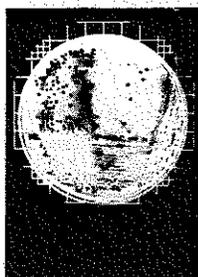


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# Radiometry and Microcalorimetry—

*Techniques for the rapid detection of foodborne microorganisms*

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□ ONE OF THE MOST COMMON of the microbiological procedures used to indicate the quality of certain foods is the standard plate count. This procedure is often used to estimate the level of aerobic mesophilic microorganisms in foods and thereby assess the sanitation and time-temperature conditions during production, transportation, and/or storage. In cases where processed foods may be exposed to undesirable time-temperature profiles due to equipment breakdown or human error, a high mesophilic count would indicate a possible public health hazard since similar conditions could have allowed the growth of infectious and toxigenic microorganisms (such as *Salmonellae* and *Staphylococci*).

It is important to know as soon as possible whether conditions have existed which may have resulted in a potential public health hazard so that timely corrective action may be taken. However, with the standard plate count, two days may elapse before the microbiological quality of a food can be assessed. The major delay in this method is the incubation time needed to enable bacterial cells to multiply to numbers adequate to form the countable colonies used to estimate microbial contamination. Such delays may pose an economic burden in terms of extended hold and storage intervals, as well as possible loss of a subsequent lot due to untimely corrective action.

It is evident that we need more rapid techniques for assessing the microbiological quality of foods. At the U.S. Army Natick Laboratories, radiometry—measurement of the intensity of radiant energy—and microcalorimetry—measurement of minute quantities of evolved or absorbed heat energy—have been explored as the basis for techniques for rapid detection of undesirable levels of foodborne microorganisms. This article will describe the operational principles, current apparatus, and capabilities of each concept.

## RADIOMETRY

Radiometry involves the measurement of radioactive CO<sub>2</sub> evolved during bacterial metabolism of <sup>14</sup>C-labeled substrates contained in septum-stoppered 50-ml serum

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vials. These vials, containing 12–36 ml of an optimum growth medium, were rendered anaerobic or aerobic by sparging either with a mixture of 85% N<sub>2</sub>, 10% CO<sub>2</sub>, and 5% H<sub>2</sub>, or with 90% air and 10% CO<sub>2</sub>, and were then inoculated with vegetative cells, spores, or meat suspensions having various levels of the normal flora and incubated at 37°C on a rotating shaker at 275 rpm. At hourly intervals, the headspace of each vial was tested for <sup>14</sup>CO<sub>2</sub> in a Bactec Model 301 instrument (Johnston Laboratories, Inc., Cockeysville, Md.). In accordance with the recommendation of the manufacturer, a reading of 20% of full scale was considered as positive evidence of the presence of viable bacteria, although background readings above 4% of scale were not observed in our laboratory.

Each 50-ml vial contained, as growth medium, either TSB, TSS, or TYT-GFG. TSB was composed of 1.42% trypticase, 0.25% phytone, 0.21% K<sub>2</sub>HPO<sub>4</sub>, and 0.42% NaCl; TSS was composed of TSB supplemented with 3.91% thiotone, 0.42% yeast extract, and 0.042% sodium thioglycolate. In addition, these media contained <sup>14</sup>C-glucose (U) at radioactivity levels indicated in the individual experiments. TYT-GFG was composed of 5.0% thiotone, 0.5% yeast extract, 0.5% trypticase, 0.25% K<sub>2</sub>HPO<sub>4</sub>, and 0.5% NaCl with a total of 3.75 μCi of <sup>14</sup>C-glucose (1.75 μCi), 5-<sup>14</sup>C-glutamate (1.0 μCi), and <sup>14</sup>C-formate (1.0 μCi). The development of these media has been described by Previte (1972) and Previte and Rowley (1973).

