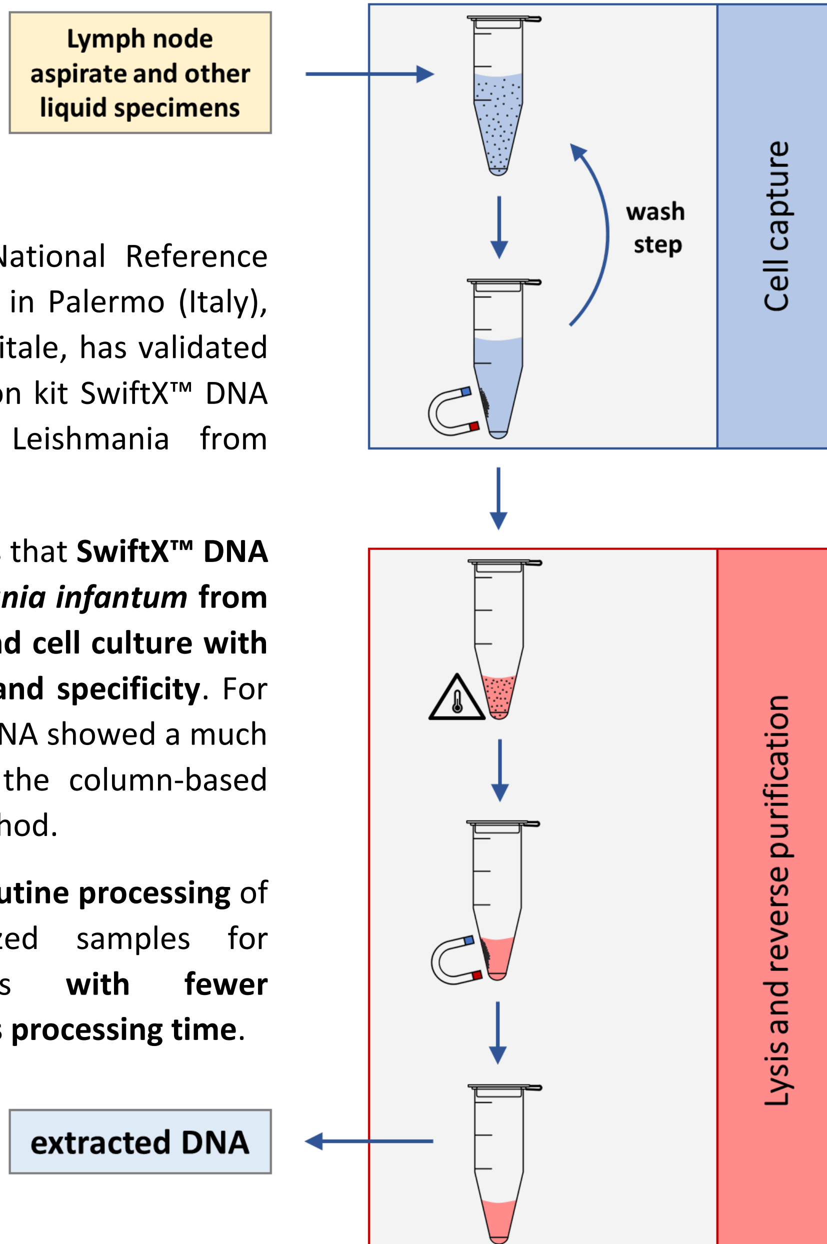
Extraction of Leishmania DNA from lymph node aspirates and cell culture

Related product

This application note and the attached validation report are related to **SwiftX™ DNA** (cat.no. SXD-25).



Summary

The WOAH-associated National Reference Center for Leishmaniasis in Palermo (Italy), headed by Dr. Fabrizio Vitale, has validated the nucleic acid extraction kit SwiftX™ DNA for the detection of Leishmania from different samples.

This report demonstrates that **SwiftX™ DNA extracts DNA of *Leishmania infantum* from lymph node aspirates and cell culture with an excellent sensitivity and specificity**. For most samples, SwiftX™ DNA showed a much higher DNA yield than the column-based reference extraction method.

SwiftX™ DNA enables routine processing of liquid and homogenized samples for Leishmania diagnostics **with fewer processing steps and less processing time**.

