

## SAMPLING CARCASSES AND MEAT PRODUCTS\*

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Numerous procedures are available to determine the microbiological condition of meat surfaces and meat products (table 1). For meat surfaces, swab, rinse and direct agar contact methods or analyses of excised skin or tissue samples often are utilized. For meat products, blending of a representative portion with sterile diluent is commonly used. For specific information on these and other tests as applied to meat and poultry, the reader is referred to reviews by Favero et al. (1968), Patterson (1971), Barnes et al. (1973), Kitchell et al. (1973) and Baldock (1974). In the selection of a bacteriological testing procedure several factors should be considered (table 2), particularly the objectives of the test. Interpretation of test results may be difficult or lead to erroneous conclusions when the limitations of the test procedures are not fully understood. For example, counts obtained with two procedures may differ considerably because with one procedure, clumps of bacteria or particles containing more than one cell are broken up more extensively, resulting in higher counts. Some of the variables and limitations for the swab, rinse and direct agar contact method are listed in table 3.

With respect to the sample, it should be recognized that considerable variation can occur in number and types of bacteria on or in different parts of the carcass and cuts. Certain areas are more likely to become and/or remain contaminated during slaughtering, dressing, chilling, fabrication and transportation. Furthermore, differences exist among different meats and various parts of the same carcass with respect to pH,  $a_w$ , and lean vs. fat, which may cause differences in microbial development. In addition, the skin surface characteristics or topography of various species (beef, pork, lamb, poultry) are different. This undoubtedly will affect not only the ability of the skin or surfaces to harbor microorganisms but also the effectiveness by which microorganisms can be removed by the various testing procedures. No attempt will be made to compare the advantages and limitations of individual testing procedures for the examination of meat and poultry. Excellent reviews on the subject are already available (Barnes et al., 1973; Kitchell et al., 1973).

Different testing procedures exert different forces upon the surface of meats and thus most likely remove microorganisms at different rates. In addition, the removal and subsequent recovery of microorganisms from meat and poultry surfaces by any of the testing procedures will depend upon the forces by which the microorganisms are held in or onto the meat. An understanding of this variable requires information about the mechanism of attachment onto and detachment of bacteria from meat surfaces.

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Table 1. Methods for bacteriological testing  
of surfaces and whole food samples

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1. Excision of skin or meat tissue	a. Maceration by blending or b. Rinsing of sampling only
2. Swab methods	a. Cotton, alginate, cellulose sponge b. Metal or disposable templates c. Specific areas swabbed or "total body swab"
3. Rinse methods	a. Immersion only or b. Immersion and agitation c. Spray-gun technique d. Dye reduction tests of rinse fluid e. Whole carcass rinse or of specific areas
4. Direct agar contact methods	a. Direct agar contact method (Rodac) b. Direct agar contact medium with titanium dioxide and tetrazolium (reflectance medium) c. Direct surface agar plating medium where growth medium is poured over surface of sample, covered, and incubated
5. Skin-scraping methods	a. Sterile cylinder is pressed against carcass and diluent within cylinder is scraped against skin
6. Impression methods	a. Adhesive tape pressed against sample is stained and examined microscopically or b. Tape is transferred to agar plating medium
7. Vacuum methods	a. Vacuum removes particles and impinges them against an agar surface
8. Light scattering, radiometric and bioluminescence	

(continued)

Table 1. Continued

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| 9. Blending with sterile diluent | a. A representative sample (50g) is blended with sterile diluent (450g)<br>b. Use of stomacher for mixing<br>c. With frozen product, thaw first or use auger type bit fitted with variable speed drill |
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Table 2. Factors that influence selection of test procedure

1. The type of sample	Size, shape, nature of surface (rough, smooth, dry, wet) wettability, frozen or not
2. The objective of the test	Shelf life General bacteriological condition Total counts or specific types Effect of one or more processing steps on count Surface flora only or internal flora or both Sampling the most contaminated areas or an overall assessment
3. Microbial levels expected	
4. Presence of bactericidal compounds on surface of sample	
5. Resources available for sampling and laboratory analysis	
6. Environmental conditions during sampling (space-time, etc.)	
7. Precision and accuracy required	

