

# MICROBIOLOGICAL ANALYSIS

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## Indicator Organisms

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### Keywords:

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## Introduction

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Routine examination of meats for a multitude of potential pathogens is impractical, yet regular testing for selected pathogens may be necessary when evidence suggests that they may be present. Difficulties in pathogen detection due to their low concentration and uneven distribution in food samples have prompted the use of 'indicator microorganisms'. Such an organism 'indicates' the possibility that a pathogen may present below the limit of detection in a given food sample (or related sample). There is still controversy over the degree to which the presence of various indicator organisms actually indicates presence of enteric pathogens.

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This article will provide a brief overview of the historical development of indicator organisms, reasons for using indicators, and the qualities of an acceptable indicator. The benefits and limitations of indicators for detecting hazardous contamination of raw materials, assessing the adequacy of processing treatments to reduce or eliminate dangerous pathogens, and evaluating the recontamination of meat products will be discussed. The article will conclude with a brief discussion of the use of indicator organisms in a Hazard Analysis and Critical Control Points (HACCP) and regulatory systems.

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## Historical Perspective

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To understand the current application of indicators in meat systems, it is desirable to understand their earli-

est use in food safety. The concept of indicator organisms had its beginnings in the early history and development in shellfish sanitation. As early as 1603, oysters and other shellfish were being implicated as the vehicle for typhoid fever and other enteric diseases, but it was not until the late nineteenth century that many European scientists began routinely collecting bacteriological and epidemiological data to conform the correlation. By 1879, it was widely accepted by European health officials that 'typhoid bacillus' (now known as *Salmonella* Serovar Typhi) was present in raw sewage. If this sewage was dumped into oyster beds, subsequent contamination could cause typhoid fever in individuals consuming oysters from those beds. Between 1904 and 1909, 124 of 855 typhoid cases in Birmingham, England, implicated mussels as the source of infection. Bacterial examination of mussels in the Birmingham markets over the course of a year found a correlation between the quantity of microorganisms and the purity of the mussel harvest sites. This in turn led to the development of a classification system that was based on (i) the total number of microorganisms, (ii) the number of *Bacillus* spores, (iii) the number of glucose-fermenting organisms, and (iv) the number of *Streptococci* detected in mussels and harvest beds.

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Concurrent with European activities, consumption of shellfish in the United States was high, interest in sanitation was low, and the number of typhoid fever outbreaks was rising steadily. A well-documented typhoid fever outbreak on a Connecticut college campus involving oysters led investigators to begin microbiological testing of shellfish in the United States.

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At that time it was very difficult to compare results between laboratories as no standard microbiological methodologies had yet been developed. The American Public Health Association charged a committee to provide the industry with a system for scoring bacteriological results from the newly developed most probable number (MPN) technique on *Bacillus coli* (now *Escherichia coli*) in 1909; however, this committee was not prepared to give any sanitary significance to these MPN scores.

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Investigations into controlling shellfish contamination continued periodically and the issue was finally given priority after outbreaks in Chicago, New York and Washington, DC in 1924. One year later, a committee appointed by the National Shellfish Sanitation Program (NSSP) in 1925 recognized that *B. coli* was often being used as an indicator of pathogens

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of faecal origin, yet it was only ‘roughly related to the presence of disease-causing germs’. The committee did agree however, that the level of *B. coli* in fresh shellfish was a fair index to the cleanliness or contamination of the waters from which they originated. Subsequently, various guidelines based on the presence and levels of *B. coli* in water were developed and implemented at the state level.

p0035 The use of *B. coli* as an appropriate indicator of contamination was by no means unanimous. Total coliforms were judged by some to be the indicator of choice on the basis of data collected on the fate of coliform bacteria within oysters. The New York State Conservation Department, for example, set acceptable limits using total coliform counts in clams. This standard was derived using an average ratio of pathogens to coliform density in drinking water supplies, the number of typhoid cases, and several other assumptions. The suitability of either standard is still an active topic of debate and the current NSSP guidelines allow a water quality standard based on either total or faecal coliform counts for shellfish waters.

