MICROBIOLOGICAL METHODS FOR SAMPLING POULTRY PROCESSING PLANT EQUIPMENT

S. M. RUSSELL[1]
Department of Poultry Science, The University of Georgia, Athens, GA 30602-2772
Phone: (706) 542-1368
FAX: (706) 542-1827
N. A. COX and J. S. BAILEY
USDA, ARS, Russell Research Center, Athens, GA 30613

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SUMMARY

This paper describes several microbiological sampling methods that may be used for determining the cleanliness of poultry processing plant equipment, including surface swabbing, agar contact, hygiene contact slides, dry medium film, impedance, and ATP bioluminescence. Advantages and disadvantages associated with each method are mentioned. This article will be helpful to anyone faced with the task of microbiologically monitoring poultry processing equipment after sanitation.

Key words: Bacterial contamination, processing equipment, sampling procedures
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DESCRIPTION OF PROBLEM

Live birds entering the poultry processing plant carry a diverse natural microflora, most of which are not pathogenic to humans. Poultry processors seek to reduce the total number of microorganisms on the finished product in order to assure adequate shelf life during transportation, distribution, and retail display, and to prevent the spread of pathogenic bacteria to the product through cross-contamination. If not properly controlled, processing plant operations can have a detrimental effect on the microbiological quality of fully processed carcasses. Carcasses may become cross-contaminated via the following routes: bird-to-bird contact, handling of the carcasses by employees, contact with processing equipment or tools, or scald and chill tank water [1]. Because birds may become cross-contaminated from improperly cleaned and sanitized equipment, it is essential that the sanitation process be monitored using microbiological means to insure that, each morning before production begins, the bacterial populations on the equipment have been lowered to acceptable levels.

Enumeration of microbial populations on poultry processing equipment surfaces is an important means of monitoring the effectiveness of plant sanitation. Crevices and joints in processing equipment often make it difficult, if not impossible, to collect adequate samples. However, for sampling horizontal surfaces or

[1] To whom correspondence should be addressed

other surfaces that are readily accessible, this paper will describe commonly used microbiological methods, the advantages and disadvantages of each method, and possible alternative methods for sampling.

Sampling Methods

SURFACE SWABBING

The swab contact method is commonly used to sample poultry processing equipment surfaces primarily because it is simple and widely recognized. The conventional swab technique involves rubbing fives areas (approximately 50 cm^2) of the surface to be sampled with a prewetted sterile calcium alginate swab. This swab is then placed into 4.5 mL of a sterile solution containing the following: 1% sodium citrate to dissolve the swab and release bacteria [2], 0.5% polysorbate to neutralize phenolic disinfectants, and 0.07% soy lecithin to neutralize quaternary ammonium compounds [2]. Portions of the swab rinse solution measuring 1 mL and 0.1 mL are then pour plated using plate count agar or other suitable media [2]. Plates are then incubated at 35°C for 48±3 hr; the number of bacteria on plates with 30–300 colonies indicates whether the sampled surface meets sanitation standards.

The disadvantage to this method is that it requires time and labor to prepare solutions and media, dilute samples, pour plate samples, and count colony-forming units on the plates. An average poultry plant would undergo two additional cleaning and sanitation cycles before the cleanliness of a particular piece of equipment could be assessed. Thus, swab results for poultry processing equipment can function as a general guide as to the effectiveness of sanitation, but not as a direct monitoring tool. Throughout the scientific literature, swab procedures have also been used to evaluate the effectiveness of chemicals for reducing bacterial populations on poultry processing equipment [3].

AGAR CONTACT

The RODAC or agar contact method estimates the overall microbial population on surfaces in processing plants. It can also enumerate specific populations of microorganisms on such surfaces. RODAC plates are small petri dishes that are slightly overfilled with an agar-based microbiological growth medium, such that the surface of the agar can be pressed against equipment [2] and removed. This technique transfers microorganisms from the equipment to the surface of the agar plate. The plate is then incubated and the microbial populations are allowed to multiply. Bacterial colonies are counted once they reach a visible biomass.

If RODAC plates (Remel, Lenexa, KS) are purchased premade, this method does not require as much labor as the swab technique because no solutions or media need to be prepared. Another advantage of the RODAC method is that the RODAC plates can be filled with a selective medium instead of plate count agar (PCA) to enumerate specific populations of microorganisms, such as coliforms, yeasts, or molds. The disadvantages to the RODAC method are that the plates are more expensive than conventional PCA plates, microorganisms on surfaces that are highly contaminated cannot be estimated because no dilutions are involved, only flat surfaces can be sampled, the agar surface is not sticky enough to pick up bacteria that have formed biofilms, and 48 hr is required for incubation [2].