Table 3. Variables within methods for bacteriological testing of food surfaces

Method	Variable or limitation
Swab method	Nature of surface (rough, smooth, wettability) Type of swab Differences in handling between technicians
Rinse method	Immersion or immersion with agitation Difficult for large carcasses except with spray gun or similar techniques Force with which microorganisms are held to surface determines detachment
Direct agar contact method	Restricted to surface with low numbers of bacteria Spreaders or fungi often cause problems Smooth surface is needed "Particles" lifted from surface may contain more than one living cell

The sorption of microorganisms to surfaces is a widespread phenomenon in nature but information about the mechanisms involved is limited. In the marine environment, for example, microorganisms often form a primary film on surfaces. This film is frequently a prerequisite for the attachment of an invertebrate fouling population. Marshall *et al.* (1971) reported that the "reversible sorption" (weakly held near the surface) of *Achromobacter* on glass cover slips depended upon the electrolyte concentration in artificial seawater. They suggested that this sorption is associated with the London-van der Waals attractive energies between two surfaces (surface and bacterium) and the electrical repulsive energies resulting from overlapping ionic atmospheres around the surfaces. "Irreversible sorption" (firmer adhesion to surface) through formation of viscous polymers (polymeric bridging) by bacteria may overcome the repulsion barrier between such surfaces. Fletcher and Floodgate (1973) demonstrated an acidic polysaccharide layer on a marine bacterium which was involved in the adhesion to surfaces. Bacteria which had attached to a surface naturally produced a secondary fibrous acidic polysaccharide. Deinema and Zevenhuizen (1971) reported exocellular cellulose fibrils on many gram-negative bacteria.

In addition, chemotaxis (Adler, 1973) may also be involved in attachment and detachment of bacteria. Chet *et al.* (1975) reported that organic compounds such as acrylamide, benzoic and tannic acid, in concentrations not toxic to bacteria, were capable of repelling motile bacteria from marine surfaces. In this paper, we direct attention to conditions which influence attachment on and detachment of bacteria from meat surfaces with special emphasis on poultry meats since more recent information is available in this area.

1. Effect of attachment time, concentration of bacteria in attachment medium, temperature, pH and bacterial types on attachment. Notermans and Kampelmacher (1974) reported on the attachment of various bacteria onto the skin of broilers. Broilers were taken after defeathering and washed with attachment medium (8.7g NaCl per liter, 0.0062M Na<sub>2</sub>HPO<sub>4</sub>, 0.0021 NaH<sub>2</sub>PO<sub>4</sub>, 0.001 M EDTA, pH 7.2). After dipping in attachment medium with bacteria and drainage for 30 sec, skin samples were taken to determine bacterial counts. The number of *Escherichia coli* k12 on the skin increased with time which indicates a constant rate of attachment. With different concentrations of *E. coli* k12 in the attachment medium, there was a direct relationship between bacterial increase per g of skin and the concentration of *E. coli* k12 in the attachment medium. Attachment of *E. coli* k12 was optimum at about 20 C, with a significant decrease in attachment rate at either side of this optimum. Comparison of the attachment of *E. coli* k12 (peritrichous flagella) with a non-flagellated mutant *E. coli* N97<sup>+</sup> indicated that the flagella and flagellar activity were associated with the attachment mechanism. The attachment rate of the non-flagellated mutant was about 20% of that of *E. coli* k12. Incorporation of glucose in the isolation medium which reduced the development of flagella also reduced the attachment. However, incorporation of glucose also reduced the pH of the growth medium to 5 which may have reduced attachment. A comparison of the attachment rates of *Lactobacillus brevis* (non-motile), *Klebsiella* (non-motile), *Pseudomonas nigrifaciens*

(monotrichous, polar flagella), Pseudomonas putrefaciens (Peritrichous flagella) and Pseudomonas EBT/2/143 (lophotrichous, polar flagella) indicated that the flagellated species attached at much higher rates than the non-flagellated bacteria. Optimum attachment was at about 20 C regardless of whether the bacteria were mesophilic or psychrotrophic. When the pH value of the attachment medium was adjusted to values ranging from 4.45-9.3, optimum attachment occurred in most cases at about pH 8.0

2. Heat resistance of attached and unattached bacteria--Effect of processing on bacteria acquired before and after scalding. Notermans and Kampelmacher (1975a) studied the heat resistance of attached bacteria by placing skin samples in physiological saline at the desired temperature-time profile. "Attachment" was carried out by placing broilers (after defeathering) in inoculated attachment medium following by washing in water. Experiments with attached bacteria showed that above 52 C counts of mesophilic species initially exhibited a rapid decrease and then remained about the same. The initial sharp reduction in count for the attached bacteria was about the same as that for the unattached bacteria. Below 51 C destruction of the psychrotrophic species was logarithmic, with no significant difference in D values between attached and unattached bacteria. Prolonged storage of skin samples with attached bacteria (4.5 hr at 17 C) showed that E. coli k12 and Klebsiella, were no more heat resistant after 4.5 hr than after 1.5 hr.