Introduction

Leishmaniasis is a Neglected Tropical Disease (NTD) caused by hemoflagellated protozoans of the family *Leishmania*. The pathogens are transmitted through bites of sandflies. Canids such as dogs as well as rodents serve as natural reservoirs. Leishmaniasis is endemic in almost 100 countries in Asia, Africa, South & Central America, and Southern Europe, where about 10 million people are infected. More than 25,000 people die from Leishmaniasis every year.

There are about 20 *Leishmania* species known to cause Leishmaniasis in humans. The most important ones are *L. major*, *L. infantum*, and *L. braziliensis*. The replication of *Leishmania* takes place in macrophages (mononuclear phagocytes). In the human body the protozoa transform from the promastigote stage into the amastigote stage.

Leishmaniasis can distinguished in two major forms: cutaneous leishmaniasis (CL) and visceral leishmaniasis. CL is characterized by skin ulcers developing around the site of the sandfly bite. Ulcers can grow significantly and resembling leprosy lesions and can destroy large skin areas. In later stages, the protozoa invade liver and spleen, which causes fever, anemia, and organ damage - the typical symptoms of visceral leishmaniasis (VL). VL has an extremely high mortality rate if left untreated.

This application note takes reference to the validation performed by the National Reference Center (NRC) for Leishmaniasis in Palermo (Italy), which is also a WOAH (formerly called OIE) laboratory. The data reported by the NRC demonstrate the smooth applicability of SwiftX™ DNA for Leishmaniasis diagnostics and validate the high performance of the cell capturing and reverse purification technology in SwiftX™ kits.

Experimental details

The following samples have been processed and tested:

- Aspirates from lymph nodes of dogs
- *L. infantum* cell culture

Extractions with the SwiftX™ DNA kit were performed essentially as described in Protocol 3 of the handbook:

1. Add 400µL Buffer EN and 30µL Beads A into a microcentrifuge tube and mix
2. Add 200µL liquid sample and mix by vortexing or overhead shaking
3. Incubate at room temperature for 3 minutes
4. Place sample tube in a magnetic stand and let the magnetic particles separate at room temperature for 2 minutes
5. Discard the supernatant
6. Add 500µl Buffer EN, remove the tube from the magnetic stand and resuspend the beads by vortexing for 10 seconds
7. Place sample tube in a magnetic stand for 1 minute
8. Discard the supernatant
9. Add 100µl Buffer DL, remove the tube from the magnetic stand and resuspend the beads by vortexing for 10 seconds
10. Incubate lysis mixture for at 95°C for 10 minutes

11. Remove condensate by shaking down or tapping the tube on the bench
12. Place sample tube in a magnetic stand for 1 minute
13. Use the supernatant for the qPCR reaction

The extractions were performed in duplicates. The extracted DNA was pre-characterized spectrophotometrically using a Qubit instrument. Quantification of the extracted Leishmania DNA was performed using an in-house real-time PCR assay of the laboratory (see Castelli *et al.* (2021) Pathogens 10(7): 865 for further details).

For separation of the magnetic particles, the **magnetic rack (cat.no. MAG-12)** can be used, which is available from Xpedite Diagnostics.

All samples have been previously characterized by DNA extraction using a spin column-based purification kit. The sample input volume was 200µL as well.

Results

The National Reference Center tested 4 *L. infantum*-positive lymph node aspirates, 6 negative lymph node aspirates, and 1 cell culture sample. The real-time PCR data show that the DNA extraction using SwiftX™ DNA led to a correct identification of the *Leishmania* infection status of all specimens leading to perfect concordance with the reference extraction method. Hence the laboratory calculated 100% sensitivity and 100% specificity for SwiftX™ DNA.

The original validation report by the laboratory is provided as an attachment to this application note (scroll down this PDF file).

Table 1 shows a detailed analysis of the PCR data provided by the NRC. The comparison of the Ct values for SwiftX™ DNA extraction and the Ct values from the pre-characterization reveal that the yield of Leishmania DNA extracted with SwiftX DNA is 3- to 60-times higher than with the reference extraction method (silica-spin column method).

Table 1: Summary of Ct values of the real-time PCR analysis as reported in the validation report by the NRC for Leishmaniasis (Palermo, Italy) and comparison with the data from the pre-characterization.

