CL *Detect*™ Rapid Test
For Cutaneous Leishmaniasis

Intended 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.

Summary and Explanation

Several *Leishmania* species are responsible for CL. In the Middle East and Central Asia, the predominant species responsible for cutaneous forms of leishmaniasis are *L. major* and *L. tropica* [1-6, 13]. *L. donovani* and *L. infantum* predominantly lead to visceral forms of Leishmaniasis. In Iraq, *L. major* is the major cause of CL. It is the primary agent for zoonotic cutaneous leishmaniasis (ZCL). In Afghanistan, the primary agent for cutaneous leishmaniasis is *L. tropica* with an active infection rate in Kabul of 2.7%, but areas of Northern Afghanistan are also endemic for *L. major* [7]. *L. tropica* is more frequently associated with anthroponotic cutaneous leishmaniasis (ACL). In Central and South America, *L. braziliensis*, *mexicana*, *amazonensis* and *panamensis*, among other species, are prevalent [8, 9] and responsible for cutaneous leishmaniasis [6, 8-11]. Ulcerative skin lesions are reservoirs of *Leishmania* amastigotes that can be used in aiding diagnosis of CL [12-16].

Principle

The CL *Detect*™ Rapid Test for CL is a qualitative, membrane-based immunoassay for the detection of antigens present in amastigotes present in skin lesions of individuals infected with *Leishmania* parasites [12-16]. The membrane is pre-coated with an affinity-purified polyclonal antibody to an amastigote antigen (peroxidoxin) [17-19] on the test line region and goat anti-mouse IgG on the control line region. During testing a sample from the skin lesion is collected with a dental broach and placed in lysis buffer. The lysed sample is applied to the test strip and reacts with the dye conjugate (monoclonal antibody to the amastigote antigen) which has been pre-coated in the test device. The mixture then migrates upward on the membrane chromatographically to react with the affinity-purified polyclonal antibody on the membrane and generates a red line. Presence of this red line indicates a positive result, while its absence indicates a negative result. Regardless of the presence of amastigote antigens, as the mixture continues to migrate across the membrane to the immobilized goat anti-mouse IgG region, a red line at the control line region is expected to appear. The presence of this red line serves as verification for sufficient sample volume and proper flow and as a control for the reagents.

Materials Provided

The kit contains the following:

1. Twenty-five (25) individually pouched test strips or twenty-five (25) test strips in a vial
2. One (1) vial of Lysis Buffer, 6mL
3. One (1) vial of Chase Buffer Type A solution, 6mL
4. One (1) vial of Positive Control solution, 6mL
5. One (1) vial of Negative Control solution, 6mL
6. Twenty-five (25) sterile dental broaches

Materials Required But Not Provided

1. 20 µL pipettor and tips, or twenty-five (25) transfer pipets (20 µL) (option)
2. Twenty-five (25) sample cups
3. Twenty-five (25) reaction cups

Warning and Precautions

- **FOR *IN VITRO* DIAGNOSTIC USE.** A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert.
- For prescription use only
- Do not use after expiration date listed on kit box label.
- Do not use serum, plasma or whole blood with this test strip.
- Do not use samples from lesions that are over 4 months old.
- Handle all samples and used supplies as if they contain infectious agents. Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for proper disposal of samples and used supplies.
- Wear protective clothing, eye protection and disposable gloves while performing the assay. Wash hands thoroughly when finished.
- Avoid all contact between hands and eyes or mucous membranes during testing.

- Do not eat, drink or smoke in the area where samples and kits are handled.
- Do not cross-contaminate kit controls by mixing bottle caps.
- Chase Buffer Type A contains a preservative; avoid all possible contact with skin and mucous membranes. Lysis buffer contains detergents and should not be in contact with skin or be ingested.

Storage

The entire kit is designed to be stored at room temperature (20°C-30°C) for the duration of its shelf life. Exposure to temperatures over 30°C can impact the performance of the test and should be minimized. The test strips should not be frozen. The test strips should be used immediately after removal from the pouch or vial to minimize exposure to humidity.

