the Middle East and Afghanistan, and which had a skin lesion consistent with cutaneous leishmaniasis, tested positive compared to the gold standard of culture and/or microscopy.

N. Instrument Name:

SmartCycler Dx System

O. System Descriptions:

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.

The SMART Leish Assay contains the following reagents packaged in a test kit for detection of Leishmania DNA.

- SmartMix™ HM Lyophilized PCR Master Mix
- Leishmania Genus Primer and Probe Set
- Leishmania major Primer and Probe Set
- Leishmania Positive and Negative Control DNA Beads

Modes of Operation:

The assay is performed on the automated Cepheid SmartCycler Dx System. The SmartCycler Dx System is a real time thermal cycler used for identifying DNA or RNA from prepared samples. Each instrument contains 16 independently programmable I-CORE modules, each with one reaction site. Thermally optimized proprietary reaction tubes combined with the unique design of the I-CORE modules allow very rapid cycling and faster amplification and detection. Up to six SmartCycler II processing blocks can be daisy-chained together,

allowing simultaneous analysis of up to 96 discrete samples.

The SmartCycler Dx System is suited to *in vitro* diagnostic assays that require automatic, repeated, rapid heating and cooling cycles for test samples, such as PCR and RT-PCR. Specific sequences can be detected using hybridization probes. The system has the capacity to store any number of runs, limited only by available disk space. All assay data and results are stored in a database.

. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes ____x____ or No _____
The software controls the operation of the I-CORE module, and collects, analyzes and interprets the acquired optical data. This software has been determined to be a moderate level of concern.

3. Specimen Identification:

- User enters the sample identifiers under the "Sample ID Column" before starting the run.
- User inserts each reaction tube into the assigned site of the SmartCycler.

4. Specimen Sampling and Handling:

- Collected specimens should be stored in 70% - 100% ethanol. During shipment, the samples can be kept at ambient temperature, 20^0 C – 29^0 C.
- Once received, samples should be stored at 2°C–8°C until tested.
- Extracted DNA samples must be stored in the AE buffer provided in the Qiagen kit and may be stored at 2°C–8°C for up to 3 days.
- DNA extracts in AE buffer may be stored for more than 3 days in a –20°C freezer. Avoid multiple freeze–thaw cycles of the samples.

5. Calibration:

The thermistors used to monitor the reaction chamber temperature are calibrated to
±0.50°C using National Institute of Standards and Technology (NIST)-traceable standards. The optical system is calibrated using standard concentrations of the individual unquenched dye-oligo standard to determine the spectral characteristics.
The instrument performs optics self-test before each run start to verify that the optical system is functioning properly.

6. Quality Control:

Positive DNA controls are provided in the assay kit. The positive extraction control (*Leishmania mexicana* parasites) and negative controls (molecular biology grade water) are materials required but not supplied. One positive DNA Control and one negative DNA Control are processed for each assay run on the SmartCycler Dx System. The software automatically assigns the positive DNA Control to the second to last position, and the negative DNA control to the last position.

The positive DNA control monitors for reagent failure and operator error. The negative DNA control monitors reagent or environmental contamination (or carry-over). The positive extraction control monitors for extraction failure and operator error. The negative extraction control monitors for reagent contamination and environmental contamination in the extraction process.

The internal control (IC) verifies functional PCR reagents and the absence of inhibition
that would prevent PCR amplification. DNA for the IC is included in one of the reagent
beads within the Smart Leish Assay. The response of the IC in the presence of inhibitors correlates with the response of target DNA in the presence of inhibitors.

Before start of PCR reaction, the SmartCycler Dx System is programmed to perform an
optical measurement or probe check on the optical channels associated with *Leishmania* target and Internal Control detection. The Probe Check control verifies reagent bead
rehydration, appropriate tube filling, probe integrity, and dye stability. Probe Check is
considered to PASS if optical measurements meet the validated acceptance criteria. If the
Probe Check fails in either optical channel, the test will not continue.

The System Control Check for Temperature Control ensures that the SmartCycler Dx
Instrument is operating within specification.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

None

Q. Proposed Labeling:

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

R. Conclusion:

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