Evaluation of Microbiological Methods Used for the Examination of Precooked Frozen Foods

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The increasingly wide acceptance of precooked frozen foods has led to the establishment throughout the United States of new frozen food plants devoted entirely to the precooked products. Among the problems facing this rapidly expanding industry is the development of adequate quality control standards for flavor, attractiveness, stability, and wholesomeness. As early as 1947 the question of wholesomeness was raised by Fitzgerald, who pointed out the importance of high quality raw materials, adequate cooking, careful plant sanitation during processing and packaging, rapid handling, and quick-freezing. Food sanitarians and public health officials presently are focusing their attention on the potential health hazards resulting from unsanitary operations and improper handling during the production and distribution of these foods. For effective sanitary control in the industry, processors may find it desirable to employ a standardized bacteriological testing program as an adjunct to rigidly supervised measures of cleanliness. The purpose of this report is to describe and evaluate laboratory methods used for estimating levels of bacterial populations surviving in the precooked frozen products.

When frozen meals were introduced into the U. S. Air Force in-flight feeding program in 1951, a military specification for these foods was published by the U. S. Army Quartermaster Corps. The sanitary requirements specified for their production included a provision for continuous plant inspection by the Veterinary Corps and imposed bacterial standards for the finished product. At present the specification (MIL-M-13966) provides for a standard plate count not to exceed 100,000 organisms per g, and a coliform plate count not to exceed 10 per gram. The two counts serve as an index of adequate cooking. Since the relatively heat-labile colon-aerogenes group of organisms cannot survive the time-temperature conditions of normal cookery, the coliform count has also been a useful indicator of post-cooking contamination. The basis for establishing bacterial standards for precooked frozen foods purchased by the military was discussed in a preliminary report (Rayman et al., 1955) and will be dealt with more extensively in a forthcoming publication (Huber et al., 1958). From those studies over a 5-year period, it became apparent that a need existed for specifying reliable, simple, and rapid methods of analysis suitable for control laboratories with limited facilities.

For determination of standard plate counts the tentative methods for the microbiological examination of frozen foods published in 1946 by the American Public Health Association, Committee on Microbiological Examination of Foods (APHA, 1946), proved to be relatively satisfactory. However, in some control laboratories where frozen foods are subjected to bacteriological testing it has been observed that a variety of procedures differing from the APHA methods have been employed. The precision and accuracy of some of these nonstandard modifications have not been determined and, particularly for specification purposes, the data so obtained do not permit ready comparisons between laboratories. Therefore, studies were made to determine whether the various details involved in the preparation of food samples for analysis, such as weight of sample and method of dispersion, would influence the bacterial counts. Previously mentioned tentative methods for frozen foods recommend an incubation temperature of 32 C for 4 days and current APHA methods for the examination of dairy products permit a choice of incubation temperature at 35 or 32 C for 48 hr. Since minimal incubation times are generally desired, comparisons of plate counts as influenced by times and temperatures were made. In addition to the total plate count and coliform plate count, the suitability of tests for enterococci and coagulate-positive staphylococci was also investigated.

Inadequate refrigeration often exists during transportation or storage of frozen precooked foods. If thawing should occur with ensuing bacterial multiplication, and the food refrozen, the consumer may not be warned of the potentially hazardous situation, although experts may recognize some evidence of
refreezing. Since the heat treatment given precooked frozen foods by the consumer is sometimes a mere overwarming which cannot be depended on for destruction of organisms, the presence of even small numbers of potential pathogens in these items poses a problem for regulatory agencies. Tests for the coagulase-positive staphylococci and for enterococci are warranted when there is reason to suspect that precooked frozen foods have been subjected to inadequate processing or to mishandling as described above. Simpler, more rapid methods for isolating and identifying these organisms are needed.