When broilers were contaminated just before scalding (10 ml of  $10^9$ /ml E. coli k12 by spraying pericloacal skin) and examined after scalding, the data showed that the counts did not decrease logarithmically during the scalding treatment.

In other trials, some broilers were contaminated with E. coli k12 before and others after scalding. Skin samples then were analyzed before defeathering, after defeathering and after spin-chilling. The results seem to indicate that bacteria which were acquired before scalding and survived this treatment were not removed as readily during the later stages of processing as those acquired after scalding.

3. Detachment of "attached" bacteria--removal of the water film-effect of spin- and spray-cooling. Notermans and Kampelmacher (1975b) reported on the attachment of E. coli k12, E. coli k12 N97<sup>+</sup> and P. putrefaciens to the skin of broilers with and without the removal of the waterfilm. After immersion in the inoculated attachment medium and drainage for 30 sec, the carcasses were rinsed for 3 min in running water. The rate of attachment with or without removal of the waterfilm was about the same. The number of bacteria removed from the skin by rinsing for 3 min was independent of the number on or in the skin.

In other trials, carcasses were contaminated (after defeathering) with E. coli k12 ( $10^4$ - $10^7$  per ml attachment medium) and others with E. coli k12 N97<sup>+</sup> ( $10^6$  per ml attachment medium) and then rinsed for 3 min in water. Then, detachment in the spin-chiller was determined. The reduction in bacterial numbers was independent of the original concentration and was about 1 log. A similar reduction was noted during spray-cooling.

4. Evaluation of sanitary procedures during processing--comparison of skin maceration, "skin-dip" and "whole carcass rinse" methods.

Notermans, Kampelmacher and van Schothorst (1975a) compared the recovery of E. coli k12 from broilers contaminated during various stages of processing. In the skin maceration method, the skin samples were macerated for 60 sec; in the skin-dip method the skin samples were placed in 9 times their weight of peptone-saline solution and shaken for 2 min at 200 rpm. Recovery by the skin-dip method was smaller than by the maceration method if the contamination occurred early in the processing line. If it occurred in the spin-chiller, the recovery by either method was about the same. When these two methods were used to evaluate the natural contamination at different stages during processing, considerable variation in counts was observed between methods. Counts determined by rinsing the whole carcass (500 ml peptone-saline solution, shaken for 30 sec) could not be related to those obtained with the skin-maceration method.

5. Effect of freezing and thawing on attachment of bacteria.

Notermans, Kampelmacher and van Schothorst (1975b) reported on studies in which broilers were contaminated after defeathering by immersion in physiological saline inoculated with bacteria ( $10^6$ - $10^7$ /ml). Samples of skin were taken before freezing and after freezing and thawing. One part of the sample was used for the maceration procedure, the other for the skin-dip method. The data show that the difference in count obtained with the maceration method and the skin-dip procedure was not significant if the counts (log total count and Enterobacteriaceae) of the spin-chiller water was low ( $<2.5$ /ml and  $<3.5$ /ml, resp.). However, if the Enterobacteriaceae count was high, the skin-dip procedure gave lower counts than the skin maceration procedure probably because of a higher death rate of the bacteria associated with the waterfilm than those attached to the skin.

In other trials, broilers were contaminated with E. coli k12 and Klebsiella after plucking. After freezing and thawing, bacterial counts were determined of (a) the skin by the maceration method, (b) the thaw water, and (c) from the rinse fluid by washing the entire carcass with 500 ml peptone saline solution and shaking for 30 sec. Of the Klebsiella only 5% were recovered in the thaw water and 17% in the rinsing fluid. Of the E. coli k12, 3.5% were found in the thaw water and 12% in the rinsing fluid. There was a high correlation between the counts determined by rinsing the carcasses and those of the thaw water.