## s0015 Objectives of Indicators

p0040 The main objective for any use of microbial indicator organisms is to assess the microbial quality of a food product. Indicators may also revealing flaws in process controls that could allow food to be contaminated or a pathogen to multiply to dangerous levels. A specific pathogen may not be present in the sample being tested, but the presence of indicator organisms is meant to suggest that pathogens have a reasonable likelihood of occurring in other samples of the same product. There are many types of organisms that may be used as indicators; [Figure 1](#) shows the relationships between such organisms.

## s0020 Qualities of a Good Indicator Organism

p0045 For an organism to be considered of optimal use in identifying potential hazards, it must fulfil many requirements. The source of the indicator organism should be known. If an organism is being used to indicate faecal contamination or pathogens of faecal origin, it must be from a faecal source. Ideally, the indicator will always be present when the hazard is present.

p0050 The indicator organism should also behave physiologically in a similar manner to the pathogen of interest; inactivation and growth rates should be similar, as should their resistance to environmental conditions. If the indicator organism multiplies rapidly at the normal storage temperature of the meat product

but the pathogen does not, indicator values will be skewed. Additionally, if the indicator organism is inactivated more quickly during processing than is the pathogen, the utility of the indicator is reduced. Ideally, a change in pathogen concentration should result in an appropriate increase or decrease of the indicator organism.

An indicator organism should be easy to culture and differentiate from any other microorganisms normally present in the food. There should be standard methods of detection for this organism and they should be simple, reliable, accurate, rapid and widely accepted. If the tests are either more difficult or less accurate than those that test for the pathogen of concern, then little is gained. An optimal indicator organism will be present in the food at levels that can be correlated with the pathogen but will be absent from foods not contaminated with the hazard. While such a ‘wish list’ is nice in principle, there is no single indicator organism capable of meeting all of these requirements. p0055

## Common Uses Indicator Organisms

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### Identification of Contaminated Raw Materials

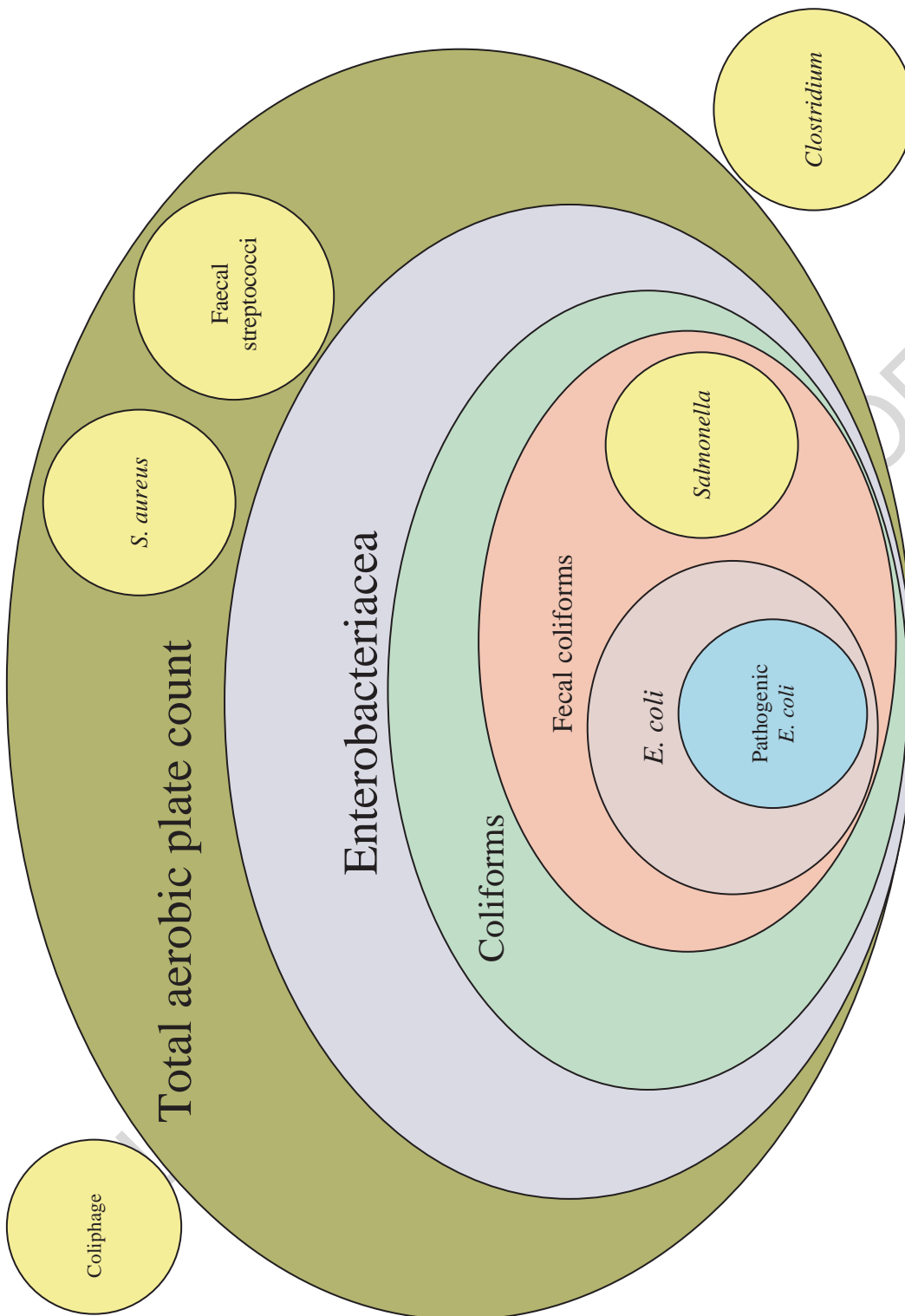
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The quality and safety of a finished meat product is highly dependent on the raw materials used. Indicator organisms are often used to assess quality and identify hazardous contamination of carcasses and raw meat products. Aerobic plate count (APC) can help evaluate the impact of time–temperature history or slaughter sanitation conditions if these factors are unknown. If counts are very high or vary widely among samples from different lots or within the same lot, inadequate microbiological control during processing or transport may be one explanation. While APC has some utility, it is generally less accurate than other indicators in indicating the presence of pathogens in meats. Normal and spoilage microflora are typically included in the APC, so APC values are skewed when these counts are high. p0060

