EN

SVLTest, Standard for Lab Verification of the Test Legipid® Legionella Fast Detection

Catalogue reference:

311-30-SV

biotica®
FAST DETECTION FOR LIFE

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I. INTRODUCTION

SVLTest, *Standard for Lab Verification of the Test Legipid® Legionella Fast Detection* (Ref. No. 311-30-SV) it's a simple and fast system for the lab to verify **the Legipid ®*Legionella Fast Detection*** test. The standard UNE-EN ISO/IEC 17025, 2017, indicates the need to "use appropriate methods and procedures for all laboratory activities" and, to do so, says that "the laboratory must verify that it can properly carry out the methods before using them, ensuring that they can achieve the required performance". The standard UNE-EN ISO 16140-1 defines the validation of a method as the "establishment of the behavioral characteristics of a method and the provision of objective evidence, demonstrating that the behavioral requirements for intended use are met". On the other hand, the UNE-EN ISO 16140-2 standard adds as an objective that validation studies "be developed by organizations dedicated to the validation of methods". The Legipid® test is a method already validated by the competent international organization AOAC-RI and it is up to the user to provide "objective evidence" that it fits the "intended use".

To carry out this verification, a protocol is proposed that applies the specific criteria of accreditation bodies belonging the European Co-operation for Accreditation Association, which allows to confirm the operational characteristics, at least, recovery and reproducibility. These parameters evaluate both the technical capability of a laboratory with the method and its robustness.

SVLTest allows you to prepare a series of standard 9 mL samples, at three different levels of **Log_{10} UFCeq**,with n-replies (up to 10) per level. In addition, it allows to inoculate those same levels to samples of the selected matrices, which are concentrated by filtration. The laboratory records the relative **absorbances** obtained for each concentration, when applying the **Legipid®** test to the samples, both in the direct samples as in the filtered ones.

The data is entered in a spreadsheet that calculates the parameters of reproducibility, recovery and uncertainties.

II. KIT REAGENTS AND COMPONENTS

Reference **311-30-SV** contains 10 vials per level (high, medium, low) with a volume of 0. 6 mL/vial of a frozen suspension of a known concentration of *Legionella pneumophila* sg 1 **Log_{10} UFCeq** and one L0 bottle with 300ml.

III. EXPIRY AND STORAGE

Once received, the vials should be immediately stored at -20ºC and the L0 bottle at 5±3°C. The expiry of the material, properly stored, is **3 months** from the date of supply. Each set of vials per level is labeled with its lot number and storage conditions. In addition, the protocol includes code, lot number and expiration, so traceability of all vials is guaranteed.

IV. MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes of 100-1000µl.

V. CAUTIONS AND RECOMMENDATIONS

- The vial should be immediately kept in freezing at reception.
- Defrost and temper the vial to be used. Once defrosted and tempered, use the content as described in VI. Protocol, and don't save any remnants.

VI. PROTOCOL

Before you start:

Take the exact number of vials you need out of the freezer for each level or range. As many as replicas per level you will use. Allow defrosting and tempering at room temperature, before preparing standard samples and analyzing them with the Legipid® *Legionella* Fast Detection kit.

Procedure:

Preparation of direct samples:

1. Defrost and temper n-vials per level, as many as replicas will use (up to a maximum of 10).
2. For each level and n-vials, **take 0.5 mL** of each vial and add it to each n-cuvettes of the Legipid ®.
3. Bring all cuvettes to a final volume of 9 mL with L0 (i.e. add 8.5 mL of L0).

Preparación de muestras concentradas:

1. Defrost and temper n-vials per level, as many as replicas you're going to use (up to a maximum of 10)
2. **Take 0.5 mL** of one vial and inoculate in 1000 ml of matrix, with n-replicates for each level of contamination and matrix. Homogenice for gentle and repeated inversions.
3. Filter each inoculated sample and elute the filter according to the Legipid® test protocol.

Analysis:

4. Perform Legipid® test on all samples (use negative control per batch)
5. Record the value of the **absolute absorbance** of negative control
6. Record the **relative absorbance values (with respect to negative control) of samples**

Interpretation:

The spreadsheet allows you to enter the reads of the replicas for **a matrix and a level.** Replicate the sheet to your file as many times as you need based on the number of matrices and levels. In the sheet header you can note the method, matrix, range or level, and microorganism.

