INTRODUCTION:

VIRAPID® LEGIONELLA CULTURE is an immunoassay for the qualitative detection of Legionella genus, Legionella pneumophila serogroup 1 and Legionella pneumophila serogroups 1-15 in samples from bacterial cultures obtained from human respiratory or environmental samples. 25 tests.

PRINCIPLE OF THE TEST:

Bacteria are resuspended in a medium to extract their wall components. When the extracted sample is added into each well of the device, the colloidal gold conjugate is solubilized and a first reaction between the bacterial components and the corresponding Mab coupled to the gold particles takes place. These complexes move along the membrane and reach the corresponding test line, where the complementary Mab, immobilized on the membrane, anchor these complexes, rendering a red line. Each strip contains a final control line for the validation of the assay, able to react even if the sample is negative.

KIT CONTENTS:

- VIRCELL LEGIONELLA CULTURE MEDIUM: 6 vials of a lyophilized buffered solution, containing sodium azide.
- VIRCELL LEGIONELLA POSITIVE CONTROL: 1 dessicated suspension of inactivated Legionella pneumophila serogroup 1.
- VIRCELL LEGIONELLA NEGATIVE CONTROL: 1 dessicated suspension of inactivated Pseudomonas aeruginosa.

Store at 2-30°C and check expiration date.

Materials required but not supplied:
- Thermoblock (optional)
- Automatic micropipette and the corresponding tips
- Timer
- Vortex

STORAGE REQUIREMENTS:

Store at room temperature or refrigerated, 2-30°C. DO NOT FREEZE.

Do not use the kit reagents beyond the expiry date. This will be valid only if reagents are capped and stored at 2-30°C.

VR002: Immunochromatographic test for the qualitative detection of Legionella genus, Legionella pneumophila serogroup 1 and Legionella pneumophila serogroups 1-15 in samples from bacterial cultures obtained from human respiratory or environmental samples. 25 tests.

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FOR IN VITRO DIAGNOSTIC USE

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STABILITY OF REAGENTS ONCE OPENED:

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>STABILITY</th>
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<tbody>
<tr>
<td>REconstituted VIRCELL CULTURE MEDIUM</td>
<td>Store at 2-8°C for 2 months</td>
</tr>
<tr>
<td>VIRCELL POSITIVE CONTROL and VIRCELL NEGATIVE CONTROL</td>
<td>Store at 2-8°C for 2 months</td>
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STABILITY AND HANDLING OF REAGENTS:
The kit is stable until the expiration date at 2-30°C. Handle reagents in aseptic conditions to avoid microbial contaminations.

VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:
1. For in vitro diagnostic use only. For professional use only.
2. Use kit components only. Do not exchange VIRCELL CASSETTES, VIRCELL MEDIUM, VIRCELL POSITIVE CONTROL and VIRCELL NEGATIVE CONTROL between lots and kits.
3. Specimens should be handled as in the case of infectious samples using safety laboratory procedures.
4. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens. Wash hands thoroughly after manipulating samples.
5. Do not use the kit after expiration date.
6. Dispose of unused reagents and waste in accordance with all applicable regulations.
7. Reagents in this kit could include substances of animal and/or human origin. Although that material is not infectious, it should be handled as potentially infectious. All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal.
8. The VIRCELL MEDIUM contains sodium azide. Sodium azide may react with metal plumbing, forming explosive components. Upon disposal, flush with plenty of water. Observe the local regulations for chemical waste disposal.
9. If the kit or its components are stored in the refrigerator, please bring them at room temperature before use.
10. The cassettes are stable in their closed pouch until the expiry date indicated on the label. Do not open until you are ready to perform a test.
11. Several tests can be performed at the same time.
12. Adequate resuspension of a sufficient amount of bacterial culture in the included medium is required for a correct performance of the test.

PRELIMINARY PREPARATION OF THE REAGENTS:

Reconstitute the amount of vials of VIRCELL MEDIUM needed for the assay with 1 ml of distilled water each one. For each sample, dispense 200 µl of reconstituted medium in microtubes of 0.5 to 1.5 ml.

Reconstitute VIRCELL POSITIVE CONTROL and VIRCELL NEGATIVE CONTROL when they are going to be used, with 200 µl of VIRCELL MEDIUM. Shake with a vortex until a complete homogenization.

SPECIMEN COLLECTION AND HANDLING:
Select two typical colonies (1-3 mm) from the culture plate, resuspend them in 200 µl of reconstituted medium, and shake with a vortex. Any of the following two alternative methods can be chosen to process and assay the sample. In the hot method, the tube is incubated for 10 minutes in a thermoblock at 95°C. In the cold method, the tube is incubated for 5 minutes at room temperature.

TEST PROCEDURE:
Both methods (hot and cool) have the same procedure except for the steps 2 and 6.
1. Bring all reagents to room temperature before use (approximately 1 hour), without removing the cassettes from the pouches.
2. For the hot method, allow the treated sample to cool at room temperature for approximately 5 minutes (not more than 10 minutes) before addition to the cassette.
3. Open the pouch and put the cassette on a flat surface.
4. Homogenize the treated sample using a vortex.
5. Add 90 µl of the sample into each well with an automatic micropipette, always beginning with the L.GENUS test.
6. The result must be read after 15 to 20 minutes for the hot method or after 30 minutes for the cold method.