Cross-contamination of meat with coliforms originating on hair, hoofs or hide during slaughter can be very common and has thus been responsible for their suggested use as an indicator of enteric pathogen contamination. Total coliforms include all aerobic and facultative anaerobic Gram-negative, non-spore-forming bacilli that have the ability to ferment lactose to acid and gas within 48 h at 35 °C. This group is classified according to biochemical reactions, not genetic relationships; thus coliforms do not represent any specific taxonomic group. Organisms included in this group are *Escherichia coli*, *Citrobacter freundii*, *Enterobacter aerogenes*, *E. cloacae* and *Klebsiella* p0065

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**Figure 1.** The relationships between typical indicator organisms

*pneumoniae*. Coliforms are relatively easy to detect, yet classical methods are targeted at ‘typical’ organisms and sacrifice accuracy for speed. Coliforms inhabit both human and animal intestinal tracts, and

have been associated with both faecal and nonfaecal matter.

It is very difficult to avoid *Escherichia coli* contamination of carcasses during slaughter owing to the

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organism's close association with animals. *E. coli* is considered the most prominent faecal coliform. Some believe it is the only indicator that should be used to suggest the presence of pathogens of faecal origin. A limitation to utilizing *E. coli* as an indicator of pathogens of faecal origin is the cost and complexity of *E. coli* testing over that of other methods. As with other indicators, the presence of *E. coli* does not guarantee that pathogens of faecal origin are present, nor does its absence ensure that contamination has been avoided. The use of *E. coli* as the sole indicator of cross-contamination events during slaughter has not been advised.

A correlation between faecal streptococci and enteric pathogens in unprocessed, raw meats has also been suggested. The correlation has been shown to be much better than with meats that are heated, cured, frozen or dried. Faecal streptococci (or enterococci) are Gram-positive, catalase-negative cocci from selective media that grow in bile-aesculin agar at 45 °C and are loosely referred to as faecal streptococci. These organisms are able to survive outside the intestine better than coliforms. Faecal streptococci include *Streptococcus faecalis*, *S. faecium*, *S. equinus*, *S. mitis*, *S. salivarius* and *S. avium* as well as numerous *Enterococcus* spp. The correlation of faecal streptococci with enteric pathogens in meat products is still controversial. Faecal streptococci may be found in the intestinal tracts of humans and warm-blooded animals and on hide, hair, hoofs and feathers of animal, and are widespread in nature. Their universal presence may restrict their use as an indicator of pathogens of faecal origin. The presence of *S. bovis* and *S. equinus* indicates animal pollution, possibly during slaughter, as neither is associated with humans.