Preliminary surveys in this laboratory indicate that coagulase-positive strains of staphylococci are not infrequently found in precooked frozen foods. Evidence that such strains are pathogenic, enterotoxin-producing types has been reported by Evans and co-workers (1950). Many methods proposed for detection of these organisms rely on selective media containing high concentrations of sodium chloride, thereby permitting the development of staphylococci while suppressing the growth of most other bacteria (Hill and White, 1929; Chapman, 1945). Liquid media of choice, reported by Maitland and Martyn (1948), containing 10 per cent added salt, proved highly satisfactory for purposes of enrichment. Zebovitz et al. (1955) devised a tellurite glycine agar which was particularly selective for isolating coagulase-positive staphylococci. Utilizing the selective properties of both sodium chloride and tellurite, the present report describes a modified method suitable for detecting small numbers of these micrococci in frozen foods.

The role of enterococci as food-borne pathogens has been a subject of much debate (Moore, 1955). In recent years bacteriologists have shown considerable interest in the use of enterococci as an index of fecal pollution in water, and some (Larkin et al., 1955) have proposed its substitution in lieu of the long established coliform index. Burton (1949) suggested that enterococci which survive for long periods of time in fresh frozen foods may be a better index of fecal contamination than the coliform group which decreases in number during processing and storage.

In 1937 Hartman found that the use of sodium azide suppressed the growth of gram negative bacteria while permitting the streptococci to grow. Since this discovery, several investigators (Hajna and Perry, 1943; Winter and Sandholzer, 1946; Rothe, 1948; Mallmann and Seligmann, 1950; and Hajna, 1951) have devised selective media for isolating enterococci. These were tested for suitability in the analysis of precooked frozen foods. Although they are highly selective for enterococci, it was found desirable to use a confirmatory medium such as the ethyl violet azide broth (Litsky et al., 1955) for verification.

**Materials and Methods**

The majority of foods used in this study were precooked frozen tray and casserole meals produced for U. S. Air Force contracts (Military Meals). In addition, commercial precooked frozen meals, pot pies, and 21/2 pound bulk pack fresh frozen vegetables were purchased either from frozen food distributors or retail supermarkets. Wherever possible, all test samples in a set were from the same lot. Generally, a minimum of 5 meals was analyzed from a lot which usually consisted of a day's production of one menu (1000 to 3000 meals).

**Sample preparation.** Each component of a precooked frozen meal was analyzed as a separate entity. The entire (85 to 170 g) frozen block of meat (or potato or other vegetable) was chopped into approximately 1-g pieces with a sterile knife. The frozen pieces were weighed either in a sterile wide-mouth glass-stoppered bottle or a tared blender jar. A 1:5 suspension was made by adding one-half of the measured volume of sterile distilled water to the pieces in the blender jar, blending for 1 min, adding the remaining diluent and blending for an additional 2 min. The 1:10 dilution was made by pipetting 50 ml of the 1:5 suspension into a sterile, 50-ml buffered water dilution blank containing glass beads. Additional dilutions were made in buffered water as required. Military casserole meals were stripped aseptically of their baked crusts and 100-g samples chopped from wedge-shaped sections of the fillings. These 100-g amounts were then prepared as described above. The unbaked top crusts of a few commercial pot pies (chicken and beef) were analyzed separately in the prescribed manner. However, for the majority of the commercial samples the crusts were discarded. In the analysis of spaghetti and meat ball casseroles, 50 g of meat plus 50 g of spaghetti were used.

When samples blended in a Waring Blender were compared with those shaken in bottles with diluent and glass beads, a 1:5 initial suspension was prepared for each method. Samples of 50 g were blended in the blender jars with 200 ml of sterile distilled water, and 25-g samples were shaken in bottles with 100 ml of sterile distilled water and a teaspoon of glass beads.

**Standard plate count.** For each appropriate dilution, 1 ml portions were inoculated into duplicate plates using milk-protein hydrolysate glucose agar (BBL) as the plating medium. Unless otherwise stated, incubation was at 32 C for 72 hr. For experiments comparing 32 and 35 C incubation, two sets of duplicate plates were prepared, one set incubated at each temperature. When incubation times were compared, plates were counted after 48 and 72 hr.

