What are coliforms and Enterobacteriaceae?

Coliforms can be found in the aquatic environment, in soil and on vegetation and are present in large numbers in the feces of warm-blooded animals. While coliforms themselves are not normally the cause of serious illness, they are easy to culture, and their presence can be used to indicate that other pathogenic organisms of faecal origin may be present.

Coliforms are a part of a Family of bacteria, Enterobacteriaceae (EB), which are ubiquitous in the environment, and were initially isolated from the gut flora of mammals (hence, the name, bacteria of the intestine). This family is considered to be a more global indicator of hygiene in food process environments.

What are *Escherichia coli*?

*Escherichia coli* are a group of mostly harmless bacteria that inhabit various ecological niches, and classically the human digestive system. They are part of the larger group of coliform bacteria. *Escherichia coli* can be beneficial to its host by producing vitamin K2 or preventing colonisation of harmful bacteria, for example, the probiotic *E. coli* Nissle 1917. There are also some isolates of *E. coli* that are highly pathogenic for the human host, such as *E. coli* O157:H7 and other related pathogenic strains (which can be called EHEC or STEC [VTEC]).

What are indicator organisms?

Indicator organisms are a group of organisms (coliforms, EB or a specific species e.g. *E. coli*) that are more likely to be found through testing because they appear at relatively high numbers or more often compared to the target microorganism of concern. They are harmless bacteria that can be easily measured and correlate with the likelihood of the presence of the microorganism of concern.

The presence of indicator organisms in foods or in the processing environment can be used to assess the hygienic status and quality of the food product or the adequacy of control measures in place.

*E. coli* as indicator organisms of faecal contamination

For many years, it was thought that *E. coli* could only survive in the environment for a limited amount of time, which made them potential indicator organisms to test for faecal contamination. However, it is increasingly being shown that *E. coli* can persist in the environment and can even increase in numbers outside the host. Therefore, not all *E. coli* enter the food chain through direct contact with faeces and trend analysis of analytical data shows that *E. coli* does not seem to be an appropriate indicator of faecal contamination.

*E. coli* carried in the intestines of ruminants are excreted in their feces, and can spoil the teats of cows, increasing the likelihood of contamination during the milking process. As a result, good hygiene practices on the farm and during milking can limit faecal contamination, without eradicating it totally, so the contamination of milk with low numbers of *E. coli* is inevitable, even under good manufacturing conditions.
**E. coli** as hygiene indicators in cheese

In pasteurised milk cheese

In the case of cheese made from pasteurised milk, the purpose of testing for *E. coli* is to verify general hygiene. As the pasteurisation of milk can be considered an effective means of control, any *E. coli* present are likely to result from heat treatment failure (e.g. organisms passing through because of fouling), biofilm development in the post-heating equipment or from other post-heating sources (ingredients, water, etc.) or the processing environment (food contact and non-food contact surfaces). Whatever the reason is for *E. coli* being present, only a root cause analysis will help to identify where and when the contamination occurred in the cheesemaking process. In firm and hard ripened cheese and in many ripened soft cheeses (pH <4.7 and high lactate contents), *E. coli* present will grow during the cheesemaking process and will peak within the first weeks of ripening; hence, an opportune time to sample is when cheese typically after 1-2 weeks of ripening. For the purpose outlined (verification of general hygiene), it makes little sense to test finished cheese (ready for sale) as the numbers decline with age. Where the numbers of *E. coli* do not decline with age, samples could be taken 1-2 weeks after ripening or later.

In unpasteurised milk cheese

In the case of cheese made from unpasteurised milk, the purpose of testing for *E. coli* would be quite different compared to cheese from pasteurised milk. It is most likely that the unpasteurised milk used will contain *E. coli* that can grow to relatively high numbers in the fresh raw cheese curd. Testing for *E. coli* in fresh cheese from unpasteurised milk is only of limited value to a manufacturer attempting to implement an appropriate HACCP plan. The usefulness of *E. coli* as a hygiene indicator for raw milk cheese is questionable. Testing for *E. coli* only makes sense if the purpose is to verify the safety of the finished cheese, for instance to verify that the decline in numbers has taken place during ageing (sampling and testing at the end of the ripening period). Whether such routine testing provides useful information to the manufacturer will depend on what other tests are performed. In this regard, it may be noted that many studies published in the scientific literature indicate that the decline rate of pathogens tends to be greater for *Listeria* and *Salmonella* than for *E. coli*.

Regulatory limits on *E. coli* numbers

To protect public health, there are regulations limiting the permissible number of *E. coli* in unpasteurised milk. Pasteurisation will inactivate *E. coli*, therefore, the regulatory limits are less for pasteurised milk. During cheesemaking, there is an approx. 10-fold increase in bacterial numbers in the cheese, not due to growth, but due to concentration of the bacteria in the curd. For hygiene monitoring, the limit of *E. coli* in cheese should therefore be 10 times higher than the limit in milk. Additionally, *E. coli* has the potential to grow in milk, irrespective of whether pasteurized or not, but not during ripening of hard cheese due to ecological competition of microbial food cultures, availability of nutrients, water activity and acidity. The regulatory limit in cheese is therefore classically higher than that for liquid milk.