

## **Advice 02-2016 of the Scientific Committee of the FASFC on challenge tests and durability tests for *Listeria monocytogenes* in cheese**

In Regulation (EC) No 2073/2005 of the European Commission of 15 November 2005 on microbiological criteria for foodstuffs, i.a. microbiological criteria are established concerning ready-to-eat foods able to support the growth of *Listeria monocytogenes*. In order to comply to this criteria, in some cases studies have to be performed. Therefore, the European reference laboratory for *Listeria monocytogenes* (EURL Lm) has established a technical guidance document for conducting challenge tests and durability tests for *Listeria monocytogenes* in ready-to-eat foods. These tests aim to evaluate the growth potential or the maximum growth rate of *Listeria monocytogenes* during the shelf-life of ready-to-eat foods placed on the market. Furthermore, the FASFC prepared a service note with further clarification in order to achieve a harmonized application of this technical guidance document in Belgium.

The Scientific Committee was asked to provide clarification concerning the above-mentioned technical guidance document and in particular concerning the conducting of challenge tests and durability tests for *Listeria monocytogenes* in cheese. For challenge tests, questions are asked about the inoculation, the measurement of the pH and  $a_w$  (water activity), the microbiological analyzes, the recommendations for the storage temperature and duration and the extrapolation of the results to other cheeses. For durability tests, questions are asked about the measurement of the pH and  $a_w$ , the microbiological analyzes, the recommendations for the storage temperature and duration, the extrapolation of the results to other cheeses and the utilization of durability tests as an alternative for challenge tests. Next to this, some questions are asked about the interpretation of the technical guidance document.

In this advice, an answer is formulated to the questions. *Listeria monocytogenes* can contaminate cheese via several contamination routes. The growth potential of *Listeria monocytogenes* can be studied by means of challenge tests and/or durability tests. An initial contamination of cheese with *Listeria monocytogenes* may come from the (raw) milk used to produce the cheese, from the production environment (equipment, tools, infrastructure, etc.) or from the staff involved in the production, ripening, affinage, cutting and packaging of the cheese.

A contamination coming from the (raw) milk can be simulated by means of inoculation of the milk that is used for the production of cheese (via a pilot study) or by means of inoculation in the core of the cheese. The choice of the way of artificial inoculation of *Listeria monocytogenes* (inoculation in the core and/or on the (cutting) surface of the cheese) depends on the most probable contamination route which depends on the type of cheese, the physical-chemical properties, the production process of the cheese and the aim of the customer. The inoculum can possibly change the intrinsic properties of the product at the level of the injection zones. Therefore, it is stated in the technical guidance document that the ratio of the inoculum and the product should not be higher than 1 % of the mass (or the volume) of the test unit. In accordance with the technical guidance document, the inoculation has to be conducted after the ripening of the cheese on the first day of the shelf-life (day 0), i.e. the moment that the cheese is placed on the market as a ready-to-eat food. Potentially, the cheese contains a label specifying the recommended storage conditions. In accordance with the technical guidance document, the initial contamination level has to be about 100 cfu (colony forming units)/g. An estimated growth potential of  $> 0,5$  log units at an initial contamination level of 100 cfu/g is not considered as being significantly different in comparison with an expected growth potential at an initial contamination level of 10 cfu/g. Challenge tests should preferably be performed with well characterized strains of *Listeria monocytogenes* as prescribed in the technical guidance document and not with strains of *Listeria innocua*.

For challenge tests as well as for durability tests, the measurements of the pH and  $a_w$  have to be performed at day 0 and at the last day of the shelf-life one time on one sample per batch. They should be done in the core and/or on the surface of the cheese, at the level of the place of inoculation for a challenge test or in accordance with the most probable contamination route for a durability test. The recommendations for storage duration and temperature are given in table 3 of the technical guidance document and are further explained in the service note of the FASFC. The results of challenge tests and durability tests can only be extrapolated to batches of the same type of cheese. Durability tests can serve as an alternative for challenge tests if sufficient analyzes are performed on day 0 as well as on the last day of the shelf-life.

The Scientific Committee recommends to work in a hygienic way, to respect the cold chain as good as possible, to avoid cross contaminations with *Listeria monocytogenes* and to strive to absence of *Listeria monocytogenes* in cheese from the good manufacturing and hygienic practices (GMP and GHP). Finally, some recommendations are made regarding the interpretation of the technical guidance document.

The full text is available on this website in dutch and in french.