### • Detection of Vegetative Cells of Foodborne Pathogens.

Lyophilized cultures of *Staphylococcus aureus*, strain Giorgio, or *Salmonella typhimurium*, strain RIA—two common foodborne pathogens—were rehydrated, inoculated into trypticase soy broth from Baltimore Biological Laboratory (BBL), and incubated for 24 hr at 37°C. The number of viable cells was estimated from colony-forming units of cultures serially diluted in distilled water and spread on plates of trypticase soy agar (BBL). Cells were inoculated into detection vials to final concentrations of 1 cell to 10<sup>4</sup> cells per ml of TSS or TSB and incubated at 37°C for up to 10 hr.

In 36 ml of TSS containing 3.0 μCi of <sup>14</sup>C-glucose, initial levels of 10<sup>4</sup> *S. typhimurium* or *S. aureus* per ml were detected within 3–4 hr, whereas 1 cell/ml required 9 hr (Fig. 1). The high sensitivity of this method, as evidenced by detection of initial levels of

## Radiometry and Microcalorimetry . . .

1 cell/ml, has also been shown by others with *Escherichia coli* ATCC 11775, *Proteus vulgaris* ATCC 6380, and *Pseudomonas aeruginosa* ATCC 9721, (Waters, 1972; Waters and Zwarun, 1973). With a lower concentration of  $^{14}\text{C}$  in TSB ( $0.5 \mu\text{Ci}/36 \text{ ml}$ ), a slightly longer detection time was required (Fig. 1). Increasing the  $^{14}\text{C}$  concentration of  $3.0 \mu\text{Ci}/36 \text{ ml}$  shortened the detection time in TSB (Previte, 1972). A further increase of the level of labeled glucose to  $9.0 \mu\text{Ci}$  in TSS shortened the detection time by only 30 min (Previte and Rowley, 1973).

• **Detection of Spores of Foodborne Pathogenic and Spoilage Microorganisms.** Spores of *Clostridium botulinum*, strain 62A, represented a foodborne pathogen, and Putrefactive Anaerobe, P.A. 3679, represented a spoilage microbe encountered in thermally underprocessed foods. Spores were prepared, stored frozen, thawed just prior to use, and heated at  $80^\circ\text{C}$  for 10 min to destroy vegetative cells, and the number of viable, heat-resistant spores was determined (Anellis and Rowley, 1970; Rowley and Feeherry, 1970). Heated spores of *C. botulinum* or P.A. 3679 were inoculated into TSS to give final concentrations ranging from  $10^2$  to  $10^7$  spores/ml and incubated at  $37^\circ\text{C}$  for up to 11 hr.

An initial level of as few as 90 spores of P.A. 3679 per ml (12 ml per detection vial) was detected in 11 hr (Fig. 2). A spore concentration of  $10^4/\text{ml}$  of either P.A. 3679 or *C. botulinum* was detected within 7 hr, a time which was 3–4 hr longer than the time required to detect this concentration of vegetative cells of *S. aureus* (Fig. 1). This difference in detection time corresponds to the time required for spores of *C. botulinum* 62A to germinate and initiate cell division (Rowley et al., 1970). Recently, others have made similar observations with *C. botulinum* 62A as well as

a variety of other anaerobic spore formers and have demonstrated that estimates of the numbers of these organisms can be obtained more rapidly with the radiometric method than with conventional methods (Evancho et al., 1974).

• **Detection of Nonfermentative Bacteria.** Some microorganisms (e.g., *Alcaligenes faecalis*, *Pseudomonas aeruginosa*) do not readily produce  $\text{CO}_2$  from glucose. Thus, to enhance the universality of radiometric detection, we screened a variety of  $^{14}\text{C}$ -labeled substrates (Previte et al., 1974). The combination of  $5\text{-}^{14}\text{C}$ -glutamate ( $1.0 \mu\text{Ci}$ ),  $^{14}\text{C}$ -formate ( $1.0 \mu\text{Ci}$ ), and  $^{14}\text{C}$ -glucose ( $1.75 \mu\text{Ci}$ ) allowed the detection of non-glucose fermentors (*A. faecalis* and *P. aeruginosa* at  $10^2/\text{ml}$ ) within 10–12 hr, and shortened the detection time of glucose fermentors (data not shown).

