

Microbial update

water

Provided as a service to the industry by Oxoid, part of Thermo Fisher Scientific.

Water is extremely important to the food industry, with many different roles to play, both in the primary production of foods and in subsequent food processing. In primary production, for example, it may be used to irrigate crops, in the watering of livestock or in aquaculture.

Later, during food processing, it may be used as an ingredient, transport medium or hygiene aid. However, like any food ingredient or aspect of the environment in food production, water is also a potential source of microbiological contamination and, therefore, risk to the consumer.

Risks to humans associated with the use of contaminated water during the production of foods include:

- Ingestion of foods to which contaminated water has been added as an ingredient.
- Ingestion of foods irrigated with, or harvested from contaminated water.
- Ingestion of foods that have come into contact with contaminated water during processing.

Waterborne micro-organisms

Water used in the food industry may come from three primary sources: it may originate from ground water or surface water sources or it may be from the main municipal supply. It is, therefore, treated or untreated (raw) prior to use. Raw water is likely to contain micro-organisms, many of which may be harmless to humans.

However, some sources, particularly surface water, can be contaminated with human or animal faecal material and is a potential source of harmful, or pathogenic, micro-organisms.

Even water that is treated to remove harmful micro-organisms, such as municipal water supplies, may subsequently become contaminated due to improper storage or distribution systems or leakage from dirty water systems to clean water systems.

Food manufacturers are therefore required to ensure that the water used within their facilities is at no stage a potential threat to the microbiological quality of their products.

Guidelines for assessing the suitability of various water sources for different uses within the food industry are available from



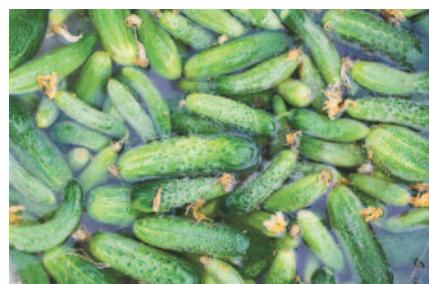
the International Life Sciences Institute. In addition, the European Directive for Drinking Water prescribes standards for the quality of water intended for human consumption (i.e. water offered for sale in bottles or containers and water used in the production of food) and the WHO Guidelines for Drinking Water Quality also sets microbial targets for potable water.

Many water sources contain micro-organisms, but these are not always harmful or detrimental to quality.

Microbiological investigations to measure total microbial counts will determine if levels fall within acceptable limits, but more specific investigations for indicator organisms or known waterborne pathogens will determine whether the water is a potential risk to consumers.

Indicator micro-organisms

The presence of certain micro-organisms in water, such as *Escherichia coli* and coliform bacteria, is used to determine the microbio-



logical quality of water. Coliforms are abundant in soil and natural vegetation, and are also present in the animal intestine. *E. coli* is seldom found outside the intestine unless faecal contamination has occurred.

These organisms indicate contamination with faecal material (sewage) from animal or human sources, and their presence (even small numbers of non-pathogenic species) in a water sample indicates that potentially dangerous pathogens may also be present.

Despite being present in large numbers in faecal material, levels of indicator organisms in water will be significantly diluted, so numbers are likely to be much lower.

Therefore, in order to isolate any contamination present, large volumes of water need to be tested.

This is usually achieved by filtering the water through a membrane. The membrane is then placed on a suitable culture medium, such as Membrane Lactose Glucuronide Agar (MLGA).

This medium contains selective agents to inhibit the growth of interfering, Gram positive micro-organisms, and identification of *E. coli* and coliforms is facilitated by two biochemical reactions within the medium:

- Lactose fermentation; detected by the dye, phenol red, which results in yellow colonies from the drop in pH.
- Glucuronidase activity; detected when the chromogen in the medium is cleaved by the enzyme, glucuronidase, producing blue coloured colonies.

Coliforms are lactose positive, producing yellow colonies, and *E. coli* is both lactose

and produces glucuronidase, resulting in green colonies, so allowing them to be easily enumerated (see photograph).

Enterococci are also used as secondary indicators of faecal contamination in water.

These organisms are used to assess the significance of coliforms in a water sample in the absence of *E. coli*.

Their presence may help to differentiate between human and animal contamination (for example, a predominance of *E. bovis* and *E. equines* would indicate pollution due to animal excrement), and they are also indicative of recent contamination, since they survive for only a short period of time outside their natural habitat.

Enterococci can be grown and enumerated on culture media, such as Slanetz and Bartley Medium.

Another useful indicator organism for determining water quality is *Clostridium perfringens*.

Although *C. perfringens* is generally present in faeces in much lower numbers than *E. coli* and enterococci, it is often used in addition to other indicators, as it produces resistant spores that survive in water and the environment for much longer periods than the vegetative cells of *E. coli* and coliforms. Its presence, therefore, is an indication of remote or intermittent pollution.

