Instructions for use. In-vitro diagnostic product. For Swine.

*Lawsonia* FIRSTtest™
for the detection of *Lawsonia intracellularis* in porcine feces

Single use field test kit

Catalog number: 609030

An ELISA-based colorimetric test
for the detection of *Lawsonia intracellularis* in porcine feces
Intended use

*Lawsonia* FIRSTTest™ is a qualitative single-use test designed to be used on swine farms or in veterinary clinics – “in the field” -for the detection of *Lawsonia intracellularis* in porcine feces.

Test description

The Enzyme-Linked ImmunoSorbent Assay (ELISA)-based FIRSTtest™ detects the *Lawsonia* bacterium after isolation from feces with the help of magnetic particles (beads). Polyclonal antibodies that are directed against the *Lawsonia* antigen (*Lawsonia p-Ab*) were bound, on the one hand, to magnetic beads used in the capture step and, on the other hand, to the enzyme peroxidase (POD), which is used in the detection step.

Figure 1 provides a schematic description of the test. First, the diluted porcine feces are placed into the collection tube containing the magnetic beads coated with *Lawsonia*-pAb which captures the Lawsonia. Following capture of the *Lawsonia intracellularis* from the porcine feces, the beads are held against the wall of the collection tube with the help of a magnet and then the supernatant is carefully discarded. Two wash cycles following the same procedure are used to remove other fecal components. The Lawsonia-pAb conjugated to POD is added to the isolated beads. The beads are held again with the magnet to the wall of the collection tube and the supernatant is discarded. Next, to remove the unbound POD conjugate, the beads are washed twice. Finally, the detection solution is added to the complex, which leads to color change. A blue solution indicates a LI-positive result while a colorless solution is a sign of a LI-negative result. The test contains positive controls to assist the user in determining color change and kit performance.
Figure 1. FIRSTtest™ principle. SA = streptavidin; LI = Lawsonia intracellularis; POD = peroxidase; pAb = polyclonal antibody.

Kit Components (for 20 tests)

Table 1: Overview of kit components

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tubes for sample material (collection tube)</td>
<td>20 tubes</td>
</tr>
<tr>
<td>2</td>
<td>Spatulas for collection of sample material</td>
<td>20 spatulas</td>
</tr>
<tr>
<td>3</td>
<td>Assay tubes containing magnetic beads coated with Streptavidin and biotinylated Lawsonia-pAb (2 μg Lawsonia-pAb per tube). Preservative: 0.09% sodium azide</td>
<td>24 tubes (20 sample tubes, 2 positive and 2 negative control)</td>
</tr>
<tr>
<td>4</td>
<td>Lawsonia-pAb POD conjugate, lyophilized. 7 mL after reconstitution (20.34 mU/mL). Preservative in the lyophilized culture: 0.01%N-methyl isothiazolone and 0.02% of 4-dimethylaminoantipyrine</td>
<td>2 bottles containing lyophilisate</td>
</tr>
<tr>
<td>5</td>
<td>One disposable pipette for each fecal supernatant (transfer pipette)</td>
<td>20 pipettes</td>
</tr>
<tr>
<td>6</td>
<td>Two disposable pipettes for each of the following: the collection of wash buffer, conjugate solution and substrate buffer</td>
<td>6 pipettes</td>
</tr>
<tr>
<td>7</td>
<td>Dilution buffer for porcine feces (phosphate butter with bovine serum albumin [BSA] and rabbit IgG). Preservative: 0.01% of N-methylisothiazolone and 0.1% of 2-chloracetamide</td>
<td>1 bottle containing 125 mL</td>
</tr>
<tr>
<td>8</td>
<td>Washing buffer (phosphate buffer with Tween). Preservative: 0.01% of N-methylisothiazolone and 0.1% of 2-chloracetamide</td>
<td>1 bottle containing 110 mL</td>
</tr>
<tr>
<td>9</td>
<td>Solution buffer for the conjugate (phosphate buffer with BSA). Preservative: 0.10% N-methylisothiazolone and 0.1% 2-chloracetamide</td>
<td>2 bottles containing 7 mL</td>
</tr>
<tr>
<td>10</td>
<td>Substrate buffer (tetramethylbenzidine, TMB)</td>
<td>1 bottle containing 15 mL</td>
</tr>
<tr>
<td>11</td>
<td>Positive control; biotinylated polyclonal antibodies directed against rabbit Fcg IgG (rabbit Fcg pAb) in phosphate buffer with BSA, bovine IgG and Tween; 1μg rabbit Fcg pAb per tube. Preservative: 0.01% of N-methylisothiazolone and 0.1% of 2-chloracetamide</td>
<td>2 tubes containing 0.5 mL each</td>
</tr>
</tbody>
</table>
Table 2: Equipment required but not included in the kit