Sample Collection

- Only ulcerative skin lesions are to be used with this kit. Human serum should not be tested with this kit.
- Samples (skin lesions) will be collected in the optionally provided sample cup or similar vessel. Samples will then be treated with 3 drops of lysis buffer in preparation for testing. Incubate samples in lysis buffer at room temperature for 5-10 minutes. Samples must not remain in lysis buffer for more than 30 minutes prior to testing. In all cases, ensure that the lysis buffer and the sample material are well mixed.
- Test should be performed as soon as possible after collection. Do not leave samples at room temperature for prolonged periods

Skin Lesion Preparation: A sample from the suspected ulcerative skin lesion should be obtained by a trained medical professional. Note that the lesion should be less than four months old. If more than one suspected ulcerative lesion is present, select one that appears most active (erythematous and edematous).

- Thoroughly clean the lesion and surrounding skin by washing with water and detergent. Betadine and mercurochrome should not be used, as even minute traces can detrimentally impact performance of CL *Detect*™.
- Debride the sore by using forceps to apply a gauze pad soaked in sterile 0.9% saline solution. Rub until the entire crust is removed, leaving the lesion looking like "baby's skin."
- Apply EMLA cream to the surrounding edge of the lesion or use injectable lidocaine if necessary to numb the area.
- A scalpel may be used to debride if necessary, only after reducing discomfort pain with EMLA or lidocaine.
- Once the lesion is prepared, a sample can be obtained by a trained medical professional. Below is a method that has been clinically proven to give good results.

Dental Broach Procedure

- Remove sterile dental broach from individual packaging.
- It is important to note that when using the dental broach sample in the area of inflammation it should be inserted near the border of the lesion and move in a direction away from the ulcerated area and towards the area of normal skin.
- At the border of the lesion, insert the barb into the lesion to a depth of approximately ½ the length of the barb from the ulcer to the inflamed area.
- Press a thumb onto the skin where the broach was inserted.
- Gently rotate (twist) the broach twice (back and forth each 2 times).
- Remove broach with a quick sharp pull, twisting slightly.
- Place it barbed-end down into a sample cup containing 3 drops of lysis buffer.
- Twist the barb to remove as much cellular material as possible.
- Use transfer pipette or 20 µL pipettor to flush tissue from broach into lysis buffer as needed.
- Make sure the lysis buffer and material are well mixed. Material should remain in lysis buffer for at least 5-10 minutes, but no longer than 30 minutes, prior to testing with CL *Detect*™.

Note: The dental broach should be disposed of once sample is obtained. Do not reuse the dental broach.

Quality Control

The user is responsible for performing quality control testing according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. 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. Avoid switching caps of positive and negative control bottles, or otherwise contaminating the tips.

Procedure:

1. Add 1 drop of positive or negative control plus 3 drops of lysis buffer to a sample cup.
2. Remove test strip from pouch or vial.
3. Using a transfer pipet or 20 µL pipettor and tips, apply 20 µL of the diluted positive or negative control to the test strip in the area beneath the arrow.

4. Add 3 drops of Chase Buffer Type A solution to the reaction cup.
5. Place the test strip into the reaction cup so that the end of the strip is facing downward as indicated by the arrows on the strip.
6. Read results in 20-30 minutes. Follow procedure below regarding interpretation of results.
7. The positive control should yield a positive result and the negative control a negative result.
8. 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.

Test Procedure

1. Collect skin lesion sample as described in the Sample Collection section of this product insert.
2. Sample should remain in lysis buffer for 5-10 minutes prior to testing with CL *Detect*™.
3. Remove test strip from pouch or vial.
4. Add 20 µL of sample to the test strip in the area beneath the arrow using 20 µL pipettor and tips.
5. Add 3 drops of the Chase Buffer Type A solution to a new reaction cup.
6. Place the test strip into the cup so that the end of the strip is facing downward as indicated by the arrows on the strip.
7. Read the results in 20-30 minutes. It is important that the background is clear before reading the test, especially when samples have low levels of antigen, and only a weak band appears in the test region (T). Results interpreted after 30 minutes can increase the likelihood of false positives. Follow procedure below regarding interpretation of results.

Note: Do not test this product with the Chase Buffer Type A solution alone. 20 µL of sample must be added first.

Note: If migration of the gold is not observed within 10-15 seconds after the addition of chase buffer, apply light pressure on the sample tape region of test strip until gold migration is observed.

