Abstract

The original use of “indicator” organisms to signal the possible presence of pathogenic bacteria dates back more than a hundred years. Since then, the use of these organisms as predictors of pathogens has been extended from water to a variety of raw and processed food products. When used to predict the possible presence of pathogens, the term “index organism” is now preferred; “indicator organisms”, in comparison, are used as a means of assessing process integrity. Various index organisms have been used, including: coliforms, “fecal” or “thermo-tolerant” coliforms, *E. coli*, *Enterobacteriaceae*, enterococci, and “aerobic plate count”. Despite the enteric connotations associated with the names of some of these organisms, they are not confined to the intestinal tract and often represent bacteria of widely divergent origin. Some coliforms and *Enterobacteriaceae* can even grow on refrigerated muscle foods. Numerous studies have failed to find solid relationships between coliforms, *Enterobacteriaceae*, or *E. coli*, and pathogens such as *Salmonella*, *Listeria monocytogenes*, and *Campylobacter* on a variety of meat and poultry products. The few studies which have actually analyzed the predictive value of various index organisms have drawn similar conclusions. Numerous international and national advisory committees, including the FAO/WHO and the National Research Council’s Subcommittee on Microbiological Criteria, have concluded that it is invalid to attempt to predict the safety of a meat or poultry product based on the levels of APC, coliforms, “fecal” coliforms, *Enterobacteriaceae*, *E. coli*, or enterococci found on the product.

Index and Indicator Organisms: History and Terminology

For more than a century, microbiologists have used indirect tests as a means of detecting possible pathogen contamination of water and foods. These indirect assays have typically involved either aerobic plate counts (APCs) or groups of organisms (coliforms, enterococci, and *Enterobacteriaceae*) which are thought to co-exist with enteric pathogens in the intestinal tracts of humans and animals. In the 1890’s, Schardinger (1892) began testing water for *Escherichia coli*. The presence of *E. coli* was used as an indication of fecal contamination, and, possibly, of the presence of *Salmonella typhi*. Over time, such testing was extended first to dairy products, then to food products, and food processors began to use indicator assays as a means of determining the adequacy of a process. The reader is referred to Mossel (1967) for a historical review of the role of indicator organisms in the determination of food safety.

In view of the dual functions which these indirect assays served, researchers attempted to clarify the situation (Mossel, 1978). The terms “index”, “marker”, “simulator”, and “surrogate” were suggested for reference to organisms whose presence at certain levels indicates the possible occurrence of pathogens (Brodsky, 1995). In contrast, “indicator organisms” or “hygiene marker organisms” are those whose detection is indicative of a failure in GMP or integrated system control which results in a food product of unacceptable microbiological quality (Brodsky, 1995). Testing for index organisms, then, serves a predictive function while testing for indicator organisms is a means of assessing process integrity (Mossel, 1978). Although the distinction between the functions of index and indicator organisms is an important one, workers in this field have often used the terms interchangeably, leading to considerable confusion. This situation is further confounded by studies in which a particular group of organisms functions as both index organisms and as indicators. This review will use the terms “index organism” and “indicator organism” as defined above; “index/indicator organism” will be used when referring to information common to both index and indicator usages.

*J.L. Johnson, Deibel Laboratories, Inc., 103 S. Second St., Madison, WI 53704.

The most common reasons for testing for index/indicator organisms rather than pathogens deal with cost, analytical complexity, and time. Simple, inexpensive index/indicator organism analyses can yield results in 1 to 3 days as opposed to more complex traditional pathogen isolation and identification procedures which may take more than a week. Also, testing for the (presumably) more numerous index organisms minimizes sampling difficulties inherent to the detection of small numbers of pathogens amongst large populations of background microflora.

**Characteristics of Ideal Index Organisms**

An ideal index organism meets a number of criteria including:
- Being easily and rapidly detectable
- Being easily differentiated from other microflora
- Having a history of constant ecological association with the pathogen in the environment where contamination originates
- Being present when the pathogen is present and absent when the pathogen is absent
- Having a reliable and defined quantitative relationship with the pathogen of concern
- Having growth requirements and a growth rate identical to those of the pathogen of concern
- Reacting in a similar manner to adverse conditions/processing as the pathogen of concern; greater resistance to adverse conditions is advantageous.

