INTENDED USE

Sensilist is a method for detection and enumeration of low concentrations of *Listeria monocytogenes* in food and production environment samples.

PRINCIPLE

Detection is based on growth of the bacterium in a selective medium (Sensilist Broth), which contain components that leads to a colour change from red to yellow (fig. 1) when the bacterium has grown to a sufficiently high density in the broth. Both *Listeria monocytogenes* and *Listeria innocua* give colour change, so a confirmation of *L. monocytogenes* is needed to distinguish between the two species.

Sensilist is both a qualitative and quantitative method. Quantification of low concentrations in the sample is possible as a large amount of sample and broth is incubated in a quantification tray which allows quantification with MPN (Most Probable Number).

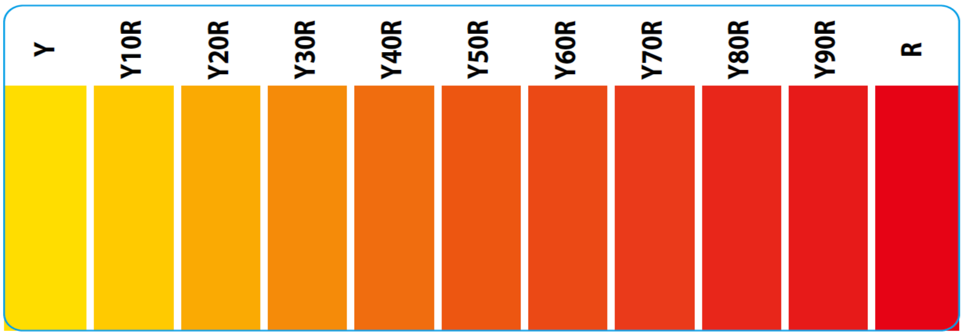

Figure 1. Colour chart for reading changes from red to yellow.

INTENDED USER

The method is designed for use by qualified personnel with appropriate training.

INSTRUCTIONS FOR USE

Qualitative analysis

<u>Food samples</u>

1. Put 25 g sample into a stomacher bag or box.
2. Add 225 ml Sensilist broth.
3. Homogenise by shaking, squeezing or with stomacher, without forming to much foam.

4. For correct reading of the colour, add a label on each unit. Mark the start colour on the label (fig. 1). The colour needs to be marked immediately after mixing, and the development be monitored during incubation.
5. Incubate the bag or box at 37^{0}C±1°C for 48 hours, or until a visible change to yellow colour, see point 6.
    a. Read colour after 24 and 48 hours.
6. The instructions for presumptive *Listeria monocytogenes* positive results are (fig. 1):
    a. A change of at least two steps towards yellow on the colour chart after incubation. (e.g., from Y60R to Y40R). Confirmation see page 3.
    b. All tests with colour Y10R or Y.

<u>Environmental samples</u>

1. Put the sample back into the bag, in a box or a bottle (water)
2. Add Sensilist broth (1:10 dilution).
    a. The amount of sample can be maximum 10 % of the total broth, which means that the broth needs to cover the sample. For instance, a swab with high amount of water will dilute the broth and make the colour less clear. In such cases, more Sensilist broth or less water in the swab is needed to obtain a detectable colour change.
3. Homogenise by shaking, squeezing or with stomacher, without forming to much foam.
4. For correct reading of the colour, add a label on each unit. Mark the start colour on the label (fig.1). The colour needs to be marked immediately after mixing, and the development be monitored during incubation.
5. Incubate the bag or box at 37^{0}C for up to two days, or until a visible change to yellow colour, see point 6.
    a. Read colour after 24 and 48 hours.
6. The instructions for presumptive *Listeria monocytogenes* positive results are (fig. 1):
    a. A change of at least two steps towards yellow on the colour chart after incubation. (e.g., from Y60R to Y40R)
    b. All tests with colour Y10R or Y

Quantitative analysis

<u>Food samples</u>

1. Put 10 g sample in a bag/ box or other container.
2. Add 190 ml Sensilist broth (1:20 dilution).
3. Homogenise by shaking, squeezing or with stomacher, without forming to much foam.
4. Transfer 100 ml to a quantification tray suitable for MPN enumeration with wells approximately 1 ml or 0.1 ml each.
5. Laminate the Q-tray in DF 15 for 4-6 sek at 90°C.

6. For correct reading of the colour, add a label on each unit. The colour needs to be marked immediately after mixing, and the development be monitored during incubation. Mark the start colour on the label (fig.1).
7. Incubate the tray/plate at 37 °C for up to 48 hours, or until a visible change to yellow colour, see point 7.
    a. Read colour after 24 and 48 hours.
8. The instructions for presumptive *Listeria monocytogenes* positive results are (fig. 1):
    a. A change of at least two steps towards yellow on the colour chart after incubation. (e.g., from Y60R to Y40R)
    b. All tests with colour Y10R or Y
9. Enumeration of *L. monocytogenes* with MPN:
    a. If you use a quantification tray suitable for MPN enumeration, read presumptive positive wells and use the MPN table suitable for that number of wells of the tray.
    b. If you apply a 96 well tray, use an MPN table suitable for that number.

Confirmation of presumptive positive samples

1. Confirm the presence of colonies according to ISO 11290, biochemical galleries, or an ISO 16140-6:2019 validated confirmation method.

2. Streak using a sterile 10µl loop from the positive (yellow) sample bag, box or well onto an ALOA or similar agar plate.

Incubate at 37°C±1°C for a total of 48h ± 2h. If colonies of presumptive *Listeria monocytogenes* or *Listeria* spp are evident at 24h ± 2h, then incubation can be stopped at this stage.

FLOW DIAGRAM OF METHOD

Qualitative method, detection level 1 cfu/25 g, sample size 25 grams

1. •Put 25 g sample in a bag/ box with 225 ml Sensilist Broth. Homogenise.
2. •Incubate at 37°C for 48 h or until colour change
3. •Change of colour from red to yellow indicate presence of presumptive Listeria
4. •Confirmation

Quantitative, detection level 0,2 cfu/g, sample size 10 grams.

1. •Put 10 g sample in a bottle/ bag with 190 ml Sensilist Broth. Homogenise.
2. •Transfer 100 ml to a quantitray with wells.
3. •Incubate at 37°C for 48 h or until colour change
4. •Change of colour from red to yellow indicate presence of presumptive Listeria
5. •Confirmation

PRECAUTIONS AND LIMITATION OF THE METHOD

1. The detection level for qualitative analysis is 0.04 cfu/g, sample size 25 g.
2. The detection level for quantitative analysis is 0.2 cfu/g, sample size 10 g.
3. Sensilist is not suitable for the following fishery products:
   a. Acid marinated products
   b. Mayonnaise and cream-based products
   c. Products with added lactic acid bacteria
   d. Shellfish
   e. Herring and mackerel
   f. Raw squid rings

CERTIFICATE OF ANALYSES (CoA)

Certificate of Analysis for Sensilist Broth can be found at www.sensilist.com

Revisjonsnummer	Dato	Endring	Godkjent av
1.0 Dokument 08.01.03 Description of Method_English	09.07.2024	First edition	Quality Manager
2.0 Dokument 08.01.03 Description of Method_English	17.11.2025	Changed the text in principle and made some adjustments to the text elsewhere in the document	Quality Manager