• **Detection of the Indigenous Flora of Meat Loaf.** Pre-cooked, frozen meat loaf was thawed 18–20 hr at  $4^\circ\text{C}$  and incubated at  $37^\circ\text{C}$ . At various time intervals (0, 4, 6, 8, 9, 10, 14, and 20 hr), 50-g samples were blended with 450 ml of buffer ( $0.066 \text{ M PO}_4$ , pH 7.2) for 2 min. Three ml of the blended sample was withdrawn with a sterile syringe and injected into vials containing 27 ml of TYT-GFG at  $37^\circ\text{C}$ . The vials were incubated at  $37^\circ\text{C}$  for up to 11 hr. Samples were also taken from the blender, and the number of viable organisms per gram of meat loaf was estimated as previously described for vegetative cells.

As demonstrated with pure cultures (Figs. 1 and 2), there was a high degree of correlation (correlation coefficient  $R = 0.97$ ) between the logarithm of the concentration of microorganisms in meat loaf and the detection time (Fig. 3). It appears that the radiometric procedure could be used in place of the laborious and

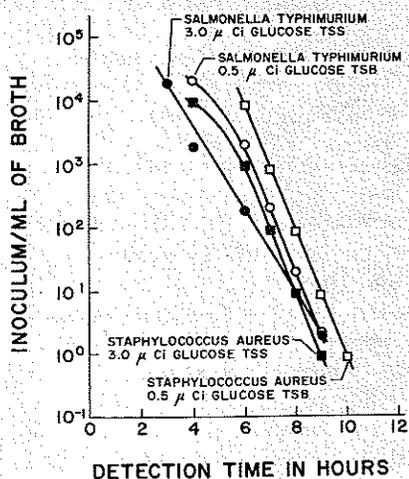


Fig. 1—RADIOMETRIC DETECTION of *Salmonella* and *Staphylococcus* with  $0.5 \mu\text{Ci}$  of  $^{14}\text{C}$ -glucose/ $36 \text{ ml}$  of TSB or  $3.0 \mu\text{Ci}$  of  $^{14}\text{C}$ -glucose/ $36 \text{ ml}$  of TSS (Previte, 1972; reproduced with permission of Am. Soc. for Microbiology)

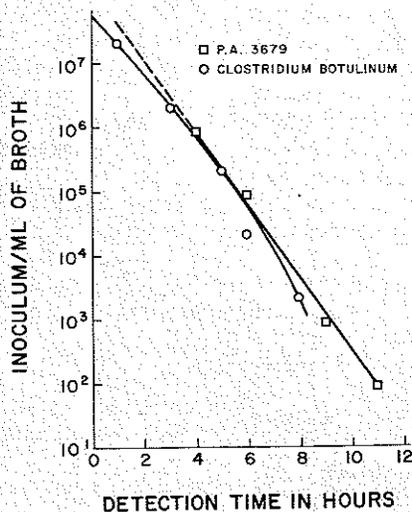


Fig. 2—RADIOMETRIC DETECTION of P.A. 3679 and *Clostridium botulinum* 62A spores in TSS with  $1.0 \mu\text{Ci}$  of  $^{14}\text{C}$ -glucose/ $12 \text{ ml}$  of TSS (Previte, 1972; reproduced with permission of Am. Soc. for Microbiology)