Interpretation of Results

A Positive Result

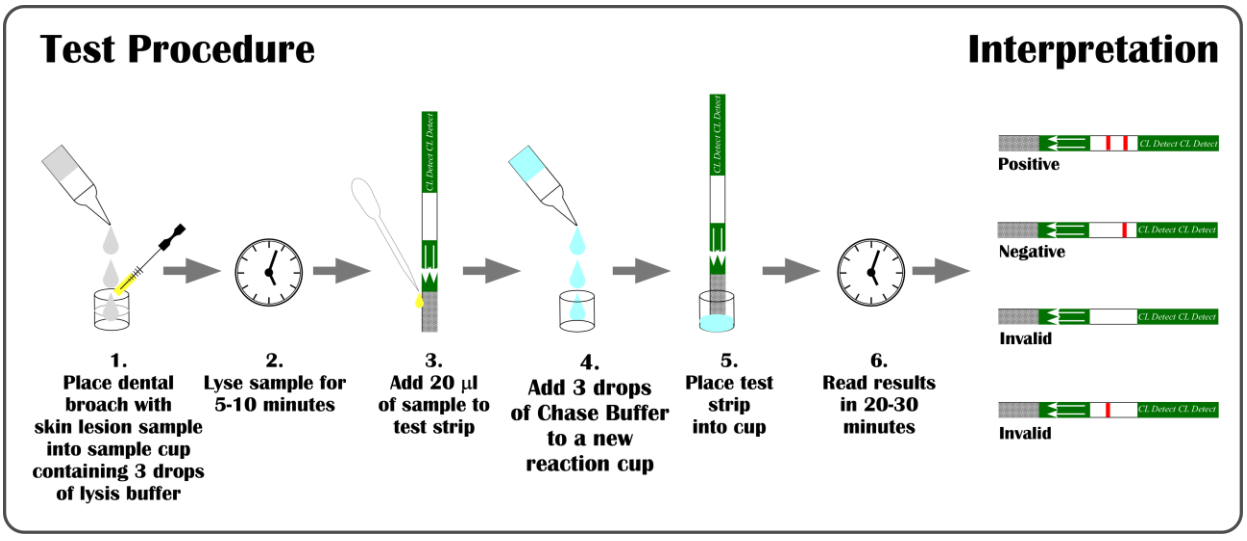
The test is positive when a control line and test line appear in the test area. A positive result indicates that the CL *Detect*™ Rapid Test detected antigens present in *Leishmania* amastigotes. A faint line of any red color intensity is considered a positive result. Neither the quantitative value nor the rate of increase in antigen can be determined from the color intensity of this qualitative test.

A Negative Result

The test is negative when only the control line appears. A negative result indicates that the CL *Detect*™ Rapid Test did not detect antigens in the sample. No test line is present.

An Invalid Result

No control line is visible. It is recommended to retest using a new CL *Detect*™ Rapid Test for Cutaneous Leishmaniasis and fresh sample.



Limitations

- This product is for prescription use only.
- This assay is not for use to diagnose Visceral Leishmaniasis or Mucosal Leishmaniasis.
- Do not use serum samples.
- Care should be exercised to ensure that bloody samples are not used. In analytical studies, whole blood at ≥ 5% final concentration showed some interference with the reading of low positive samples, possibly leading to false negative results.
- Betadine and mercurochrome should not be used on the lesion, as even minute traces can detrimentally impact performance of CL *Detect*™. Betadine contamination can lead to false positive results and mercurochrome contamination can lead to false negative results.
- This test will only indicate the presence of *Leishmania* parasite antigens in patients with cutaneous leishmaniasis and should not be used as the sole criterion for the diagnosis of leishmaniasis. This test alone must not be used for any clinical treatment decision. As with all diagnostic tests, all results must be considered with other clinical information available to the doctor.
- If the result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is recommended. A negative result does not preclude the possibility of Leishmaniasis.
- A false positive result may occur. Confirmatory testing (microscopy) is advised especially in cases where no symptoms exist.
- The CL *Detect*™ rapid test is intended for use with ulcerative skin lesions and has not been evaluated with purely verrucous or nodular skin lesions which may occur with cutaneous leishmaniasis.
- Clinical testing was performed in a region known to be endemic for *L. major*, and the infecting parasite species was not confirmed for every patient. 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.
- In the analytical studies, four mammalian cell lines [HeLa (human cervical cancer), MCF-7 (human breast cancer), WI-38 (human fibroblast) and WM-115 (human melanoma)] produced false positive results at high concentrations (refer to "Cross-Reactivity" section for further details.)
- The CL *Detect*™ Rapid test has not been evaluated for possible cross-reactivity with the following organisms: *Bacillus anthracis, Brucella melitensis, Clostridium botulinum type A, Clostridium botulinum type B, Francisella tularensis, Yersinia pestis.*