HYGIENE CONTACT SLIDES

Difco (Detroit, MI) has recently developed a contact slide called HYcheck. HYcheck is a double-sided slide with a hinged paddle that has nutrient or selective agar-based microbiological growth medium attached to both sides of the paddle. This paddle is enclosed in a sterile plastic test tube and attached to a screw top that serves as the top of the test tube. To use the HYcheck contact slide, the top of the test tube is unscrewed and the paddle is removed from the tube. The paddle is placed on the surface to be tested, removed, then replaced in the sterile tube and incubated. Currently available types of HYcheck slides include: D/E-neutralizing agar; disinfection control; Enterobacteriaceae; *Pseudomonas*; total count; and yeast and molds. The D/E-neutralizing agar slides are especially useful for monitoring sanitation because this substance neutralizes the chemicals used during sanitation that will interfere with microbial enumerations. These slides are inexpensive, greatly reduce the time and labor required to sample surfaces, and can be used to indi-

cate levels of pathogenic bacteria, spoilage bacteria, and molds; however, as with RODAC plates, the agar surface is not sticky enough to pick up bacteria that have formed biofilms, and a relatively long period of incubation (24–48 hr) is required before results can be obtained.

DRY MEDIUM FILM

Dry medium film (Petrifilm, 3M, St. Paul, MN) is rapidly becoming a popular means of estimating microbial populations on equipment surfaces. Petrifilm consists of standard methods (SM) nutrients and a cold-water-soluble gelling agent [4]. The Petrifilm slide consists of two films, a bottom film coated with SM nutrients and a top film coated with a gelling agent and triphenyltetrazolium chloride (TTC) indicator dye. This dye facilitates counting by staining the colonies red as the microorganisms grow [4].

To use the Petrifilm Aerobic Count Plate for direct contact enumeration, 1 mL of sterile 0.1% peptone water is placed onto the paper, the upper film containing the growth medium is allowed to contact the liquid on the bottom film, and the medium is allowed to rehydrate for 30 min. After 30 min, the top film is lifted and the hydrated agar medium is pressed against the equipment surface, then lifted to pick up microorganisms. The Aerobic Count Plate is then incubated at 30°C for 24 hr. Colony-forming units on the plates are then counted, and results are reported as count/20 cm^2.

Populations of coliforms and *Escherichia coli* on equipment surfaces can also be estimated using Petrifilm Coliform Count Plates and *E. coli* Count Plates, respectively. In comparison to conventional plate count methods, this technique is inexpensive, requires less labor, and produces results in 24 instead of 48 hr. The disadvantages associated with this method are that peptone water must be prepared, the medium on the film is difficult to keep from smearing, and it does not produce results as rapidly as other techniques, such as impedance or ATP bioluminescence.

IMPEDANCE

Impedance microbiological techniques are commonly used to estimate microbial numbers on equipment surfaces. Impedance is defined as the opposition to flow of an alternating electrical current in a conducting material. During growth, bacteria convert large molecules into smaller metabolites which change the ability of the medium to conduct electricity by increasing its conductance and decreasing its impedance. When microbial populations reach a level of 10^6–10^7 cells/mL, the medium's change in impedance is sufficient to be detected by the instrument. Firstenberg-Eden [5] refer to the time required for this exponential change to occur as the impedance detection time (DT).

DT can be obtained in much shorter periods of time than standard aerobic plate counts (APC). Results can usually be obtained in less than 12 hr, depending on the microbial load of the sample. There are several fundamental differences between impedance microbiological techniques and APC [5]. When conducting plate counts, all bacteria that are able to reach a visible biomass are counted [6], whereas the impedance technique relies on the measurement of metabolic changes [5]. Factors such as media, time, and temperature are critical parameters in the assay.

When using impedance as a means of estimating total aerobic bacterial populations, it is essential to use a medium, temperature, and environment that minimizes the differences in generation times of the species in the sample [7]. If generation time differences are minimized, the microorganisms will multiply at a similar rate and will reach the detection threshold of 10^6–10^7 at approximately the same time. Using this method, correlations between impedance and APC will be highest. By correlating DT to APC, future APC can be estimated based on impedance readings.

Media, temperature, and environmental conditions may be manipulated to selectively enumerate one species or one group of bacteria. For example, in a mixed sample containing 100,000 pseudomonads and 1 coryneform, incubating the sample at 30°C would cause the coryneform to reach the detection threshold level first [7]. At 30°C, the generation time of the pseudomonads is four times that of the coryneform; thus the coryneform can reach 10^6 cells/mL before the pseudomonads. Therefore, using selective media, impedance could be used to rapidly enumerate coliforms,

Escherichia coli, and yeast and molds from equipment surfaces.

ATP BIOLUMINESCENCE

A promising rapid method for monitoring the effectiveness of sanitation of poultry processing equipment has been developed. This method is called the firefly bioluminescent/adenosine triphosphate (ATP) assay and is referred to as a "real time" procedure because results can be obtained in less than 1 hr.