It is also used to assess the effectiveness of the chlorination process, since bacterial spores (and protozoan cysts) are more resistant to chlorination than enteric viruses and enteric bacteria.

A suitable culture medium for the growth of *C. perfringens* is Membrane-Clostridium perfringens (m-CP) Agar, which is a simple membrane-filtration method.

The use of indicator organisms, such as *Escherichia coli* and coliform bacteria, as a means of assessing the potential presence of pathogens in water, is key to protecting public health.

However, the inclusion of tests for other bacteria, such as enterococci and *Clostridium perfringens*, can give vital clues as to the

source of any contamination or breakdown in processing efficiency.

Practices and procedures for sampling and methods for the isolation and enumeration of coliforms, *E. coli*, enterococci, and clostridia are published by the UK Environment Agency.

Pathogenic micro-organisms

Water that has been disinfected adequately should be free from pathogenic micro-organisms. However, raw water or subsequently contaminated water may be a potential source of disease.

Waterborne pathogens can be divided into three main groups:

- Bacteria – such as *E. coli* O157, *Salmonella* spp, *Shigella* spp, *Campylobacter* spp and *Vibrio cholerae*.
- Viruses – including enteric viruses (for example, rotavirus, Norwalk-like viruses, calcivirus and adenovirus) and hepatitis A.

- Protozoa (parasites) – such as *Giardia* spp, *Cryptosporidium* spp, *Cyclospora* spp and *Entamoeba histolytica*.

A number of selective culture media are recommended for the isolation and identification of bacterial pathogens.

In addition, toxins produced by certain micro-organisms, such as the enterotoxin produced by certain toxin producing strains of *E. coli* and *V. cholerae*, can be detected using techniques like reversed passive latex agglutination (RPLA).

Viral and protozoan pathogens can be detected using a number of techniques, including microscopy, molecular methods (such as PCR) and immunological assays.

Prevention of contamination

Water is essential to food production/processing. It is important, therefore, to minimise any risks that it may present by adopting appropriate control measure, such as HACCP.

It is the responsibility of the food manufacturer to understand the water supply, to ensure its fitness for use and to ensure that contamination cannot occur during storage or distribution.

One way in which water can be made safe from microbiological hazards is by using disinfectants, for example chlorine dioxide or ozone.

● Chlorine dioxide is commonly used as a disinfectant for surface waters with odour and taste problems. It is an effective biocide at low concentrations (as low as 0.1 ppm) and over a wide pH range. Chlorine dioxide disinfects according to the same principle as

chlorine, but with less harmful effects on human health.

● Ozone is a very strong oxidation agent. It consists of oxygen molecules with an extra oxygen atom. When ozone comes into contact with odours or micro-organisms, it breaks them down by means of oxidation.

The third oxygen atom of the ozone molecules is lost in this process, leaving harmless oxygen molecules.

Such water disinfectants are used in various industries. Ozone is used in the preparation of ultra-pure water, for example in the pharmaceutical industry, whereas chlorine dioxide is used primarily for drinking water preparation and for the disinfection of piping. Other disinfectants used in the treatment of water include UV and chloroisocyanurates.

Safeguarding consumers

The presence of indicator or pathogenic organisms in water used within the food industry warrants further investigation and appropriate action to remove any risk to consumers. This may require investigation of treatment, storage and distribution systems, and initiation of thorough disinfection procedures.

Whatever stage in the food production process that it is used, untreated or incorrectly handled water should be considered a potential source of microbiological contamination, and every care should be taken to ensure its safety. This is important, not only for protecting the health of the public, but also for protecting the reputation of the food manufacturer. ■

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References

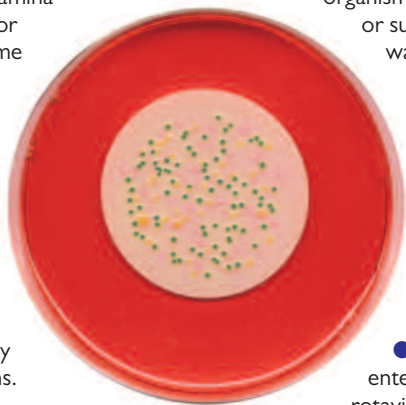
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● European Commission, the Drinking Water Directive (DWD), Council Directive 98/83/EC http://ec.europa.eu/environment/water/water-drink/index_en.html

● WHO Guidelines for drinking water quality http://www.who.int/water_sanitation_health/dwq/guidelines/en/

● UK Environment Agency. The microbiology of Drinking Water, parts 1-10 <http://www.environment-agency.gov.uk/>

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***E. coli* and coliform colonies differentiated on MLGA.**