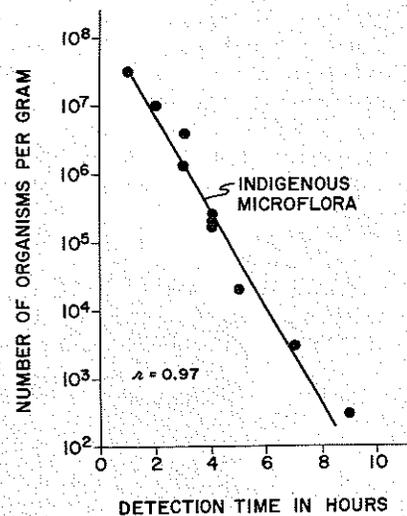


Fig. 3—RADIOMETRIC DETECTION of the indigenous microflora of precooked meat loaf incubated at  $30^\circ\text{C}$  for 0, 4, 6, 8, 9, 10, 14, and 20 hr. The number of microorganisms per gram of meat loaf increased from  $3.0 \times 10^2$  at 0 hr to  $3.3 \times 10^7$  at 20 hr. The detection medium (30 ml) was TYT-GFG with a total of  $3.75 \mu\text{Ci}$  of  $^{14}\text{C}$ -glucose ( $1.75 \mu\text{Ci}$ ),  $5\text{-}^{14}\text{C}$ -glutamate ( $1.0 \mu\text{Ci}$ ), and  $^{14}\text{C}$ -formate ( $1.0 \mu\text{Ci}$ )

time-consuming (48-hr) standard plate count as a rapid screening procedure to estimate the concentration of microorganisms in meat loaf.

**A RAPID QA TOOL**

High microbial counts may indicate poor sanitation and/or improper time-temperature conditions during production or storage of a food and suggest that conditions favoring the growth of foodborne pathogens could have prevailed (Thatcher and Clark, 1968). During an experiment at Fort Lewis, Washington, we found in a centralized food service system that more than 99% of all properly produced precooked chilled foods (entrees, soups, sauces, and gravies) that were tested had 10,000 or fewer microorganisms per gram (Powers et al., 1973).

By using the appropriate standard curve for each food, a rapid (5-6 hr) determination of the microbial level may be possible with the radiometric technique. In the case of meat loaf (Fig. 3), a detection time equal to or greater than 6 hr by the radiometric technique ( $\leq 10,000$  microorganisms/g) would suggest proper food production and storage; and a detection time of less than 4 hr would indicate a sufficient concentration of microorganisms to warrant rejection of the meat loaf as a potential health hazard. Food samples showing detection times of 4-5 hr would require more extensive microbiological analyses. It is likely that the latter samples would amount to only a small percentage of the total produced.

**MICROCALORIMETRY**

The potential of microcalorimetry as a sensitive technique for determining the microflora of foods was suggested by Insalata et al. (1967). Sachs and Menefee (1972), using thermistors, studied thermal changes in canned foods undergoing bacterial spoilage. They discussed the possible use and limitations of microcalorimetry as a nondestructive technique for detecting cans which were not properly processed.

At Natick Laboratories, we have initiated an investigation of the feasibility of utilizing microcalorimetry as a rapid screening procedure to estimate the level of microorganisms in model systems and processed foods, by measuring the exothermic heat production rate

(HPR) of bacteria in a model system. A Mound Laboratories Differential Resistance Microcalorimeter (Anonymous, 1969) was used in a custom Tronac isothermal bath. The total system consisted of dual calorimeter vessels suspended in the Tronac isothermal bath, a constant current source, an amplified, and a strip-chart recorder.

The calorimeter system (Fig. 4) had one sample vessel interconnected, as indicated, as  $R+\Delta R$  forming two arms of a Wheatstone bridge. An identical control vessel formed the other two arms (R). When a constant current (5 milliamps) was applied through the bridge, the output voltage ( $V_2$ ) represented a difference in resistance (in this instance, proportional to temperature difference) between the two vessels.

In use, one vessel held a sterile sample or standard, and the other held the sample containing the independent variable. The samples (13.4 ml) were contained in heat-sterilizable pouches (polyester/aluminum foil/modified polyolefin) fitted with a septum of silicone rubber sealant on the pouch surface. Reactions common to both samples or any minor temperature fluctuations in the constant-temperature water bath did not contribute to background or extraneous signals. The microvolt output, which specifically represents total exothermic HPR, was amplified and graphically recorded on a strip-chart with  $1 \mu v$  corresponding to  $1.6 \times 10^{-2}$  cal/hr.