Discard any reading made after 60 minutes.

INTERNAL QUALITY CONTROL:
Each batch is subjected to internal quality control testing before releasing, complying with highly strict specifications. Final quality control results for each particular lot are available. Positive and negative controls are included to check the correct performance of the kit in your laboratory. In order to use them, after their reconstitution (see “Preliminary preparation of the reagents”), shake with a vortex and then incubate in a thermoblock at 95°C for 10 minutes (hot method) or incubate for 5 minutes at room temperature (cold method). The assay has to be performed as indicated in “Test Procedure”.

INTERPRETATION OF RESULTS AND VALIDATION PROTOCOL FOR USERS:
In order to perform the reading of the test, the intensity card included in the kit should be used. 4 levels of color intensity ranging from 0.5 to 3 can be read. When the intensity is lower than 0.5, the result is considered negative. When the intensity is higher than or equal to 0.5, the result is considered positive. If the sample contains Legionella bacteria, the corresponding test lines will show a positive reaction. A culture is considered positive if one or several test lines plus the corresponding control lines react.

The interpretation of the results is carried out according to the following chart and table:

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sp specimens from a Spanish Hospital.

comprising 8 colonies from 64 water samples received in an accredited water analysis laboratory for microorganisms may be present in the sample. A negative result may correspond to the incubation steps are essential for accurate results. The test was checked with the following bacterial clinical isolates: 

Pseudomonas aeruginosa, Pseudomonas sp (8 isolates), Salmonella sp, Proteus mirabilis, Xantomomas sp, Aeromonas sp, Enterobacter aerogenes Staphylococcus aureus (2 isolates), Escherichia coli, Serratia sp, Staphylococcus epidermidis, Bacillus cereus, Klebsiella sp, Citrobacter sp, Acinetobacter sp, Haemophilus sp, Streptococcus sp (2 isolates) and Streptococcus viridians, all specimens coming from a Spanish Hospital. No false positive results were obtained.

LIMITATIONS:

1.-This test has been verified to be used with bacterial cultures obtained from human respiratory or environmental samples. This test has not been verified with other types of samples.

2.-The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful handling, timing and temperature control of the incubation steps are essential for accurate results.

3.-This test has not been designed nor evaluated to identify whole bacteria or bacterial components directly from biological samples such as urine, respiratory secretions or water samples.

4.-As with any diagnostic test, results must be interpreted with consideration of all clinical and laboratory findings. The kit results may be used in conjunction with clinical evaluation and other diagnostic procedures.

5.-The test provides qualitative results.

6.-Reliable results are dependent on adequate specimen collection, transport, storage, processing and culturing procedures.

7.-A negative result does not exclude the possibility of a Legionella strain present in the sample. A negative result may correspond to the microorganism present in the sample at a concentration below the sensitivity limit of the test.

8.-Although the test has been evaluated with a large number of Legionella isolates, it cannot be excluded that some Legionella strains present antigenic differences, making them unreactive with the test.

9.-A positive test does not rule out the possibility that other microorganisms may be present in the sample.

PERFORMANCE

SENSITIVITY AND SPECIFICITY:

Three groups of samples have been analyzed to evaluate the test performance: Group I) Bacteria strains from reference collections (NCTC, DSMZ, ATCC) and 13 non-clinical Legionella; Group II) 141 isolated colonies from 64 water samples received in an accredited water analysis laboratory for Legionella testing; and Group III) a group of clinical isolates comprising 8 Pseudomonas sp, 1 Acinetobacter sp, 1 Staphylococcus aureus, 1 Streptococcus viridians, 1 Haemophilus sp and 2 Streptococcus sp specimens from a Spanish Hospital.

The following results were obtained:

INTRA-ASSAY PRECISION:

4 samples (one positive close to the detection limit for each test line and one negative) were tested 5 times in a single assay performed by the same operator in essentially unchanged conditions. Equivalent results were observed in all the assays.

INTER-ASSAY PRECISION:

4 samples (one positive close to the detection limit for each test line and one negative) were individually tested on 3 consecutive days by 2 different operators. Equivalent results were observed in all the assays.

CROSS REACTIVITY AND INTERFERENCES:

The test was checked with the following bacterial clinical isolates: Pseudomonas aeruginosa, Pseudomonas sp (8 isolates), Salmonella sp, Proteus mirabilis, Xantomomas sp, Aeromonas sp, Enterobacter aerogenes Staphylococcus aureus (2 isolates), Escherichia coli, Serratia sp, Staphylococcus epidermidis, Bacillus cereus, Klebsiella sp, Citrobacter sp, Acinetobacter sp, Haemophilus sp, Streptococcus sp (2 isolates) and Streptococcus viridians, all specimens coming from a Spanish Hospital. No false positive results were obtained.

SYMBOLS USED IN LABELS:

BIBLIOGRAPHY:


For any question please contact:
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