Index organisms used to indicate the presence of enteric like *Salmonella* must also originate only in the intestinal tract, occur in feces in high numbers, and be incapable of sustained growth outside the intestinal tract.

**Types of Index Organisms Used and Their Limitations**

Six groups of index/indicator organisms have seen service in assessing the microbiological safety of raw meat and poultry. These groups of organisms are “total” aerobic count (APC), coliforms, “fecal” or thermotolerant coliforms, *E. coli*, enterococci (sometimes referred to as “fecal” or “Group D streptococci”), and *Enterobacteriaceae*.

Fecal matter has long been regarded as the main source of *Salmonella*, *Campylobacter*, and some pathogenic *E. coli* on raw meat and poultry. Although it is commonly assumed that coliforms, “fecal” coliforms, *E. coli*, enterococci, and *Enterobacteriaceae* (a group which includes *E. coli* and many coliforms) originate in the animal gut or feces, there is little direct proof for this with respect to foods (FAO/WHO, 1979). Despite the enteric and fecal connotations of their names, these organisms are not confined to the intestinal tract, making them unreliable direct indices of fecal contamination (Chordash and Insalata, 1978; Hartman et al., 1992; Jay, 1992; Roberts, 1976). The fact that *Enterobacteriaceae*, coliforms, and enterococci are common on raw meats, even those produced under the most hygienic of conditions (Jay, 1992; Roberts, 1976; Tompkin, 1982), casts doubt on their value as index organisms (Cox et al., 1988).

Some members of the coliform and *Enterobacteriaceae* groups are capable of growth at refrigeration temperatures (Buchanan et al., 1992; Gustavsson and Borch, 1993; Jay, 1992; Roberts, 1976), making them unsuitable for use as index/indicator organisms on stored muscle food products (Goepfert, 1976; Zeitoun et al., 1994). Nortjé et al. (1990) have even stated that “*Enterobacteriaceae* might be common psychrophils in the meat production chain, maybe originating from the abattoir and wholesale environments.” In contrast, *E. coli* generally does not grow at refrigeration temperatures, and growth of this organism may be indicative of improper storage conditions (Gill and McGinnis, 1993). On vacuum-packed mutton, Australian researchers observed an increase in coliform counts of more than 10⁶ CFU/cm² over a 2 week period at 5°C with no corresponding increase in *E. coli* (Grau, 1979). Numbers of enterococci usually do not increase during handling of raw meats as long as proper storage temperatures are maintained (Stiles et al., 1978).

Much of the controversy over the use of index/indicator tests, especially those targeting organisms thought to be indicative of “fecal” contamination, can be traced to the relatively non-specific nature of such tests. The majority of these assays depend on simple, easily-detectable biochemical reactions which give no information about the ecological origin of the reactive organism or about the taxonomy of member species. The coliforms in general, and the “fecal coliforms” in particular, are ill-defined agglomerations of organisms with vastly divergent ecological associations (Mossel and van Netten, 1991). Newton (1979) cautioned against the use of coliform assays for detecting fecal contamination on raw meats, noting that positive reactions could be obtained from a variety of non-fecal coliforms as well as from psychrotrophic coliforms and *Aeromonas* spp. Media, methods, and incubation temperatures all have a tremendous impact on assays for index/indicator organisms, and results are often highly dependent on the method used (Hitchins et al., 1992). Since coliform, “fecal” coliform, *Enterobacteriaceae*, and enterococci assays are often used without additional confirmational testing, methodology has a tremendous impact on the sorts of organisms which will be enumerated from a particular sample (Mossel, 1978). When different testing methodologies for a particular group of index organisms enumerate different microbial populations, it is unrealistic to expect that a defined relationship will be maintained between numbers of index organisms and pathogens.