No.	Sample type	Reference extraction		SwiftX™ DNA		ΔCt SwiftX™ DNA
		mean Ct	CV [%]	mean Ct	CV [%]	vs. Reference
1	Positive lymph node aspirate	23.45	2.1%	17.57	0.9%	-5.88
2	Positive lymph node aspirate	26.85	0.6%	22.83	1.4%	-4.02
3	Positive lymph node aspirate	32.67	1.6%	33.51	1.9%	0.84
4	Positive lymph node aspirate	30.23	1.1%	28.62	1.1%	-1.61
5	Negative lymph node aspirate	neg.		neg.		n.a.
6	Negative lymph node aspirate	neg.		neg.		n.a.
7	Negative lymph node aspirate	neg.		neg.		n.a.
8	Negative lymph node aspirate	neg.		neg.		n.a.
9	Negative lymph node aspirate	neg.		neg.		n.a.
10	Negative lymph node aspirate	neg.		neg.		n.a.
11	Positive culture	16.08	0.9%	12.5	3.2%	-3.58

Discussion

The data presented here demonstrate the excellent sensitivity and suitability of SwiftX™ DNA for the detection of *Leishmania* cells in liquid samples. This is enabled by the proprietary technology in SwiftX™ kits:

- the magnetic bead-based capturing of *Leishmania* parasites and host cells from liquid samples leads to exceptionally good recovery of parasite DNA
- the reverse purification mechanism provides efficient removal of inhibitory substances and avoids DNA loss as sometimes observed with silica-based purification methods.

Furthermore, SwiftX™ DNA enables much higher sample input volumes of 1mL or more, which would significantly increase the amount of extracted DNA and, thus, the sensitivity of the diagnosis. Interestingly, this does not require higher amounts of magnetic beads to be used for the extraction. It only requires additional Buffer EN, which is available as an accessory product.

Attachment

Validation Report "RV02/22 Leish" from Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Centro di Referenza Nazionale per le Leishmaniosi, Palermo, Italy

World Organisation
for Animal Health
Founded as OIE

ISTITUTO ZOOPROFILATTICO SPERIMENTALE
DELLA SICILIA "A. MIRRI"

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VALIDATION REPORT *RV02/22 Leish*

LEISHMANIA:

EVALUATION OF **SwiftX™ DNA** EXTRACTION KIT ON MATRICES FOR IDENTIFICATION OF *LEISHMANIA INFANTUM*

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1. Introduction

National Reference Centre for Leishmaniasis, at the request of Dr. Ludovic Ebert, Technical Sales & Marketing, Xpedite Diagnostics GmbH carried out the evaluation of the new extraction technique, called reverse purification of SwiftX^{TM} DNA EXTRACTION KIT.

2. Materials and Methods

2.1 Samples

A panel of 11 samples from different biological matrices included: lymph node aspirates and culture extracts of Leishmania infantum at a concentration of 1000000 Leishmania/ml.
All samples analysed are field matrices that have already been tested for extraction, using commercially kits (with columns) and real time PCR at the National Reference Centre for Leishmaniasis. The concentration of the extracted DNA was determined spectrophotometrically using Qbit®, while the real time PCR test was performed following the protocol used by CRENAL (Castelli G, Bruno F, Reale S, Catanzaro S, Valenza V, Vitale F. Molecular Diagnosis of Leishmaniasis: Quantification of Parasite Load by a Real-Time PCR Assay with High Sensitivity. Pathogens. 2021 Jul 9;10(7):865. doi: 10.3390/pathogens10070865. PMID: 34358015; PMCID: PMC8308825.)

Sample	Matrix	C.Re.Na.L. Result
1	**lymph node aspirates**	**P**
2	**lymph node aspirates**	**P**
3	**lymph node aspirates**	**P**
4	**lymph node aspirates**	**P**
5	**lymph node aspirates**	**N**
6	**lymph node aspirates**	**N**
7	**lymph node aspirates**	**N**
8	**lymph node aspirates**	**N**
9	**lymph node aspirates**	**N**
10	**lymph node aspirates**	**N**
11	**Cell culture**	**P**

Legend	
N	Leishmania negative
P	Leishmania positive

3. Statistical analysis

For the purpose of statistical analysis, the results of the testing of the individual samples were classified as correct when conform to expected result.
For the evaluation of **accuracy** were considered two categories of results: "Negative" and "Positive";
The accuracy was evaluated through the calculation of the accuracy (proportion of correct outcomes) and the sensitivity and specificity values obtained by each laboratory. While the degree of agreement between laboratories (concordance) was evaluated by the Cohen index K.
All calculations were performed with the WinEpiscope program.