The total ATP present on a surface indicates its general cleanliness. ATP is found in bacterial, yeast, and mold cells and in extracellular fluids associated with raw and cooked poultry. A high level of ATP on a surface, even if the source is extracellular fluid, indicates that the surface must be recleaned before production begins. Because extracellular fluids are an excellent growth medium for bacteria, populations of bacteria may utilize the nutrients in the fluid and multiply to high concentrations within a few hours.

To begin the procedure, the surface to be assayed is swabbed. The swab is then immersed and rotated in a releasing reagent to release the microbial ATP from the microorganisms present in the sample. Approximately 500 μL of reconstituted ATP monitoring reagent is added to the cuvette. The cuvette is then placed into a luminometer that measures the light emitted when firefly luciferin/luciferase in the ATP monitoring reagent reacts with the ATP from the equipment surface. The light released is measured as relative light units (RLU) [8]. RLU are related to the total amount of ATP present on the surface.

Because many sources of ATP are consistently found on poultry processing equipment, extraneous sources of ATP such as extracellular ATP from damaged microbial cells and somatic (nonmicrobial) ATP must be eliminated if estimations of bacterial numbers are to be made based on RLU readings. Various techniques are used to remove extracellular and somatic ATP from swab samples. One method involves enzymatic degradation of extracellular ATP as described by Kennedy and Oblinger [9]. Another technique reported by Littel *et al.* [10] involves double filtration sampling, in which a prefilter traps food particles and a second filter retains the bacteria. The second filter is treated with an enzyme reagent to hydrolyze nonmicrobial ATP [10]. The type of ATP luminometer used determines whether or not microbial ATP can be separated from total ATP. Systems that utilize swab assemblies containing all of the bioluminescent chemicals in the swab housing can determine general cleanliness only. To estimate microbial populations using ATP bioluminescence, a luminometer that accepts cuvettes containing the luminescent reagents is required.

Historically, a major impediment to monitoring sanitation using bioluminescence was that these systems were not portable. Currently, a number of companies are producing portable devices that may be carried into the processing plant to sample equipment. These devices are easy to carry, simple to use, compact, and employ assays requiring little or no preparation. Most ATP luminometers are computerized and can collect large amounts of data. After collection, the data can be downloaded to a computer for analysis. Many food processing operations use bioluminescent techniques to complement Hazard Analysis Critical Control Point (HACCP) programs.

CONCLUSIONS AND APPLICATIONS

1. Poultry processing equipment is difficult to sample microbiologically.
2. Surface swabbing or some variation of this is most commonly used, but these methods are labor intensive and require excessive time to obtain results.
3. When sanitation monitoring becomes part of a HACCP program so that microbiological data must be obtained quickly, ATP bioluminescence seems to offer a practical alternative.

REFERENCES AND NOTES

1. **Bailey, J.S., J.E. Thomson, and N.A. Cox,** 1987. Contamination of poultry during processing. Pages 193–211 in: The Microbiology of Poultry Products. F.E. Cunningham and N.A. Cox, eds. Academic Press, Orlando, FL.

2. **Sveum, W.H., L.J. Moberg, R.A. Rude, and J.F. Frank,** 1992. Microbiological monitoring of the food processing environment. Pages 62–76 in: Compendium of Methods for the Microbiological Examination of Foods. C. Vanderzant and D.F. Splittstoesser, eds. Amer. Publ. Health Assn., Washington, DC.

3. **Bailey, J.S., J.E. Thomson, N.A. Cox, and A.D. Shackelford,** 1986. Chloride spray washing to reduce bacteria on poultry processing equipment. Poultry Sci. 65:1120–1123.

4. **Swanson, K.M.J., F.F. Busta, E.H. Peterson, and M.G. Johnson,** 1992. Colony count methods. Pages 75–95 in: Compendium of Methods for the Microbiological Examination of Foods. Amer. Publ. Health Assn., Washington, DC.

5. **Firstenberg-Eden, R.,** 1985. Electrical impedance method for determining microbial quality of foods. Pages 679–687 in: Rapid Methods and Automation in Microbiology and Immunology. K.O. Habermehl, ed. Springer-Verlag, Berlin, Germany.

6. **Firstenberg-Eden, R.,** 1983. Rapid estimation of the number of microorganisms in raw meat by impedance measurement. Food Tech. 37:64–70.

7. **Firstenberg-Eden, R. and G. Eden,** 1984. Impedance Microbiology. Research Studies Press LTD, Letchworth, Hertfordshire, England. pp. 48–49.

8. **Eilers, J.R.,** 1990. Microbial analyzer cuts inventory hold time. Food Processing, October, pp. 86, 88.

9. **Kennedy, J.E., Jr. and Oblinger, J.L.,** 1985. Application of bioluminescence to rapid determination of microbial levels in ground beef. J. Food Prot. 48:334–340.

10. **Littel, K.J., S. Pikelis, and A. Spurgash,** 1986. Bioluminescent ATP assay for rapid estimation of microbial numbers in fresh meat. J. Food Prot. 49(1):18–22.