Sensilist Method Protocol

Version: 001
Date: 31.03.2024

1. PRINCIPLE

Sensilist is a method for detection (qualitative) and enumeration (quantitative) of low concentrations of *Listeria monocytogenes* in: Environmental sample - Fishery products* - RTE meat and poultry products.

*There are some exclusions for the qualitive method that apply to the fishery products category and these are detailed below. The following matrices have been removed from the scope of the fishery products category: Acid marinated products - Mayonnaise and cream-based products - Products with added lactic acid bacteria – Shellfish - Herring and mackerel - Raw squid ring

- The detection level for qualitative analysis is 0.04 cfu/g, sample size 25 g.
- The detection level for quantitative analysis is 0.2 MPN/g, sample size 10 g.

The 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 *L. monocytogenes* and *L. innocua* give colour change, so a confirmation of *L. monocytogenes* is needed to distinguish between the two species.

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.

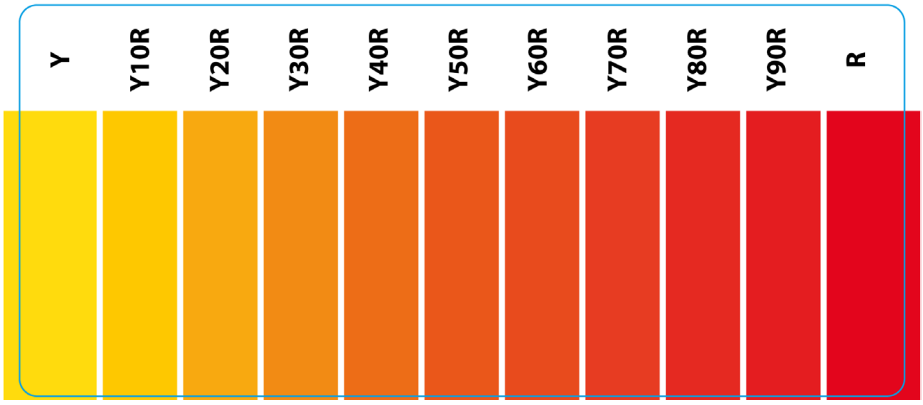

Figure 1. Colour chart for reading changes from red to yellow

2. MATERIALS NEEDED

Growth-medium:

1. Sensilist broth

Equipment:

1. Balance weight 10-250 g
2. Stomacher bag, closed bottle with cap or box with cap.
3. For quantitative analysis in addition deep well plates, 96 squares well or quantification tray suitable for MPN enumeration.

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3. Food Samples

Quantitative analysis (detection level 0,2 MPN/g):

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, or deep 96 well plates, each well 1 ml. Attaché an adhesive sealing film for microplates
5. For correct reading of the colour, add a label on each unit (fig. 1). The colour needs to be marked immediately after mixing, and the development be monitored during incubation.
6. Incubate the tray/plate at 37 °C for 48 ± 2 hours, see point 7.
7. The instructions for presumptive *L. monocytogenes* positive results are (fig. 1):
    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

8. 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.

9. Confirmation, see chapter 5.

Qualitative analysis (detection level 1 cfu/25 g or 0,04 cfu/g):

1. Put 25 g sample into a stomacher bag or box.
2. Add 225 ml Sensilist broth (1:10 dilution).
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. The colour needs to be marked immediately after mixing, and the development be monitored during incubation.
5. Incubate the bag or box at 37°C for up to 48 hours, or until a visible change to yellow colour, see point 6.
6. The instructions for presumptive *L. 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

7. Confirmation, see chapter 5.

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4. ENVIRONMENT & WATER/PROCESS WATER samples

(swabs, cloth etc.)

Qualitative analysis (detection 1 cfu/sample in the incubated volume)

1. Put the sample into a stomacher bag, box or bottle.
2. Add Sensilist broth (1:10 dilution) or it need to cover the cloth/swab .
    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 (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°C for up to 48 hours, or until a visible change to yellow colour, see point 6.
6. The instructions for presumptive *L. 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

7. Confirmation, see chapter 5.

Information about the samples:

a. Surface samples just after cleaning but before rinsing with water will, in case of soap left overs in the swab, increase the pH in the Sensilist broth so much that growth will be inhibited, and false negative results can be the result. Soap should therefore be rinsed away before the sampling.

5. CONFIRMATION OF PRESUMPTIVE POSITIVE SAMPLES

- Plate out from positive (yellow) sample (bag or box) or well on ALOA plate according to the ISO 11290 (1 or 2) method.

or

- Apply another method which distinguish between *L. monocytogenes* and *L. innocua*, e.g:
    - plating on Rapid L'mono
    - analysis with PCR
    - Vidas Malditof
    - Other suitable and verified methods.

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6. ALTERNATIVE METHODS

Preparation and incubation of food samples (for the combined method):

1. Into a stomacher bag with filter, weigh up 35 grams of the sample.
2. Add 35 ml Sensilist broth (same amount as the sample). (This gives a 1:2 dilution of the sample)
3. Mix sample by squeezing and rubbing (if you do not have a stomacher), so that it doesn`t form to much foam.
4. Divide the mix in two:
   - **a. Quantitative analysis (detection level 0,2 MPN/g).**
     - i. Put 20 ml into a bottle/bag (represents 10 g sample).
     - ii. Add 180 ml Sensilist broth (gives a 1:20 dilution of sample). Mix well.
     - iii. Distribute 100 ml into either.
       1. Quantification tray suitable for MPN enumeration or
       2. Deep well plates, 96 wells, each well 1 ml. Attach an adhesive sealing film for microplates.

   - **b. Qualitative analysis (detection level 0,04 cfu/g)**
     - i. To the remaining 50 ml (represent 25 g sample) add 200 ml Sensilist broth and mix (gives a 1:10 dilution of sample).

5. For correct reading of the colour, a label will be added on each unit. The colour needs to be marked immediately after mixing, and the development be monitored during incubation.
6. Incubate the tray/plate, bag or box at:
   a. Qualitative analysis: 37°C for up to 48 hours. Incubate the whole amount. The stomacher bag can be used as is, or the sample + Sensilist broth solution can be transferred to a transp. box or bottle.
   b. Quantitative analysis: 37°C for 48 +/- 2 hours

7. The instructions for presumptive *L. 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

8. 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.

10. Confirmation, see chapter 5.