4. Confidentiality

To ensure the confidentiality of data, operators were identified by a code (RV 02/22 Leish), which was used for all communications regarding the operators themselves.
The data are used by the validation organizer only for the analysis and evaluation of results.

5. Results

The concentration of the extracted DNA was determined spectrophotometrically (Qbit®)
The following table shows the DNA quantification results of the extraction provided by the participating kit:

Sample	ng/µl (SwiftX™)	260/280 ratio (SwiftX™)	260/230 ratio (SwiftX™)
1	8,88	0,63	1,34
2	4,88	0,56	1,5
3	3,04	0,54	1,27
4	7,30	0,59	1
5	7,48	0,58	2,81
6	0,408	0,54	1,26
7	0,616	0,53	1,14
8	0,382	0,53	1,43
9	14,1	0,54	1,02
10	20	0,71	1,27
11	31,6	0,82	2,55

The DNA obtained was used for the real time PCR assay. Each sample was evaluated in duplicate. Below are the Ct values measured for each sample

Sample	Ct Plate 1 (kit SwiftX™)	Ct Plate 2 (kit SwiftX™)
1	17,68	17,46
2	23,05	22,61
3	33,05	33,97
4	28,85	28,39
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	12,22	12,78

5.1 Results presentation

The results were received by all the operators involved.
The following table shows the results provided by the operators of C.Re.Na.L. and expected results.

Samples	Expected result (C.Re.Na.L.)	SwiftXTM
1	**P**	**P**
2	**P**	**P**
3	**P**	**P**
4	**P**	**P**
5	**N**	**N**
6	**N**	**N**
7	**N**	**N**
8	**N**	**N**
9	**N**	**N**
10	**N**	**N**
11	**P**	**P**

5.2 Accuracy

The following table shows the values of sensitivity, specificity and accuracy obtained by the different participating laboratories.
By analyzing the results provided by each laboratory is highlighted:
- the sensitivity is high for participating laboratory (0.96 threshold value, OIE 2014 Manual Chapter 3.1.11. — Leishmaniosis).
- the specificity is average for participating laboratory (0.98 threshold value, OIE 2014 Manual Chapter 3.1.11. — Leishmaniosis).

Comparing the results obtained with those reported in the literature, sensitivity and specificity values are fully fall within expected parameters.
The acceptance criterion is established when the value of accuracy is ≥ 0,90

Lab	P/P expected	Se	N/N expected	Sp	Accuracy
SwiftXTM DNA EXTRACTION KIT.	5/5	**1,00**	6/6	**1,00**	**1,00**
Totals	**5/5**	**1,00**	**6/6**	**1,00**	**1,00**

5.3 Concordance

To assess the degree of concordance in terms of reproducibility was carried out to calculate the index K among the participating laboratories.
The degree of agreement between C.Re.Na.L. and SwiftXTM DNA EXTRACTION KIT can be considered **very good** according to the Altman classification (0,61 – 0,80).

Operators	Cohen K
C.Re.Na.L. / SwiftXTM DNA EXTRACTION KIT.	**1**

The acceptance criterion is established when the value of K is ≥ 0,61 and then falls in the categories good or very good.

K value	Accordance
< 0,20	insufficient
0,21 – 0,40	mediocre
0,41 – 0,60	average
0,61 – 0,80	good
0,81 - 1	**very good**

6. Conclusions

The data reported confirm that the method provides DNA of suitable quantity and quality for subsequent PCR based detection applications. The method is therefore considered fit for the intended purpose.

By all operators it was confirmed the high sensitivity (with values of 1.00), high specificity of the test (with values of 1.00) and high accuracy with the value of 0,96.

The RV02/22 Leish can be considered successful since exceeded the minimum acceptance criteria for the accuracy (≥ 0.95) and concordance (≥ 0.61).

Palermo, 11/11/2022

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