*Aeromonas* spp. have been investigated for use as an indicator of hygiene in a processing plant. Increasing numbers of *A. caviae* or *A. hydrophila* on processing equipment such as dehairing machines, mesh gloves and belts could indicate inadequate sanitation. *Aeromonas* spp. were also identified as possible indicators for assessing carcass dressing procedures during swine slaughter.

The presence of spore-forming bacteria suggests the possible presence of *Clostridium perfringens*, *C. botulinum* or *Bacillus cereus*, all of which have been associated with meat and meat-containing products. Spores present in raw meat have the ability to survive thermal processing and proliferate in finished meat products.

A high degree of correlation between faecal coliforms and F<sup>+</sup>RNA coliphage counts has been seen while monitoring the microbiological quality of raw meat and poultry. Since methodologies for con-

centrating and detecting coliphages are relatively simple and rapid, this new technology has shown promise as a future indicator of pathogens of faecal origin.

#### Adequacy of Processes to Destroy Pathogens

APC is often used to test sanitation on a processing line during production. Ingredients may be sampled before or after addition to the product, or a complete product may be sampled before and after processing or during or after a period of delay. In addition, APC may be used to identify key steps involved in contamination during processing; APC will generally be low or at baseline levels prior to the processing step causing the problem, but will be significantly higher after contamination has occurred.

Additionally, thermal processing will inactivate a great number of vegetative organisms present in raw materials, but some spore-forming pathogens are highly heat-resistant and will probably survive most cooking operation. Most microorganisms found on meat are also very sensitive to drying and freezing, and are thus less useful as indicator organisms for frozen or dried products. It may not be advisable to use APC as a microbial indicator in fermented meats because these products may have high APC from the starter cultures used in their manufacture. That being said, starter organism colonies do have a different appearance on agar plates, so experienced technicians may still be able to differentiate high counts due to contamination versus there due to the starter culture itself.

Another group utilized to indicate inadequate processing is the family. The *Enterobacteriaceae* have been classified as Gram-negative, glucose-fermenting, oxidase-negative, usually catalase-positive, and nitrate-reducing organisms. This includes many bacteria commonly associated with faecal matter, but also includes many nonfaecal organism. Common genera of this family include *Citrobacter*, *Enterobacter*, *Erwinia*, *Escherichia*, *Hafnia*, *Klebsiella*, *Proteus*, *Providencia*, *Salmonella*, *Serratia*, *Shigella* and *Yersinia*. The detection of any member of the *Enterobacteriaceae* family present in a meat product has been used to imply the presence of enteric pathogens, but a direct correlation between *Enterobacteriaceae* presence and concentration to enteric pathogen presence and concentration is not assured.

*Enterobacteriaceae* levels have been shown to be very similar to coliform concentrations, as there is a great deal of species overlap between these two groups. *Enterobacteriaceae* may also be used to evaluate hygiene and sanitation in processing plants, as they are readily inactivated by sanitizers and capable

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of colonizing a variety of niches when sanitation is inadequate. However, the presence or absence of high concentrations of these organisms cannot confirm the presence or absence of enteric pathogens.

p0115 Faecal coliforms, a subset of the total coliforms group, have the ability to multiply in hospitable environments, are destroyed by pasteurization and normal cooking, and are typically inactivated by freezing conditions, thus making them a candidate indicator for analysis of process controls. Faecal coliforms are defined as Gram-negative bacilli that ferment lactose within 48 h at 44.5–45.5 °C. Multiplication at higher temperatures often results in this group being referred to as ‘thermotolerant coliforms’. This group includes *E. coli*, *Enterobacter* spp., *Klebsiella pneumoniae* and *Citrobacter freundii*. Testing for faecal coliforms arose from attempts to find rapid, dependable methods to detect *E. coli* without the need to isolate a pure culture. Faecal coliforms are generally specific to the intestinal environment of warm-blooded animals and thus high levels are found in faecal matter, but some organisms of non-faecal origin will occasionally show up in testing as faecal coliforms, so care must always be taken in interpreting test results. Faecal coliforms are relatively easy and reliable to detect.