The individual calorimeter vessel consisted of an inner aluminum can with a four-terminal calibration heater bifilarly wound around it. Plastic ends supported the sample and resisted axial heat flow. Two nickel resistance wires (the two  $R+\Delta R$ 's and the two R's) in Figure 4 were wound over the entire length of each of the two vessels acting as heat flux integrators. Each inner vessel was separated from an outer brass jacket by an annular air gap. Any resistance change in the nickel coils was proportional to the temperature gradient and therefore to the heat flux from the contents of that vessel.

**DETECTION OF S. AUREUS**

In order to detect vegetative cells of *Staphylococcus aureus*, pouches containing 13 ml of BBL trypticase soy broth with 0.5% Difco yeast extract (TSY) were inoculated with 0.4 ml of a suspension of *S. aureus* (inoculated pouch) or sterile TSY broth (sterile control pouch). Inoculation was made with a hypodermic syringe through the septum on the pouch surface. The pouches were then placed into the calorimeter vessels.

Removal of aliquots from the calorimeter for quantitating the concentration of viable *S. aureus* would cause serious interference with the HPR signal. Consequently, replicate samples of inoculated medium were prepared simultaneously and held in another water bath at the same temperature as the calorimeter bath (37°C). At the desired intervals, samples were removed from this auxiliary bath and the number of viable cells determined as previously described. It was assumed that these counts corresponded to their counterpart in the calorimeter.

Figure 5 shows the HPR related to bacterial growth (inoculated pouch). Virtually no heat was liberated by the sterile control pouch within a 22-hr test period. During 8 hr of growth, the initial  $3.2 \times 10^8$  *S. aureus* cells/ml of TSY broth in the inoculated pouch increased to about  $10^9$  cells/ml, and the HPR increased

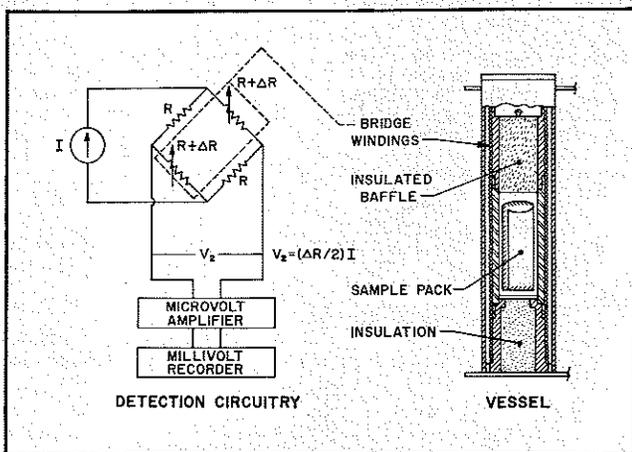


Fig. 4—SCHEMATIC DIAGRAM of detection circuitry and calorimeter vessel for the Mound Microcalorimeter (differential resistance bridge)

to a peak of 87  $\mu\text{V}$  (1.4 cal/hr). The minimum HPR detectable with the present system was 2  $\mu\text{V}$  (0.03 cal/hr), and routinely required the presence of about  $10^4$  bacteria/ml. Until signal amplification, recorder range spans, and calorimeter operational modes were optimized, this HPR ( $\mu\text{V}$ ) was considered to be the sensitivity of the system.

The data for a series of initial concentrations of *S. aureus* in TSY broth are summarized in Figure 6. The lower left curve represents the minimum time to detection (time for HPR to reach 2  $\mu\text{V}$ ) as a function of the log initial concentration. Detection time ranged from 2 hr for initial levels of  $10^7$ - $10^8$  cells/ml to 12-13 hr for 2 cells/ml. The minimum attainable detection time in the present system was 2 hr, since the introduction of a pouch into the calorimetry vessel caused a slight endothermic upset (Fig. 5) and the re-equilibration time was almost 2 hr.