Expected Values

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.

Performance Characteristics

Clinical Studies:
Clinical Performance – Non-endemic Population

A prospective clinical study was conducted at Icahn Medical School at Mount Sinai in New York City, NY, USA. 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, the reference standard. One hundred forty-four (144) samples tested negative for CL with the CL *Detect*™ Rapid Test and microscopy. Six samples were false positives. Therefore, the specificity of the CL *Detect*™ Rapid Test in this non-endemic population was 96.0%, with a 95% confidence interval (C.I.) of 91.5%-98.5%. Note that all six false positives had very faint test lines using the CL *Detect*™ Rapid Test.

Table 1: Non-Endemic Population

		Microscopy (Reference standard test)		
		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.: 91.5% - 98.5%)		

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

Clinical Performance – Endemic Population

A prospective clinical study was conducted in two sites in areas endemic for cutaneous leishmaniasis in Tunisia - Sidi Bouzid and Gafsa. These regions are known to be endemic for *L. major* [2]. One hundred sixty-eight (168) patients with suspected CL lesions were tested with the CL *Detect*™ Rapid Test and with the reference standard, 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*™. Therefore, the sensitivity of the CL *Detect*™ Rapid Test in this endemic population was 100%, with a 95% confidence interval of 97.6%-100%. Out of 19 microscopy-negative samples, three samples tested positive with CL *Detect*™, so that the specificity in the endemic population was 84.2% with a 95% confidence interval of 62.4%-94.5%. One microscopy-negative and CL *Detect*™-positive sample was positive with subsequent culture analysis.

Table 2: Endemic Population

		Microscopy (Reference standard test)		
		Positive	**Negative**	**Total**
CL *Detect*™ Rapid Test	**Positive**	149	3*	152
	Negative	0	16**	16
	Total	149	19	168
Sensitivity:		100.0% (149/149, 95% C.I.: 97.1%-100.0%)		
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.

Reproducibility Study:

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 spiked with varying amounts of *L. major* promastigotes (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 *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.

Cross-Reactivity Study:

A cross-reactivity study was performed to determine the effects of potentially cross-reactive species with the CL *Detect*™ Rapid Test kit. These species include potentially cross-reactive organisms and those that cause the majority of secondary infections in CL. Species were tested in duplicate, and are listed in Table 3 below.

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.

Of all strains tested, 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 strains, testing was negative once cells were diluted to 10^5 cells/mL. Importantly, the most closely related parasite species, as well as bacterial, viral and fungal species commonly known to cause secondary infections in CL patients, did not yield positive results on CL *Detect*™ regardless of concentration.

