



Speedy Breedy - Lab Memo 44

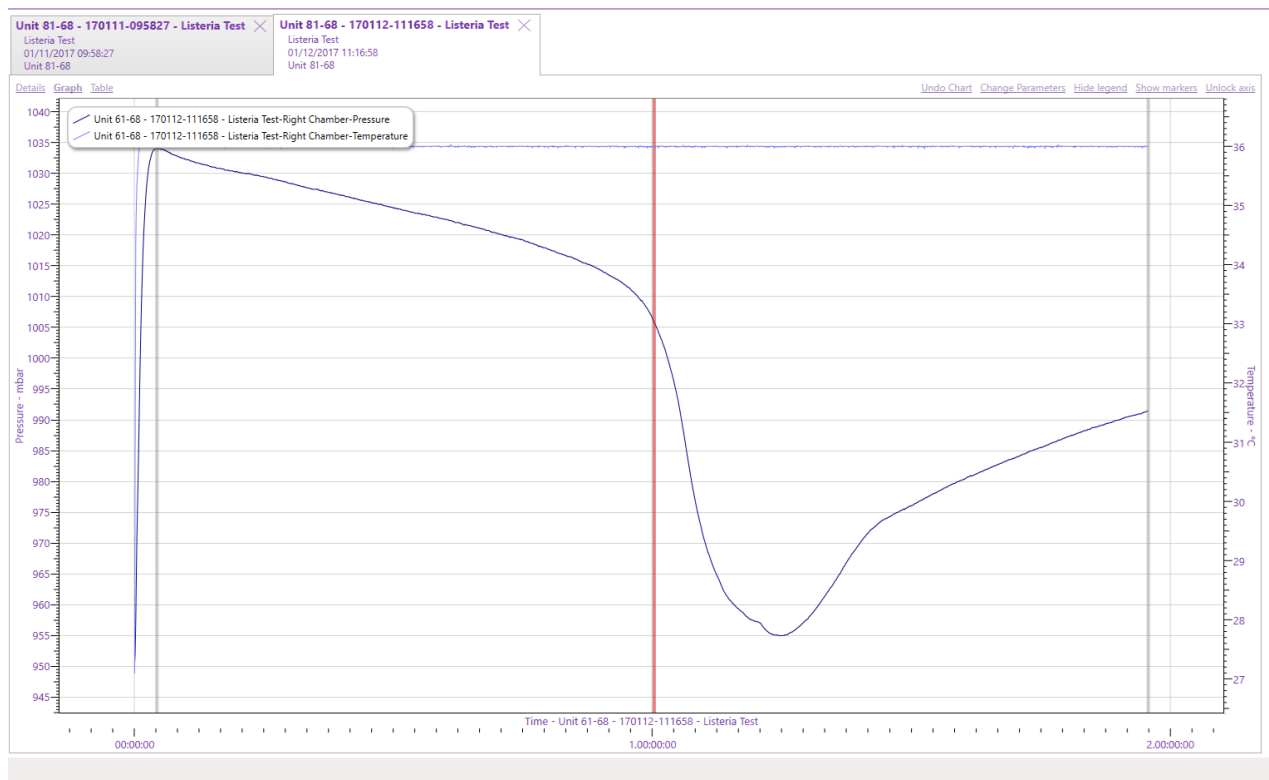
Listeria Detection in Milk and Cheese Samples

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Summary

A soft cheese manufacturer tested milk samples with Speedy Breedy and received positive results for *Listeria*, while when the samples were sent to a laboratory they were negative.

The customer sent one of the used, positive *Listeria test* vessels, a soft cheese sample and a milk sample to Bactest for testing. The used *Listeria* vessel tested positive for *Listeria monocytogenes* on an agar plate, confirming the Speedy Breedy test result made on the premises of the soft cheese manufacturer, but the cheese and milk samples were negative both in Speedy Breedy and on an agar plate.