For a given matrix and level, enter in the spreadsheet:

- In the column titled STEP 1: STRAIN TITRATION
  - The **recorded absolute absorbance** reading of negative control.
  - Calculate and enter **relative absorbance** readings of **the replicas of the direct samples**.
- In the column titled STEP 2: INOCULATED MATRIX (*)
  - The **recorded absolute absorbance** reading of negative control.
  - Calculate and enter relative **absorbance** readings **of replicas of** concentrated **samples**.

(*) We do not recommend the nylon filter because of its low recovery compared to other types of filter.

The sheet will return in CALCULATION:

- The **concentration values (UFCeq and Log_{10}UFCeq)** for each replica.
- **Statistics (mean and deviation)** for both titration and matrix level or range.
- **Uncertainty** and its components.
- **Precision** and **recovery**.

METHOD:									
MATRIX:									
LEVEL:									
MICROORGANISM:									
PART DOCUMENTARY									
PACKAGE INSERT	Introduction Technology of the test Legipid@ Reagents and components of the test Expiry and Storage Cautions and recommendations Protocol Reference certification (AOAC)			VALIDATION	Certification AOAC Scientific papers				
PART EXPERIMENTAL									
PRINCIPLE	Verify the technical competence of the user to perform the test with the Legipid test			MATERIAL	SCV Standard tubes, elution bottles, membrane filters, vacuum system, pipettes				
PROCEDURE									
STEP 1: STRAIN TITRATION					**STEP 2**: INOCULATED MATRIX				
Enter negative control (u.a.)=					Enter negative control (u.a.)=				
Replies	relative ABS	absolute ABS	CFUeq	Log_{10} CFUeq	Technician	Replies	relative ABS	absolute ABS	CFUeq	Log_{10} CFUeq
1		Add negative control			Alvaro	1		Add negative control		
2		Add negative control				2		Add negative control		
3		Add negative control				3		Add negative control		
4		Add negative control				4		Add negative control		
5		Add negative control				5		Add negative control		
6		Add negative control				6		Add negative control		
7		Add negative control				7		Add negative control		
8		Add negative control				8		Add negative control		
9		Add negative control				9		Add negative control		
10		Add negative control				10		Add negative control		
CALCULATION					Enter negative control (u.a.)=					
Titration statistics			**Mean Xr =**		Technician	Replies	relative ABS	absolute ABS	CFUeq	Log_{10} CFUeq
			Deviation Sr=		Vicky	1		Add negative control		
			n=	0		2		Add negative control		
Matrix/Level statistics			**Mean XR =**			3		Add negative control		
			Deviation SR =			4		Add negative control		
			n=	0		5		Add negative control		
COMPONENT	**FORMULA**	**RESULT**				6		Add negative control		
STRAIN UNCERTAINTY (STEP 1)	$I_{cepa} = \frac{Sr}{\sqrt{n}}$	Istrain =				7		Add negative control		
REPRODUCIBILITY UNCERTAINTY	$I_{rep} = \frac{SR}{\sqrt{n}}$	I rep =				8		Add negative control		
ROUTINE UNCERTAINTY	$I_{rut} = \frac{SR}{\sqrt{n}}$	Irout =				9		Add negative control		
RECOVERY UNCERTAINTY	$I_{rec} = \frac{\lvert XR - Xr \rvert}{\sqrt{3}}$	Irec =				10		Add negative control		
MEASUREMENT UNCERTAINTY	$I_{medida} = \sqrt{I_{cepa}^2 + I_{rep}^2 + I_{rut}^2 + I_{rec}^2}$	Imeasurement =	0,000		Enter negative control (u.a.)=					
EXPANDED UNCERTAINTY	$I_{exp}(log) = 2 \times I_{medida}$	Iexp =	0,000		Technician	Replies	relative ABS	absolute ABS	CFUeq	Log_{10} CFUeq
PRECISION (log), P (%)	P = (SR/XR) * 100	P (%) =				1		Add negative control		
RECOVERY, R (%)	R = (XR/Xr) * 100	R (%) =				2		Add negative control		
						3		Add negative control		
						4		Add negative control		
						5		Add negative control		
						6		Add negative control		
						7		Add negative control		
						8		Add negative control		
						9		Add negative control		
						10		Add negative control		

lot number: _____

Concentration (magnitude order, CFU_{eq}):

- **Level 1:** _____
- **Level 2:** _____
- **Level 3:** _____

Expiration from date of supply:

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For **technical assistance** please contact:
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Note to the user: Use this product for internal testing only

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