Table 3: Cross-reactivity

Bacteria	**Mycobacteria**	**Trypanosoma**
Acinetobacter baumannii	*Mycobacteria abscessus*	*Crithidia fasciculata*
Bacillus cereus	*Mycobacteria fortuitum*	*Trypanosoma cruzi*
Bacillus subtilis	*Mycobacteria marinum*	*Trypanosoma gambiense*
Bacillus thuringiensis	*Mycobacteria tuberculosis* (attenuated)	*Trypanosoma lewisi*
Clostridium perfringens	*Mycobacteria ulcerans*	*Trypanosoma rangeli*
Clostridium sordelli		*Trypanosoma rhodesiense*
Enterobacter aerogenes	**Fungi**	
Enterococcus durans	*Arthroderma benhamiae*	**Viruses**
Enterococcus faecalis	*Cladophialophora carrionii*	Herpes Simplex Virus Type I
Escherichia coli	*Fonsecaea pedrosoi*	Herpes Simplex Virus Type II
Haemophilus influenzae	*Microsporum canis*	Varicella Zoster Virus (Ellen and Isolate D)
Klebsiella oxytoca	*Microsporum gypseum*	
Klebsiella pneumoniae	*Phialophora verrucosa*	**Mammalian cell lines**
Moraxella catarrhalis	*Rhinocladiella compacta*	WI-38
Neisseria lactamica	*Sporothrix schenckii*	MCF-7
Pasteurella multocida	*Trichophyton soudanense*	HeLa
Proteus mirabilis	*Trichophyton tonsurans*	HEK293
Proteus vulgaris		WM-115
Providencia stuartii		U937
Pseudomonas aeruginosa		
Staphylococcus aureus		
Staphylococcus hominis		
Stenotrophomonas maltophilia		
Streptococcus pyogenes		
Streptococcus agalactiae		
Streptococcus sp. ATCC 12392		

Interference Study:

Blood components and topical treatments were evaluated to determine if they have detrimental effects on the CL *Detect*™ Rapid Test kit. Potentially interfering substances tested in this study are listed in the table below. Concentrations in this study are expected to exceed those encountered in customer usage, demonstrating "worst case scenario" of interference likelihood. In addition to the kit's positive and negative controls, a panel of simulated clinical specimens was tested. Lysed *L. major* promastigotes were diluted to generate one negative sample (0 parasite equivalents/µL), and three positive samples (500 parasite equivalents/µL, 50 parasite equivalents/µL, and 25 parasite equivalents/µL, final concentrations after dilution with interfering substances). Final concentration of interfering substances in each sample was 5% in lysis buffer.

Table 4: Interference

Substance	Results
Whole Blood	Interference observed*
Buffy Coat (wound stimulant)	No interference
Plasma	No interference
Betadine (10% povidone-iodine)	Interference observed**
Hydrogen peroxide (3%)	No interference
70% isopropyl alcohol	No interference
Saline	No interference
Mercurochrome (2%)	Interference observed***
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

* 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.

** False positives observed with betadine at >0.0008% final concentration.

*** False negatives observed with mercurochrome at >0.025% final concentration.

Skin lesions should be cleaned and prepared as described in the "Sample Collection" section of this insert. Betadine and mercurochrome should not be used on lesions, as even minute traces can detrimentally impact performance of CL *Detect*™. Small amounts of other topical treatments, blood, or blood components remaining on lesion samples should not impact performance of CL *Detect*™.

Limit of Detection:

The limit of detection of the CL *Detect*™ was estimated by an expert operator on various culture isolates of *Leishmania*. *Leishmania* parasites were serially diluted in simulated matrix and kit lysis buffer and tested with the CL *Detect*™. The estimated limit of detection for each culture isolate (detected at least 95% of the time) is reported in Table 5.

Table 5: Estimated limit of detection

Species	Parasite equivalents per test strip
L. tropica (WR-2995)	187
L. major (WR-2986A)	200
L. donovani (WR-378)	374
L. panamensis (WR-2307)	1080
L. mexicana (WR-2798)	1440
L. braziliensis (WR-2353)	1440

Hook effect:

Purified *Leishmania* peroxidoxin antigen produced reduced intensity test line 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.

Analytical Reactivity:

The analytical reactivity of the CL *Detect*™ was evaluated on various *Leishmania* culture isolates. Table 6 contains a summary of the parasite equivalents per test strip that were detected by the CL *Detect*™ in three out of three replicates.

Table 6: Analytical reactivity

Species	Parasite equivalents per 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)	1440
L. donovani (WR-2712)	626
L. donovani (WR-2801)	626
L. infantum (WR-2719)	626
L. infantum (WR-2455)	1250
L. infantum (WR-2317)	1440
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)	374
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)	1440
L. peruviana (WR-2771)	2500
L. peruviana (WR-2851)	5000

Guide to Symbols (EN 980:2003 / ISO 15223:2000)

IVD In Vitro Diagnostic medical device

REF Catalog number

LOT Batch code

Use by

Temperature limitation

CE CE marking of conformity

EC REP Authorized representative in the European community

Manufacturer

Consult instructions for use

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