6. Maceration and rinse-drip procedures for the determination of total counts, Enterobacteriaceae, E. coli and Salmonella on frozen broilers. Van Schothorst, Northolt, Kampelmacher and Notermans (1976) showed again that the evaluation of the bacteriological conditions of broilers may be influenced greatly by the testing procedure. The percentage contamination with Salmonella ranged from 25-70% depending upon the sampling procedure. With respect to the total counts, Enterobacteriaceae and E. coli counts, no correlation could be detected between the results obtained with the maceration and the skin-drip

procedures. They postulated that the rinse-drip method reflected sanitary conditions at the end of the processing line (washing and cooling) whereas the skin-maceration method revealed microbial build-up on the skin during the entire process.

In summary, the studies with broilers have shown that the attachment of bacteria to the skin is related to the numbers and types of bacteria in the attachment medium, attachment time, and temperature and pH of the attachment medium. There is also reference (Notermans and Kampelmacher, 1974) to the possibility that under certain environmental conditions (higher temperature for example) losses of "attractant substances" from the skin into the environment may cause decreased attachment. In this respect, Anderson *et al.* (1975) who studied the removal of yeasts from meat surfaces with water sprays noted that more yeasts were removed when the meats passed under the sprays at reduced speeds. Although other factors probably were involved, accumulation of water to a greater depth and increased contact time may have caused greater solubilization of substances which cause microorganisms to adhere.

Mesophilic bacteria attached to the skin were more heat resistant than unattached bacteria. Their location in the skin surface rather than polymer formation may have been responsible for this protective action. Bacteria which survived scalding could not be as easily removed during the later stages of processing. That portion of the microbial flora associated with the waterfilm on the skin could be removed relatively easy by washing, however, removal of the portion more firmly attached to the skin was more restricted. The distribution of the microflora between the skin proper and the waterfilm also affected the bacterial counts obtained with different procedures. The microflora firmly attached to the skin could be evaluated more effectively by the skin maceration procedure than by the skin-rinse method. In addition, the position of bacteria on or in the skin may affect their survival in processing steps such as heating, freezing and thawing. The objective(s) of the bacteriological analysis must be considered in the selection of the proper testing procedure.

It would be an oversimplification if we consider the data on attachment and detachment of bacteria as related to broiler skin fully applicable to carcasses of other species such as beef, lamb and pork. There exist large differences in skin and meat surface characteristics between various animals. However, the data reported by Notermans, Kampelmacher, van Schothorst and Northolt (1974, 1975a,b, 1976) and those of Marshall *et al.* (1971), Deinema and Zevenhuizen (1971), Fletcher and Floodgate (1973), Adler (1973) and Chet *et al.* (1975) are useful (a) to explain discrepancies in bacteriological counts and types when different sampling and testing procedures are used, and (b) to develop a tentative model system to relate the association of bacteria with beef, pork and lamb carcasses. Some bacteria may be more or less firmly trapped mechanically in skin or meat crevices or in hair follicles such as on hogs which are scalded and dehaired. Others may be more firmly associated with the skin or meat surfaces, for example,



through polymer bridging with the aid of polysaccharides. The degree of association may be influenced by "attractant substances" which would direct the bacteria toward the skin or meat surface (positive chemotaxis). In addition, there are bacteria associated with the moisture film on the skin or meat surface. These, probably, are less firmly associated with the surface. Changes in the electrolyte concentration and other physical-chemical properties of this layer may be responsible for changes in the degree of attachment or detachment to surfaces. A better understanding of the basic mechanisms of attachment and detachment may be helpful in designing techniques to reduce numbers of bacteria on meat surfaces, maintaining them at reduced levels, in order to improve shelf life and reduce possible public health hazards.

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Bruce Langlois: We will entertain questions at this time. At the end of each presentation, we will take questions. Are there any questions?

Tom Bidner, LSU: From the data you have presented this morning, what sampling technique, and so on, would you recommend for determining the shelf life of a beef carcass.

Carl Vanderzant: That is just exactly why I tried to approach this problem from more of a philosophical standpoint. But, you go to the height of the problem, namely: is it just one nice little technique that I can use to do away with my problem? There is no such thing. In the first place, you have got to set your objectives for what you really want from your data. Now, I can give you an answer from my own personal opinion, namely: I think you've got to sample a rather large area, because some of the data by Ingram and Roberts, for example, show very clearly, that you're wrong if you think microbial distribution is even over the carcass or that distribution of the most contaminated areas are even from carcass to carcass or from day to day. So, if you swab, let's say, five 100 square centimeter areas, you're still swabbing what, one to three percent of the total carcass. So, the larger the area that

you can swab, the better off you are. But everything is again dictated by the investments and by the objectives of your particular procedure. Now, one technique, for example, that I have seen is a swab technique using a cellulose sponge, that John Silliker advocates. You can even use sanitary napkins to swab half of a carcass. I think what you want to avoid is the pitfalls of placing too much significance on the numbers that you find on very small areas. You can't do that. So, there is really no one answer to your question. The approach to my paper this morning is somewhat my own answer to my frustrations of not being able to come up with a neat little package which will be a perfect answer for all occasions.

