

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

Catalogue reference:

311-30-SV

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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 **Log₁₀ 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 **Log₁₀ UFCeq** and one L0 bottle with 300ml.

III. EXPIRY AND STORAGE

Once received, the vials should be immediately stored at -20°C and the L0 bottle at ± 5°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. Homogenize 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 Log₁₀UFCEq)** 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		
Matrix/Level statistics			n =	0		2		Add negative control		
			Mean XR =			3		Add negative control		
			Deviation SR =			4		Add negative control		
			n =	0		5		Add negative control		
						6		Add negative control		
						7		Add negative control		
						8		Add negative control		
						9		Add negative control		
						10		Add negative control		
COMPONENT	FORMULA	RESULT		Enter negative control (u.a.)=						
STRAIN UNCERTAINTY (STEP 1)	$t_{exp} = \frac{Sr}{\sqrt{n}}$	Istrain =		Technician	Replies	relative ABS	absolute ABS	CFUeq	Log ₁₀ CFUeq	
REPRODUCIBILITY UNCERTAINTY	$t_{rep} = \frac{SR}{\sqrt{n}}$	I rep =			1		Add negative control			
ROUTINE UNCERTAINTY	$t_{rut} = \frac{SR}{\sqrt{n}}$	I rout =			2		Add negative control			
RECOVERY UNCERTAINTY	$t_{rec} = \frac{ XR - Xr }{\sqrt{\beta}}$	I rec =			3		Add negative control			
MEASUREMENT UNCERTAINTY	$t_{meas} = \sqrt{t_{exp}^2 + t_{rep}^2 + t_{rut}^2 + t_{rec}^2}$	I measurement =	0,000		4		Add negative control			
EXPANDED UNCERTAINTY	$t_{exp(99)} = 2 \times t_{meas}$	I exp =	0,000		5		Add negative control			
PRECISION (log), P (%)	$P = (SR/XR) * 100$	P (%) =			6		Add negative control			
RECOVERY, R (%)	$R = (XR/Xr) * 100$	R (%) =			7		Add negative control			
					8		Add negative control			
					9		Add negative control			
					10		Add negative control			

<p>lot number: <input style="width: 100px; height: 20px;" type="text"/></p> <p>Concentration (magnitude order, CFU_{eq}):</p> <p><input type="radio"/> Level 1: <input style="width: 100px; height: 20px;" type="text"/></p> <p><input type="radio"/> Level 2: <input style="width: 100px; height: 20px;" type="text"/></p> <p><input type="radio"/> Level 3: <input style="width: 100px; height: 20px;" type="text"/></p> <p>Expiration from date of supply: __/__/__</p>	<p>For technical assistance please contact: Biótica, Bioquímica Analítica, S.L. Parque Científico y Tecnológico, Universidad Jaime I Edificio Espatec 2, planta baja, lab 2 E12071 – Castellón, España www.biotica.es info@biotica.es Tel.: +34 964108131 Fax: +34 964737790</p>	 <p>biotica[®] FAST DETECTION FOR LIFE</p>
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Note to the user: Use this product for internal testing only