The fact that *Listeria* is present in samples processed at the customer's site, as indicated by Speedy Breedy curves above, and the positive *Listeria* plate taken from the Speedy Breedy test vessel, but wasn't found by the external laboratory, shows that *Listeria* contained within the original sample may not be surviving the transportation and/or storage of samples before being processed by the external laboratory.

Background

A soft cheese manufacturer was sending samples of unpasteurised milk delivered to his facility to a Laboratory for bacterial contamination testing, but results were taking five days to return. If positive contamination was found the whole working week's production needed to be scrapped.

By using Speedy Breedy the soft cheese manufacturer was able to obtain results within 24 hours, thus only losing one day's production and associated time and costs.

A soft cheese manufacturer tested milk samples for *Listeria* with Speedy Breedy, and in doing so unexpectedly got two positive test results. In order to evaluate these unexpected results, they sent the original milk samples to an external laboratory, which could not confirm the result.

Bactest wanted to evaluate the Speedy Breedy *Listeria* protocol using another milk sample, and requested the client to send a 100ml milk sample.

Samples

The Customer sent us a sample of soft cheese, a used *Listeria* vessel, and a sample of milk

A sample was taken from the used vessel, cheese and milk, streaked on a *Listeria* selective agar plate and incubated at 37°C for 48hours.

Two 30g samples were aseptically taken from the cheese and diluted in a sterile 100ml container with 60ml of sterile water and mixed through agitation. 50ml was then taken and added to a *Listeria* selective vessel and run in Speedy Breedy using the *Listeria* protocol.

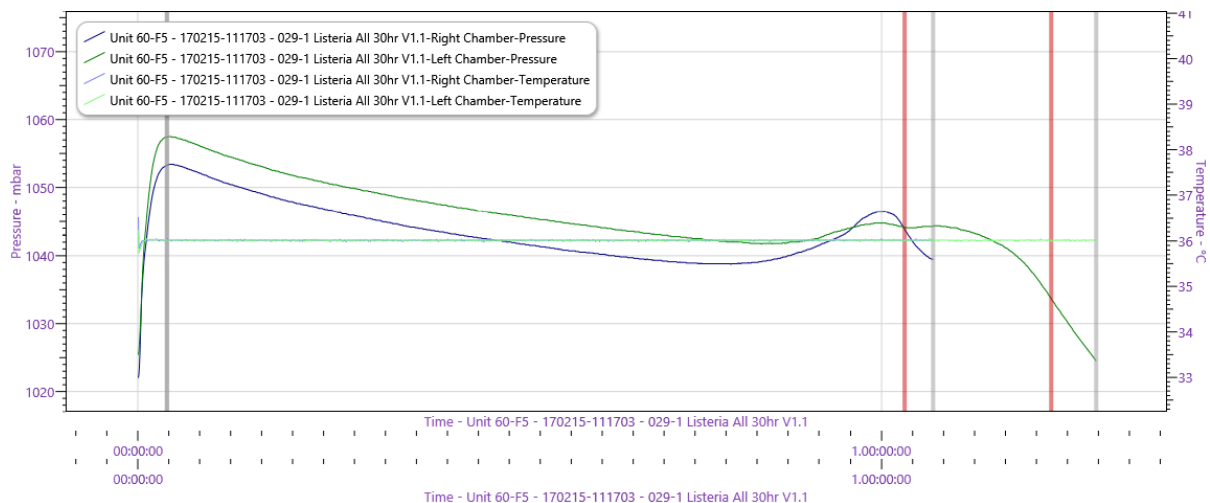
25ml of milk and 25ml of sterile water were added to a *Listeria* selective vessel and run in Speedy Breedy using the *Listeria* protocol.

Once the Speedy Breedy tests completed, the samples were taken from the vessels and diluted from 10⁸ (neat) to 10¹ and plated on *Listeria* selective agar plates.