Question: Carl, your point is well taken concerning the attachment of the bacteria. Many of the organisms that you have on carcasses have a temperature optimum of 18 or 20. So, wouldn't you ordinarily expect counts to be lower at higher temperatures if 18 to 20 C is ideal for attachment?

Carl Vanderzant: No. Some of these organisms will grow well even in temperatures of 25 degrees, and so on. The reasons that they attach best at about 18 to 20 degree centigrade are given by the authors of this paper as follows: one, that as the temperature increases, the mobility of these bacteria increases also, and the balance of the activity goes into detachment rather than attachment. It is not an all or nothing affair. It is really a balance between the two. They feel that over 20 to 25 degrees centigrade, you're actually going to a detachment mechanism.

Another possibility is that at higher temperatures, materials from the surface of the carcass are absorbed onto the flagella of the bacteria, and decrease their attachment. So, you have a detachment rather than an attachment. So, the temperature phenomena is very difficult to explain except by loading of substances on the flagella or going to a detachment mechanism at the increased temperature.

But you were right that the types of bacteria that we are greatly worried about are those that are very active in that particular range.

Marchello, North Dakota State: Your paper this morning dealt mostly with poultry where we leave the skin attached. The normal procedure for slaughtering cattle and sheep is to remove the skin. Is there any indication that we may have a different setup with these animals where there might be less chance of detachment to say the connective tissue or fatty tissue?

Carl Vanderzant: Right. Topography and all the characteristics associated with the skin and this meat surface will have enormous influence on this. For example, lamb surfaces, where the fell is left on would probably give a more even surface. Scalding and de-hairing of hogs would present an entirely different situation than you will have on beef animals. I also want to point out the enormous lack of information that exists in such an important area for beef, pork and lamb, compared with broilers. I've taken broilers as an

example because most of the fundamental information is available in that particular area. But, I'm quite certain that similar phenomena could be found on beef, pork and lamb. I mentioned in one instance concerning attracting substances, the paper from Missouri, where surfaces were subjected to water sprays. Although they did not mention it per se, some of the data indicates that chemotoxins may be involved.

Ronald Rea, Armour: Dr. Vanderzant, do you have a pretty substantial attachment of a bacteria to the skin surface, say with beef cattle or even hogs? We put a lot of emphasis in the packing plant at the time of the wash. A lot has been done, even though we try to reduce the water. We've also gone to quite a large gallon per minute rate to actually try to remove bacteria. Is it possible due to the force of the water spray to more firmly attach these bacteria or to impregnate them into the fat surface of the carcass?

Carl Vanderzant: I'm not too worried about that. What I'm worried about is that we may have to do something in addition to water. Some work by these people that I quoted this morning have shown that, for example, with broilers only a certain amount of bacteria can be removed mechanically during the spin chilling at the end of processing. So, we have to go to other means in order to remove the firmly attached bacteria. So, although I'm not going to advocate at the present, modification chemical of the water supply, I can see that if we do have a better understanding of the attachment and detachment process, then we may be able to detach some of the more firmly attached bacteria by changes in the chemical composition of the water which would change the electrical forces that hold them to the surface.

Ronald Rea: I hope that we can, because we're relying right now in the slaughtering industry almost 100 percent on the final wash and just the mechanical removal of the bacteria.

Bruce Langlois: Thank you for an excellent presentation, Dr. Vanderzant. I'm sure if anyone else has a question for Dr. Vanderzant, you can find him later this morning. I'm sure he'll be glad to answer your question.

We'll now continue with our next topic, "Sample Transport, Media and Incubation," which will be presented by Dr. Bruce Tompkin. Dr. Tompkin was born, raised and educated in Ohio. Since graduating from Ohio State, he has been employed by Swift and Company. He is their Chief Microbiologist. His main activities and research have been in the prevention of food-borne illness and how to maximize microbial quality in foods. Dr. Tompkin.