Despite the general simplicity of index/indicator assays, much effort has been expended to further streamline the tests and decrease the time interval before results are obtained. Development of the “fecal” coliform test, for instance, arose from a desire to assay *E. coli*-like organisms without having to do the time-consuming IMViC tests necessary to confirm *E. coli* (NRC, 1985). The usefulness of the “fecal” coliform test as a rough means of estimating numbers of *E. coli* varies...
with the product, the point in the processing or distribution chain at which the product is sampled, and the methodology used (Buchanan et al., 1992; Stiles and Sheena, 1987; Weiss et al., 1983).

Apart from difficulties specific to microbial physiology and detection methodologies, the efficacy of index/indicator assays is also impacted by the non-homogeneous distribution of both index organisms and pathogens in/on meat and poultry. Bacterial contamination on carcasses is often focused in “hot spots” (Ingram and Roberts, 1976), and foci of index organisms may differ from pathogen foci. The inability to collect a truly representative sample extends from carcasses to primals to ground products. Goepfert (1976) demonstrated that E. coli are not homogeneously distributed in ground beef. Numerous scientists (including Goepfert, 1976; Ingram and Kitchell, 1970; Ingram and Roberts, 1976) have also issued cautions about the imprecise nature of quantitative microbiological assays and the impact that such imprecision can have on the validity of index organism testing. Kilsby and Pugh (1981) questioned whether differing levels of variance associated with counts of index organisms and pathogens might make it impossible to reliably determine the relationship between the two groups of organisms.

**Use of Index Organisms in Assessing Food Safety**

Since the original use of index organisms for indicating possible water contamination, the use of index organisms has been extended to a variety of raw and processed products. The question of whether broadening this practice was scientifically valid seems to have drawn little attention (Cox et al., 1988; Dack, 1956). Likewise, while the presence of index/indicator organisms in a heat-treated food may reliably indicate recontamination or abuse, it may not be valid to extrapolate this to a raw muscle food which has received no bactericidal treatment (Banks and Board, 1983; Goepfert, 1976). While it is generally agreed that the selection of an index organism must be based on the microbial ecology of the particular food being analyzed, this condition has rarely been met in practice.

**Relationship Between Index Organisms and Bacterial Pathogens**

*E. coli* is often regarded as being the best index organism of fecal contamination among the commonly-used fecal/enteric index organisms. This statement, however, does not apply to raw meat and poultry (Goepfert, 1976; Holland, 1979; Tompkin, 1983; NRC, 1985). Numerous studies have found no evidence of a relationship between *E. coli* and enteric pathogens like *Salmonella* on raw meat and poultry (Childers et al., 1977; Miskimin et al., 1976; Roberts, 1976; Solberg et al., 1977). Similarly, no relationship was observed between *Salmonella* and *E. coli*, coliforms or APC on ready-to-eat meat products (Childers et al., 1977; Miskimin et al., 1976; Solberg et al., 1977). Paradis and Stiles (1978) found no relationship between APC, coliform count, *E. coli*, or enterococci and *Salmonella*, *Clostridium perfringens*, or coagulase-positive *Staphylococcus aureus* in vacuum packaged sliced bologna. An examination of pathogen naturally contaminated with *Listeria monocytogenes* revealed no correlation with coliforms (Morris and Riberio, 1991). The use of “enteric” index organisms to indicate the presence of *L. monocytogenes*, *Yersinia enterocolitica*, and *S. aureus* may not be valid as these pathogens have numerous reservoirs other than the intestinal tract. Also, *L. monocytogenes* and *Y. enterocolitica* are capable of growth under refrigeration, making selection of an appropriate index organism even more difficult.

It has been shown that *Enterobacteriaceae* are poor indices of *Salmonella*, pathogenic *Y. enterocolitica*, and *Campylobacter* spp. in raw ground meat products (Beumer et al., 1983). A number of studies have failed to uncover a relationship between levels of *Enterobacteriaceae* or coliforms and *Salmonella* on broiler chickens, turkey meat, and various raw beef and pork sausage materials (Banks and Board, 1983; Hagberg et al., 1973; Mercuri et al., 1978). In a study on hamburgers, Tamminga et al. (1982) concluded that even when *Enterobacteriaceae* counts were low, a “considerable percentage of samples” may still contain *Salmonella*. Brodsky (1995) reported data from the analysis of more than 70,000 ready-to-eat food samples in Ontario. No correlation was evident between the levels of APC, coliforms, *E. coli*, and *Enterobacteriaceae* and a variety of food borne pathogens (*S. aureus*, *Bacillus cereus*, *Salmonella*, *C. perfringens*, *Campylobacter jejuni*, *Y. enterocolitica*, and *E. coli* O157:H7).

