DEFINITION AND SCOPE OF THE TEST

The verify of the hygienic condition of the eggs used in the preparation of egg products, is based on the analysis of indicators of microbial metabolism such as lactic acid. The total bacterial count is not a suitable index because it is reduced by the pasteurization treatment. To ensure that the handling of eggs and egg products is made in respect of the rules of hygiene, the REGULATION (EC) No 853/2004 states that the lactic acid content must not be greater than 1000 mg / kg of dry matter.

TEST PRINCIPLE

L-Lactic acid reacts, by an enzymatic reaction, with a phenolic derivate and forms a pink colored complex. The absorbance of the complex, read at 505 nm is directly proportional to the concentration of L-lactic acid in the sample.

COMPOSITION OF THE KIT AND REAGENTS

Reagent test kit *300385, suitable for 100 tests, contains:
- 1 box includes: 5 x reagent test kit *300388.

Reagent test kit *300388, suitable for 20 tests, contains:
- R1: 2 packages with 10 pre-filled cuvettes with 1 mL of buffer.
- R2: dropper with 1,5 mL of enzymatic solution.
- polymer: bottle with a proper polymer for sample treatment.

For information on the hazards associated with reagents, consult the product’s safety data sheet.
Storage: reagents are stable up to the expiry date. Store at 2-8°C.

SAMPLE TREATMENT

Powdered egg: reconstitute the powder with water according to product specifications. Use the liquid sample obtained, following the instructions for the mixed egg.

Mixed egg or egg white: take 10 grams of mixed sample in a plastic glass and add 1 gr of polymer. Stir for a few minutes (e.g. with magnetic stirrer). Transfer part of the solution obtained in a centrifuge tube and centrifuge for 5 min at around 5000 rpm. Use the clear solution. If the solution is not sufficiently clear, continue in this way: add directly into the centrifuge tube, about 0,5 gr of polymer. Shake well and centrifuge again.

Yolk: Dilute one part of sample with one part of distilled water and proceed as described for the mixed egg.

It’s possible to test both Lactic and D-3-hydroxybutyric acid on the sample prepared.

SAMPLE VOLUME – MEASURING RANGE

<table>
<thead>
<tr>
<th>Test</th>
<th>Measuring range (ppm)</th>
<th>Sample volume</th>
<th>Resolution (ppm)</th>
<th>Accuracy</th>
<th>Repeatability</th>
</tr>
</thead>
<tbody>
<tr>
<td>LactAc.mix.egg</td>
<td>5 - 1030</td>
<td>10 µL of clear solution</td>
<td>0,1</td>
<td>+/- 5%</td>
<td>CV &lt;3%</td>
</tr>
<tr>
<td>LactAc.yolk</td>
<td>5 - 2100</td>
<td>10 µL of clear solution</td>
<td>0,1</td>
<td>+/- 5%</td>
<td>CV &lt;3%</td>
</tr>
</tbody>
</table>

For samples with a value of lactic acid >1030 ppm (mix) or >2100 ppm (yolk) use half of the sample volume (5 µL) and multiply the result by 2.
TEST PROCEDURE

Reagent preparation

Reagent R1: ready to use.
Reagent R2: ready to use.

Selection of the test, addition of the sample and incubation of the blank

1. Press Key 2 on keyboard to display available analysis on reading cell 2, or 0 to display all available analysis. Select the proper Lactic acid mix or Lactic acid yolk curve, confirm your selection by pressing ENTER (on display shows INCUBAT. 5 MIN).
Remove the cap of a cuvette with R1 and add in 10 µL of clear solution (see “sample treatment” session). Close the cuvette and mix it gently. Place the cuvette in the incubation cells. Repeat the operation for each sample to test. It is possible to analyze a maximum of 14 samples for each test session.
2. Press ENTER to start the incubation.

Note: Homogenize the sample in the bottle before taking it.
To prevent cross-contamination between samples, take the sample with pipette and discard it. Repeat the procedure for 2-3 times before transferring it to the reagent.
Remove excess sample by wiping the outer surface of pipette tip gently using a blotting paper.
Immerse the pipette tip in the reagent while dispensing sample. Press and release the piston of pipette several times to ensure all sample has been transferred.
Mix the sample with reagent, after adding, by inverting the cuvette several times.

Reading of the blank

3. At the end of the incubation press ENTER. The display shows INSERT BLANK.

4. Invert the incubated cuvette to mix before inserting in the reading cell with the green light. Press ENTER to start the reading. The green light turns to red for a few seconds until the reading is completed. Repeat this operation for each sample.

5. To stop the blanks reading session press ARROW KEY UP. The display shows INCUBAT. 3 MIN.

Addition of R2 and incubation of the sample

6. Add 1 drop of R2 reagent to each cuvette, mix it by inverting the cuvette 2-3 times and place it in one of the incubation cells. Repeat this procedure for each sample to test.
7. Press ENTER to start the incubation.

Reading of the sample

8. At the end of the incubation, press ENTER. The display shows INSERT SAMPLE.

9. Invert the incubated cuvette 2-3 times to mix and place it in the reading cell with the green light. Press ENTER to start the reading. The green light turns to red for a few seconds until the reading is completed. Repeat this operation for each sample to test.

10. The results, expressed in ppm of lactic acid, will be automatically printed and displayed at the end of the session.
The result is expressed in ppm of lactic acid in the sample.
In order to refer the result to dry matter it’s necessary to know the % of dry weight of the sample and calculate the result with the following formula: (e.g. sample of 23% of dry weight) result in dry matter = (result/23) x 100.

11. Press ENTER and ARROW KEY DOWN to return to the test menu.

SYSTEM STANDARDIZATION

The system is supplied pre-calibrated and ready for use.
Results are expressed in accordance with the reference method.
It is also possible to standardize the system using samples with a known titration.
For information on the operating procedure, see the manual provided with the system.

For in-vitro use only

Lactic_acid_egg_02.doc