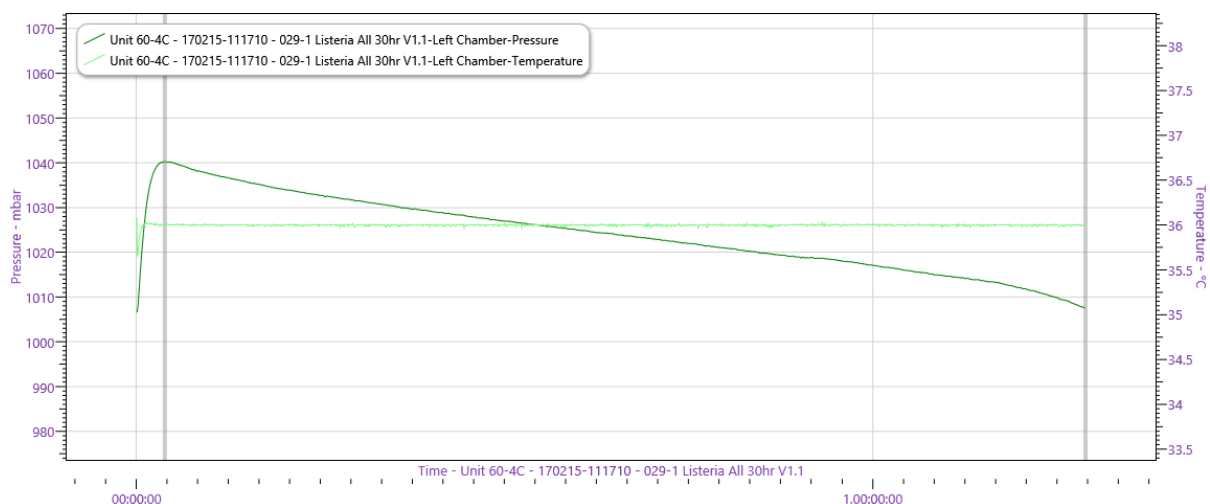
Results

Speedy Breedy

Both cheese samples gave an event in Speedy Breedy, however the curve looked different from the standard *Listeria* curves by exhibiting a rise in pressure followed by a drop in measured pressure.



The milk sample cultured in the Speedy Breedy, showed no measured event by the end of the test.



Plates

The neat cheese and milk samples showed no growth after 48h at 37°C on a *Listeria* selective plate.

The sample taken aseptically from the customer's used vessel showed several small, white colonies and two blue colonies on *Listeria* selective plates after 48h incubation at 37°C. Blue colonies are indicative of positive *Listeria monocytogenes* growth.

For both cheese samples neither of the *Listeria* selective plates showed any growth at any dilution (neat to 10¹).

The milk sample showed some small, white colonies at the three most concentrated dilutions (neat to 10⁶), but the plates were negative for *Listeria monocytogenes*, as no blue colonies were observed.

Conclusion

Listeria monocytogenes was confirmed by plating from the customer's test vessel. Since the customer's vessel did not change colour it is very likely that the whiteness of the milk masked the normal accompanying colour change indicative of a positive *Listeria* test. It is therefore strongly recommended to dilute the sample in order to observe the colour change more distinctly.

There was no indication that *Listeria* was present in either of the cheese samples. The curves looked different to curves seen at Bactest or at the customer's site and the *Listeria* selective agar plates also showed no *Listeria* growth.

When the milk sample was plated, small white colonies were seen at the top 3 dilutions, similar to the results from the customer's vessel, but no blue *Listeria* colonies were observed.

The fact that *Listeria* is present in samples processed at the customer's site, as indicated by Speedy Breedy curves and the positive *Listeria* plate, but not found by the external laboratory, shows that *Listeria* contained within the original sample may not be surviving the transportation and/or storage of samples before being processed by the external laboratory.

- It is important to observe the colour change from yellow to brown to confirm the presence of *Listeria* alongside the positive Speedy Breedy curves. This is of particular importance when uncharacteristic curves (such as curves with a rise in pressure) are encountered.
- To be able to observe the required colour change, we strongly recommend to dilute the sample by half, i.e. 25ml milk and 25ml water.