Only relatively few studies have found positive relationships between index organism counts and the presence of pathogens. Further, those pathogens which did exhibit a positive correlation were generally *C. perfringens* or *S. aureus*, rather than “true” enteric pathogens such as *Salmonellae*. Hagberg et al. (1973) reported that coliforms isolated from various turkey products tended to be “associated with higher numbers of *C. perfringens* and *S. aureus*.” Miskimin et al. (1976) found a high correlation between *C. perfringens* and coliforms in ready-to-eat foods, and *C. perfringens* and *E. coli* in both raw and ready-to-eat foods, as well as a high correlation between *S. aureus* and both coliform counts and *E. coli* counts in ready-to-eat foods. These authors cautioned, however, that “the number of indicator organisms present has essentially no relationship to the number of pathogens which may be present in a food sample.” Dempster (1978) reported that *E. coli* levels associated with 8 *Salmonella*-positive samples of raw and processed meat were higher than levels on *Salmonella*-negative samples. Nesbakken et al. (1985) found a significant correlation between high coliform counts and the presence of *yersinia* in raw pork products, but only one of the *yersinia* isolates was found to be potentially pathogenic.

In light of the limitations associated with the use of common index/organisms, some attention has been given to the possibility of one pathogen being used as an index of an-
other. Turnbull and Rose (1982) found no correlation between isolations of Salmonella and Campylobacter from raw red meats. The fact that various pathogens react differently to slaughtering, processing, or sanitation interventions further complicates the issue. Oosterom et al. (1985) attributed the differences in the prevalences of Salmonella and Campylobacter in a pork slaughterhouse to better survival of Salmonellae on surfaces and equipment.

Relationship Between APC and Bacterial Pathogens

The notion that APC may serve as an indication of the presence of pathogenic bacteria may have its origin in common mathematics - higher numbers of generic bacteria would include higher numbers of pathogens. Evidence for this idea is lacking, however (Elliott and Michener, 1961; Roberts, 1976; Silliker, 1963). Vorster et al. (1991) found no relationship between APC of broiler chickens or ground beef and the presence of Salmonella or L. monocytogenes. In a subsequent study, Vorster et al. (1994) found no relationship between APC of a variety of raw and processed meat and poultry products and the occurrence of S. aureus and/or E. coli. Doubtless, the lack of correlation between APC and most bacterial pathogens (excluding psychrotrophic types such as L. monocytogenes and Y. enterocolitica) is at least partly due to temperature — APCs on meat and poultry increase with time in refrigerated storage while levels of most bacterial pathogens do not.

I am aware of only one paper reporting a relationship between APC and enteric pathogens on red meats. Turnbull and Rose (1982) found some evidence of an association between high APC and the presence of Campylobacter in raw red meats but cautioned that only 22 samples were involved.

Interestingly, there is some evidence of a relationship between APC values and levels of C. perfringens and/or S. aureus in raw meat products (Solberg et al., 1977; Beumer et al., 1983). Some of this relationship may be attributable to the fact that assays for C. perfringens and S. aureus often involve little or no confirmational testing, meaning that other organisms may also be enumerated. Gilbert et al. (1993) reported an association between high APC counts and the presence of L. monocytogenes in refrigerated patés. Similarly, Vorster et al. (1993) found that processed meat products having APCs of between 10^5 and 10^7 CFU/g were more likely to contain Listeria spp. than were products having higher or lower APCs; L. monocytogenes was not isolated, however.