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnet</td>
<td>1</td>
</tr>
<tr>
<td>Tube rack</td>
<td>1 rack</td>
</tr>
<tr>
<td>timer or stopwatch</td>
<td>1</td>
</tr>
</tbody>
</table>

Storage
Storage temperature: between 2°C and 8°C (35.6°F and 46.4°F)

Precautions and Warnings
- Use only fresh feces. Aged samples may produce false positive or negative results.
- The positive control must be well shaken before use.
- Do not re-use assay tubes, spatulas, collection tubes and transfer pipettes and dispose of all material after use.
- As with any microbiological/clinical material, the relevant safety precautions must be observed when handling and disposing of processed test material.
- Sodium azide (used as preservative in assay tubes) may react with lead and copper plumbing to form highly explosive metal azide. Therefore, flush liberally with water during disposal.
- The reagents have been tested as a unit. Do not mix with reagents from other kit lots.
- Do not use kit components beyond the indicated use-by date.
- Do not dilute the reagents. This would have an impact both on test sensitivity and stability.
- After reconstitution, the conjugate is only meant to be used once and must be used immediately after reconstitution.
- Read the result after 7 minutes. Later interpretation may produce false-positive results due to continuing color change.
- Do not expose the substrate buffer to direct sunlight, as this may lead to oxidation which will result in a color development. The substrate buffer is not sensitive to artificial light. It is, however, still recommended to store the bottle with the remaining buffer in the kit box after use.
- Do not freeze kits.

Specimen collection and preparation
The fecal samples should be fresh (no older than one day after collection). Around 1g of fecal sample is required per test. This quantity approximately equals the 1 mL marking line in the assay tube.
All samples and collection tubes must be labeled prior to the start of the test. The cap of the required collection tubes can be removed.

**Test Procedure**

The following overview shows the procedure of the *Lawsonia* FIRSTtest™ in the form of a diagram (see figure 2). The execution of the test is presented in figure 3.

![Diagram of the Lawsonia FIRSTtest™ protocol procedure.](image)

**Figure 2:** The *Lawsonia* FIRSTtest™ protocol procedure.
Sample collection
Use the spatula to place approximately 1 g of the sample into the collection tube. This equals approx. the 1 mL mark line which must not be exceeded.

Sample dilution
Pour ~5 mL of dilution buffer into the collection tube filling it to the 6 mL-markline. After all tubes have been filled, shake well and place tubes in the rack.

Sample Sedimentation
Wait (5 min.) for passive sedimentation to occur.

Sample Transfer
Using a pipette add ~0.5 mL of the fecal supernatant from the collection tube to the assay tube containing the beads. Use 1 pipette per tube. Shake the positive control and pour the content of the positive control directly into an unused assay tube. As soon as all tubes are filled shake them one after the other to ensure even distribution of the beads and place the tubes in the rack.

Incubation 1
Leave the tubes in the rack for 30 minutes. Agitate 2-3 times during incubation by giving each tube a brief shake.

Bead Separation and Washing 2
Remove assay tube from rack and place on the magnet for bead separation for 3 min.

Washing 1/1
Carefully discard supernatant liquid by turning the magnet upside down. Use tissue paper to dab rim of the tube on. Using a pipette add ~ 0.8 mL of washing buffer to each tube. Remove each tube from the magnet one by one, shake until the beads have completely come off the wall and place back onto the magnet. Wait for separation to take place in all tubes (<1min.). Carefully discard the supernatant by turning the magnet upside down. Use tissue paper to dab tubes on.

Washing 1/2
Repeat washing process 1/1.

Addition of Conjugate
Using a pipette, add ~ 0.5 mL of reconstituted conjugate to each assay tube. Remove each tube one after the other from the magnet, shake well and place onto the rack.

Reconstitution of Conjugate
Shortly before use, pour whole conjugate solution into the bottle containing the lyophilized culture and shake well for reconstitution.

Positive Result:
Blue liquid

Negative Result:
Clear liquid

Result
Place tubes onto the magnet and check the solution immediately for visual changes.

Incubation 3
Leave tube in the rack for 7 minutes. Incubation time must not be exceeded!