The relationship represented by the lower left curve

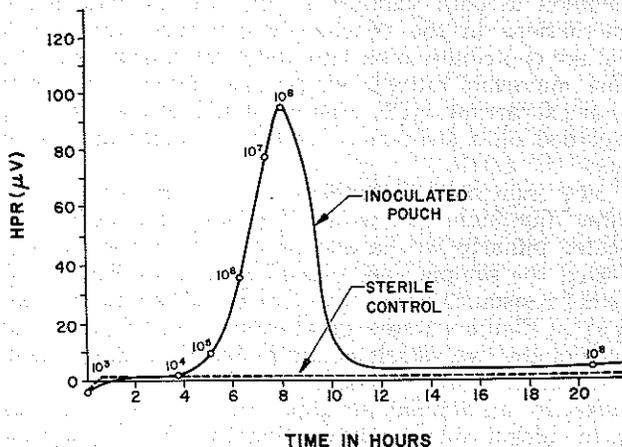


Fig. 5—HEAT PRODUCTION RATE (HPR) for *Staphylococcus aureus* in TSY broth (13.4 ml) contained in heat-sterilizable pouches. Numbers on the solid line (inoculated pouch) represent the approximate number of viable cells present at the indicated incubation times.

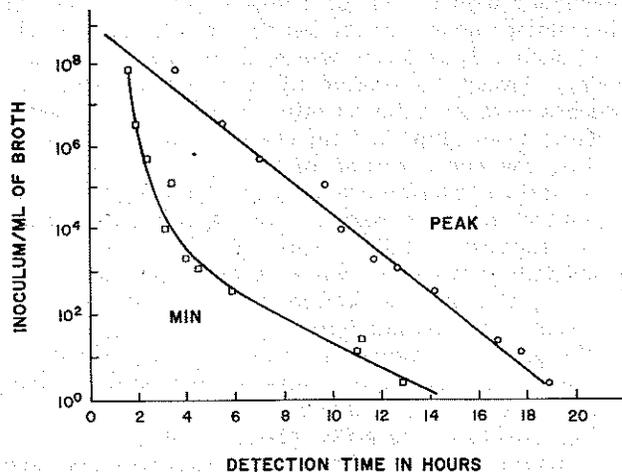


Fig. 6—EFFECT OF THE INITIAL INOCULUM (cells/ml of TSY) of *Staphylococcus aureus* on the minimum detection time (MIN) and the time to attain the peak heat production rate

in Figure 6 permits utilization of the calorimeter to estimate relative degree of contamination. If the relationship shown for a model system in Figure 6 also applies to foods, it would indicate that if there is no heat detectable within 3 hr, the number of microbes per gram may be considered to be less than 10,000 and, as discussed earlier under radiometry, that the food was produced and stored under proper conditions. Heat detection times of less than 3 hr would suggest a potential hazard which would have to be verified by longer calorimetry runs to a maximum HPR peak (the straight, upper right line in Fig. 6) or by more extensive microbiological analyses. However, this latter situation should involve only a small percentage of the total samples examined.

**BOTH TECHNIQUES PROMISING**

Our data indicate that either radiometry or microcalorimetry may eventually serve as a rapid screening procedure for estimating the level of microorganisms in foods. Both methods are simple, sensitive, rapid, can be automated, and are adaptable to situations where personnel must decide whether foods should be accepted, rejected, or tested further because of their borderline microbiological quality. Such automated techniques should reduce laboratory testing and storage time of foods under examination and would shorten the lag before initiation of appropriate corrective action.

We are presently engaged in studies to determine the reproducibility of each technique and the application of each as a potential screening process to determine the quality of precooked meats.

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Based on a paper presented at the Army Science Conference, West Point, N.Y., June 18-21, 1974.