Relationship Between High APCs and Foods Likely to Cause Illness

To date, there are few data to indicate that a raw product containing high levels of generic microflora would be more likely to cause food borne illness than a product with less total contamination. Since most food borne pathogens are poor competitors (Goepfert and Kim, 1975; Mattila-Sandholm and Skyttä, 1991), a raw product containing high levels of spoilage organisms may be unlikely to serve as a vehicle of food borne illness because pathogens would be unable to multiply to hazardous levels before the consumer rejected the product as spoiled. This is the argument put forth by Goepfert and Kim (1975) and Jay (1994). The situation with processed products differs, especially in the case of products contaminated with psychrotrophic pathogens such as L. monocytogenes. If storage conditions are such that L. monocytogenes can grow, it is likely that other psychrotrophs present on a ready-to-eat product will also grow. Whether growth of non-Listeriae will be detected by APCs will depend on the packaging treatment since lactic acid bacteria growing on vacuum-packaged products will often not register on APCs.

The Predictive Value of Index Organism Assays

For the most part, those few studies which have simultaneously examined meat or poultry samples for pathogens and index organisms have analyzed the data only for correlations between levels of index organisms and the presence or absence of a particular pathogen or pathogens. The mere fact that certain levels of an index organism are correlated with the presence or absence of the pathogen tells relatively little about how well index organisms predict the presence or absence of pathogens. That determination requires a stratified analysis of the index organism data where different index standards are imposed in an attempt to determine the number of correct or incorrect decisions which would subsequently be made with respect to the pathogen-contaminated food samples. Unfortunately, very few studies have used this approach to determine the predictive utility of index organism assays. This approach mimics a "real world" situation in which levels of index organisms would be used to determine whether a sample was deemed to be acceptable or unacceptable from the standpoint of microbiological safety.

I am aware of only two published papers (Miskimin et al., 1976, and Solberg et al., 1977) which have taken this predictive approach to the analysis and interpretation of data from meat and poultry. The conclusion of both papers was that imposition of standards based on index organism levels would not guarantee the safety of either ready-to-eat or raw meat and poultry products. Not only would pathogen-containing product be released as "acceptable", but a large amount of meat and poultry would be deemed "unacceptable" based on levels of index organisms alone and removed from the food supply.

The Use of Index/Indicator Organisms

The utility of a particular index/indicator organism is determined by a variety of factors including: the product to be examined, the length of time a product has been refrigerated, and the target pathogen(s) of particular interest. Also,
the relationship between index/indicator organism numbers and pathogens may change at different points in the slaughtering or processing line (Roberts, 1980; Oosterom et al., 1983a and b; Notermans et al., 1977). Reuter (1994) indicated that the composition of the Enterobacteriaceae found on carcasses changes as a result of different operations in the slaughtering and dressing line from mesophilic organisms deriving from the live animals to ubiquitous psychrotrophic species and strains. This finding has important implications to the validity of the Enterobacteriaceae as indices/indicators at different points in the slaughter line.

Numerous attempts have been made to use index/indicator organisms for assessing the impact of chilling on pathogen growth. Grau (1979) concluded that growth of E. coli and Salmonella during chilling of red meat carcasses was sufficiently similar that increases in E. coli counts on carcasses could indicate concurrent Salmonella growth if that organism were present. As noted above, however, the utility of E. coli for assessing adequacy of refrigeration does not extend to coliforms or Enterobacteriaceae.

One important consideration in the use of index/indicator organisms is their relative survival or inactivation rate when exposed to decontamination treatments such as organic acids. Research in the Netherlands indicates that Enterobacteriaceae are slightly more resistant to lactic acid than Salmonella, Y. enterocolitica, enteropathogenic E. coli and C. jejuni, making Enterobacteriaceae valid index/indicators of decontamination treatments designed for enteric pathogens (Smulders et al., 1986). The acid-tolerant nature of E. coli makes it a good candidate indicator organism when evaluating the efficacy of organic acid decontamination treatments on carcasses (Anderson and Marshall, 1990; Greer and Dilts, 1992). Van Netten et al. (1994) concluded that aerobic colony counts were an unreliable index/indicator and Dilts, 1992). Van Netten et al. (1994) concluded that E. coli content of raw ground beef at the retail level. J. Milk Food Technol. 39:175-178.