Incubation 2
Keep the tubes in the rack for 20 minutes. During incubation, agitate each tube 2-3 times by brief shaking.

Addition of Substrate
Using a pipette, add approx. 0.5 mL of substrate to each assay tube. Remove each tube from magnet one after the other, shake well and place onto the rack.

Washing 1/1
Carefully discard supernatant liquid by turning the magnet upside down. Use tissue paper to dab rim of the tube on. Using a pipette add ~ 0.8 mL of washing buffer to each tube. Remove each tube from the magnet one by one, shake until the beads have completely come off the wall and place back onto the magnet. Wait for separation to take place in all tubes (<1min.). Carefully discard the supernatant by turning the magnet upside down. Use tissue paper to dab tubes on.

Washing 1/2
Repeat washing process 1/1.

Sample collection
Use the spatula to place approximately 1 g of the sample into the collection tube. This equals approx. the 1 mL mark line which must not be exceeded.

Sample dilution
Pour ~5 mL of dilution buffer into the collection tube filling it to the 6 mL-markline. After all tubes have been filled, shake well and place tubes in the rack.

Sample Sedimentation
Wait (5 min.) for passive sedimentation to occur.

Sample Transfer
Using a pipette add ~0.5 mL of the fecal supernatant from the collection tube to the assay tube containing the beads. Use 1 pipette per tube. Shake the positive control and pour the content of the positive control directly into an unused assay tube. As soon as all tubes are filled shake them one after the other to ensure even distribution of the beads and place the tubes in the rack.

Incubation 1
Leave the tubes in the rack for 30 minutes. Agitate 2-3 times during incubation by giving each tube a brief shake.

Bead Separation and Washing 2
Remove tubes from the rack and place ~ 1 min. onto the magnet to separate the beads. Wash beads as described under ‘Washing 1/1’ and ‘Washing 1/2’. For negative control add ~0.8 mL of washing buffer into an unused assay tube and carry along in parallel.

Incubation 2
Keep the tubes in the rack for 20 minutes. During incubation, agitate each tube 2-3 times by brief shaking.

Addition of Substrate
Using a pipette, add approx. 0.5 mL of substrate to each assay tube. Remove each tube from magnet one after the other, shake well and place onto the rack.

Washing 1/1
Carefully discard supernatant liquid by turning the magnet upside down. Use tissue paper to dab rim of the tube on. Using a pipette add ~ 0.8 mL of washing buffer to each tube. Remove each tube from the magnet one by one, shake until the beads have completely come off the wall and place back onto the magnet. Wait for separation to take place in all tubes (<1min.). Carefully discard the supernatant by turning the magnet upside down. Use tissue paper to dab tubes on.

Washing 1/2
Repeat washing process 1/1.
Figure 3. Execution of the \textit{Lawsonia FIRSTtest™}

**Interpretation of the Test Results**

<table>
<thead>
<tr>
<th>Color intensity:</th>
<th>Transparent</th>
<th>Light blue</th>
<th>Mid blue</th>
<th>Dark blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Result:</td>
<td>Negative</td>
<td>Mildly positive</td>
<td>Positive</td>
<td>Strongly positive</td>
</tr>
</tbody>
</table>

A sample is negative if the intensity of the color is the same or less than the negative control or if the result is transparent. The test is considered invalid if there is no change of color in the tube with the positive control.

For examples of negative, mildly positive, positive and strongly positive test results see Figure 4 on back cover of the Package Insert booklet.

**Limitations of the procedure**

- The results obtained from this test should be used in combination with other data (symptoms, possible further test results) as a diagnostic aid.
- FIRST\textit{test™} has been validated for use with fecal specimens only. The test has not been confirmed for use with other specimens such as urine, saliva, or wound exudates.
- FIRST\textit{test™} establishes the existence of \textit{Lawsonia} bacteria. The test can detect both viable and non-viable bacteria and may produce a positive result even after successful treatment.

**References**


Customer Support
For support or further information contact your local sales representative. Contact numbers are on the inside back cover of the package insert.

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Figure 4: Examples of negative, mildly positive, positive and strongly positive test results (from left to right). In the background of the assay tubes, the beads that have been separated at the magnet can be seen.

<table>
<thead>
<tr>
<th>Transparent</th>
<th>Light blue</th>
<th>Mid blue</th>
<th>Dark blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Mildly positive</td>
<td>Positive</td>
<td>Strongly Positive</td>
</tr>
</tbody>
</table>

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