INTRODUCTION:

Tularemia is a zoonotic infection caused by Francisella tularensis. F. tularensis is considered to have four subspecies, of which the tularensis subspecies or type A, predominantly present in North America, is the most virulent subspecies in both animals and humans. Type B is found in Europe and Asia and causes a less virulent form of the disease. The bacterium circulates in populations of rodents and lagomorphs, and outbreaks in humans often parallel outbreaks in animal populations. The incubation period is 3–5 days normally (range 1–21 days). Most cases of naturally occurring tularemia are ulceroglandular disease, involving an ulcer at the inoculation site and regional lymphadenopathy. Lymphadenopathy can take a significant period to resolve, even with treatment. Other presentations (oculoglandular and oropharyngeal disease) may occur. Occasionally patients with tularemia present with a nonspecific febrile systemic illness (typhoidal tularemia) without evidence of a primary inoculation site. Pulmonary disease can occur naturally (pneumonic tularemia), but is uncommon. Tularemia can be transmitted by direct contact with infected animals or their tissues, ingestion of undercooked infected meat or contaminated water, animal bites or scratches, arthropod bites, and inhalation of an aerosol or contaminated dust. Tularemia responds well to antibiotic therapy and the mortality rate of the more acute forms of the disease is reduced significantly if the patient receives suitable antibiotics.

The diagnosis of tularemia by culture can be challenging because the organism grows poorly on routine culture medium. The low sensitivity of culture methods together with the lack of standardization in PCR methodology for the direct identification of the pathogen make serological assay the most widely used tool for the diagnosis of tularemia. Levels of antibody may be measurable within 1 week after infection, although significant levels usually appear in 2 weeks. Antibody levels against F. tularensis can remain detectable for years.

PRINCIPLE OF THE TEST:

When the sample is added into the well of the cassette, the colloidal gold is solubilized and the first immunological reaction between the specific antibodies of the serum/plasma and the protein coupled to the gold particles takes place. These complexes move along the membrane to the reaction line (test line), and a colored band will appear if the analyte to be detected is present in the sample. Each strip contains a control line for the validation of the assay. This line has to appear always even if the sample is negative.

STABILITY OF REAGENTS ONCE OPENED:

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.

STORAGE REQUIREMENTS:

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

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.

RECOMMENDATIONS AND PRECAUTIONS:

1. For in vitro diagnostic use only. For professional use only.
2. Use kit components only. Do not exchange VIRCELL CASSETTES and VIRCELL DEVELOPER SOLUTION 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 genetic material or 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 developer solution contains sodium azide. Sodium azide may react with metal plumbing, forming explosive components. Upon disposal, flush with plenty of water.

9. If the kit or its components (cassettes or developer solution) 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 in the label. Do not open until you are ready to perform a test.

11. Several tests can be performed at the same time.

12. Do not let that the tip of the developer solution bottle touch the sample well in order to prevent contaminations.

13. A good performance of the test depends on the correct size of the drops of developer solution. For this purpose, push the dropper smoothly, allowing the air to pass through into the bottle between each two samples.

14. Avoid the use of samples subjected to repeated freezing-thawing cycles as well as hemolized samples. Both can produce erroneous results (signal decrease) or reading failures (lack of visibility).

**SPECIMEN COLLECTION AND HANDLING:**

Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum samples are to be refrigerated (2-8°C) upon collection or frozen (-20°C) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolized or contaminated sera. Samples containing particles should be clarified by centrifugation.

**PRELIMINARY PREPARATION OF THE REAGENTS:**

All reagents supplied are ready to use.

**TEST PROCEDURE:**

1. Bring all reagents to room temperature before use (approximately 1 hour), without removing the cassettes from the pouches.

2. Open the pouch and put the cassette on a flat surface.

3. Add 20 µl of sample into the well with an automatic micropipette. Let the droplet be absorbed.

4. Add 2 drops of developer solution onto the well. Do not wait more than 5 minutes after the sample addition.

5. The result must be read after 15 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.

**INTERPRETATION OF RESULTS AND VALIDATION PROTOCOL FOR USERS:**

In order to perform the reading of the test and to determine the positivity of the samples, 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 antibodies against Francisella tularensis, a coloured line will appears in the corresponding place. The control line must be always positive and legible if the test has been performed correctly. If this line does not appear, the test must be considered invalid.

**LIMITATIONS:**

1. This kit is intended to be used with human plasma/serum.

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.

3. This test will not indicate the site of infection. It is not intended to replace isolation.

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. No correlation can be drawn between the magnitude of a positive result and the titer of antibodies in the sample.

6. This test has been verified to be used with human plasma/serum. This test has not been verified with other kinds of samples.

7. Reliable results are dependent on adequate specimen collection, transport, storage and processing procedures.

8. A negative result does not exclude the possibility of infection.

9. A positive test does not rule out the possibility that other pathogens may be implicated in the disease.

10. The kit has not been evaluated to follow up the disease after a treatment.

**PERFORMANCE:**

**SENSITIVITY AND SPECIFICITY:**

256 samples were assayed against an available microagglutination kit. The results were as follows:

<table>
<thead>
<tr>
<th>Line TULAREMIA</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>99.13</td>
<td>98.58</td>
</tr>
</tbody>
</table>

**FOR IN VITRO DIAGNOSTIC USE**

Manufacturer: VIRCELL, S.L. Pza. Domínguez Ortiz 1. Polígono Industrial Dos de Octubre. 18320 Santa Fe *GRANADA* SPAIN *Tel.+34.958.441264* Fax+34.958.510712

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INTRA-ASSAY PRECISION:
2 samples (one positive close to the detection limit and one negative) were tested 5 times in a single assay performed by the same operator in essentially unchanged conditions.
The same results were observed in all the assays.

INTER-ASSAY PRECISION:
2 samples (one positive close to the detection limit and one negative) were individually tested on 3 consecutive days by 2 different operators.
The same results were observed in all the assays.

CROSS REACTIVITY AND INTERFERENCES:
8 samples known to be positive for other specimens (cytomegalovirus, Toxoplasma gondii and Epstein-Barr virus) were assayed. 8 samples known to be positive for rheumatoid factor were assayed.
The negative results of the test demonstrated the specific reaction of the kit with no cross reaction or interferences with the referred specimens.

SYMBOLS USED IN LABELS:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVD</td>
<td>In vitro diagnostic medical device</td>
</tr>
<tr>
<td>Y°C</td>
<td>Store at X-Y°C</td>
</tr>
<tr>
<td>≤</td>
<td>Contains sufficient for &lt;X&gt; tests</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch code</td>
</tr>
<tr>
<td>REF</td>
<td>Catalogue number</td>
</tr>
<tr>
<td>€</td>
<td>Consult instructions for use</td>
</tr>
<tr>
<td>S(-)</td>
<td>Negative result</td>
</tr>
<tr>
<td>S(*) LOW</td>
<td>Positive result: Low intensity</td>
</tr>
<tr>
<td>S(*) HIGH</td>
<td>Positive result: High intensity</td>
</tr>
<tr>
<td>VRIC</td>
<td>VIRAPID® interpretation chart</td>
</tr>
</tbody>
</table>

SUMMARY OF THE ASSAY PROCEDURE:
Add 20 µl of sample into each well
Add 2 drops of Developer solution
Reading results

BIBLIOGRAPHY:

For any question please contact: customerservice@vircell.com

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