p0120 *E. coli* has been shown to have similar rates of inactivation during thermal processing as enteric pathogens including *Salmonella* spp. and thus has been suggested as an indicator of process control. As new meat processing technologies (e.g. irradiation) are developed, care will need to be taken to ensure that any difference in sensitivities is considered before determining the final suitability of *E. coli* as an indicator.

p0125 Faecal streptococci have been used as evaluators of frozen-food plant sanitation owing their ability to survive freezing. They have also been used as indicators of adequate sanitation for their ability to resist inactivation by thermal processes, drying, detergents and disinfectants. However this increased resistance can lead to undesirable stringency, as they outlast less durable pathogens such as *Salmonella* and *Shigella*.

#### s0040 Identification of Recontamination of Processed Products

p0130 High numbers of coliforms may be representative of improper handling or storage of meat products, which allows for the multiplication of any coliforms present. High counts of coliforms on cooked meat products would indicate post-processing contamination. High levels of *Enterobacteriae* suggest post-processing contamination or microbial proliferation due to inadequate storage conditions.

The association of *Staphylococcus aureus* with human skin and oral–nasal cavities has prompted its use as an indicator of inadequate employee sanitation and contamination due to handling. It has also been suggested as an indicator for detecting multiplication of bacteria during prolonged storage of processed meats without refrigeration. p0135

#### Microbial Indicators and HACCP

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HACCP plans are currently required in all meat and poultry plants within the United States. The use of indicator organisms can offer assistance for establishing, monitoring and verification of critical control points in HACCP operations. Indicators can best be used within a HACCP system to control processes that have the greatest influence on the level of microorganisms rather than on determining whether to accept or reject a given lot of product. When used for critical control point evaluation, a large number of samples can be progressively collected throughout a process. HACCP can use index organisms to assess the integrity of an evisceration procedure or thermal process by determining levels of indicator organisms before and after each process is completed. Indicator organisms can be used to establish upper limits for pathogen numbers; thus, actions should be implemented that strive to reduce indicator organism numbers to the lowest level possible. It is widely accepted that the regular monitoring of process controls in the meat industry must replace end product testing. p0140

#### Regulatory Issues

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Current regulations require that meat and poultry processing plants test for both *Salmonella* spp. and generic *E. coli* within their HACCP plans. Testing for *Salmonella* spp. has been mandated to confirm that plants are controlling pathogens within meat plants. Slaughter plants are also required to sample carcasses for generic *E. coli* to verify the prevention and removal of faecal contamination from raw meat. Generic *E. coli* was chosen because of its suggested association with pathogens of faecal origin and the relative ease and low cost of enumeration. Although most global regulatory authorities do not require microbiological testing of raw meat products, processors must meet USDA regulations for import into the United States. Australia and New Zealand, which do require testing, were able to establish regulations for food quality and safety through government and industry collaboration. p0145

s0055 **Conclusions**

p0150 None of the indicators mentioned above are obligate inhabitants of the intestinal tract of humans or warm-blooded animals. Environmental reservoirs for each have been identified. Many of the organisms mentioned here are commonly present in meat manufacturing facilities and may become part of the natural microflora. Since no one indicator can be applicable for all pathogens or all situations, it has been suggested that several indicator organisms can be used for several different pathogens. Whatever decision is made regarding the use of indicator organisms in the meat processing plant, it should be made with the full knowledge of both the benefits and limitations of the microbial indicators. No microbiological specification, no matter how perfect, should ever be used blindly or without common sense. Finally, a trained food microbiologist should be consulted when implementing or changing the use of microbial indicators in a food processing environment.

See also: **Microbiological safety of meat:** salmonella spp. (00051); *Escherichia coli* 0157:H7 (00052); *Clostridium botulinum* (00053); *Clostridium perfringens* (00054); Thermostolerant campylobacter (00055); *Listeria monocytogenes* (00056); *Yersinia enterocolitica* (00057); *Staphylococcus aureus* (00058); *Bacillus cereus*

(00059); Yeasts and moulds (00060); Prions and viruses (00061); Parasites (00062). **Microbiological analysis:** Sampling/testing (00090). **Irradiation** (00174). **Microbiological safety of meat:** *Aeromonas hydrophila* (00251).

**Further Reading**

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