**Coliform plate count.** Coliform counts were made in duplicate on serial dilutions prepared as described above, in accordance with *Standard Methods for the Examination of Dairy Products* (APHA, 1953), using desoxycholate lactose agar (BBL).
Detection of coagulase-positive staphylococci. Tubes of trypticase soy broth (BBL) containing 10 per cent added NaCl were inoculated in duplicate with 1 ml amounts of the 1:5 and 1:10 suspensions of sample and incubated for 48 hr at 32 C. From these enrichment cultures, phenol red mannitol agar (Difco) containing 10 per cent salt (PRMS-10 per cent) and/or tellurite glycine (TG) agar plates were streaked and held 24 hr at 37 C. Typical staphylococcus colonies were picked to Bacto-brain heart infusion broth and incubated at 37 C. After 18 to 20 hr, cultures were stained for verification as staphylococci, and coagulase tests were made. Directions for the test are provided with ampules of Bacto-coagulase plasma. When TG agar was used alone, the staphylococcus cultures from brain heart infusion broth were streaked on phenol red mannitol agar to demonstrate mannitol fermentation.

Detection of enterococci. The following broth media (Difco) were selected for comparison: S F, enterococcus presumptive (EP), azide dextrose (AD) and B A G. For qualitative studies, duplicate tubes of each test medium were inoculated with 1 ml quantities of the 1:10 dilution. Incubation at 45 C for 3 days was used for all except azide dextrose broth which was incubated at 37 C for 48 hr. Growth in the azide dextrose and acid production in the other three media was indicative of a presumptive positive test. Confirmation as enterococci was accomplished by subculture in ethyl violet azide (EVA) broth. Two drops from each presumptive positive tube were inoculated into EVA broth and incubated at 37 C for 48 hr. The presence of enterococci was demonstrated by turbidity and formation of a purple “button” at the bottom of the tube. Examination of smears made from the positive EVA broth cultures invariably confirmed the presence of streptococci. In preliminary trials, cultures from more than 100 positive EVA tubes were studied, employing the following confirmatory tests for enterococci described by Sherman (1937): (1) growth at 10 and 45 C, (2) growth in broth containing 6.5 per cent NaCl, and (3) survival in sterile milk after heat treatment at 63 C for 30 min. Since the presence of enterococci was continually reaffirmed by these tests in the early stages of this investigation, a positive EVA test was subsequently accepted as sufficient confirmation.

Quantitative studies were also employed for a comparison of azide dextrose broth with EP broth using the most probable number technique. In these studies, EP broth was preheated at 45 C before inoculation. Serial decimal dilutions (10⁻¹ to 10⁻⁵) were prepared, and for each dilution 3 replicate tubes were inoculated with 1 ml portions. Most probable numbers (MPN), based on confirmed positive tests, were computed according to the McGrady probability tables, (Bur- chan and Fulmer, 1928).

**RESULTS**

**Standard Plate Count**

Methods of sample preparation. Commercial samples of precooked frozen beef pot pies, precooked frozen Mexican corn (whole kernel corn combined with pimiento and green pepper) and of fresh frozen cauliflower, green peas, and lima beans were analyzed by the blending and shaking methods described previously. Results are given in table 1. The bacterial counts on the blended samples were higher, as was expected, in the majority of the samples. One shaken sample of Mexican corn, however, gave a much higher count. Each set of five samples was drawn from a single lot. This may have been due to the unequal distribution of corn, pimiento, and pepper; however, an attempt was made to choose equal proportions.

**Time of incubation**: 48 versus 72 hr. Standard plate counts were made on 114 components of precooked frozen meals (19 meats and 95 vegetables), to compare the effect of 48- and 72-hr incubation periods. The majority of the counts on the vegetables (92 per cent)

**TABLE 1**

<table>
<thead>
<tr>
<th>Product</th>
<th>Standard Plate Count, Thousands per g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Beef pot pie</td>
<td>32</td>
</tr>
<tr>
<td>Shaken</td>
<td>36</td>
</tr>
<tr>
<td>Blended</td>
<td>39</td>
</tr>
<tr>
<td>Mexican corn</td>
<td>60</td>
</tr>
<tr>
<td>Shaken</td>
<td>16.5</td>
</tr>
<tr>
<td>Blended</td>
<td>35</td>
</tr>
<tr>
<td>Peas</td>
<td>64</td>
</tr>
<tr>
<td>Lima beans</td>
<td>6.4</td>
</tr>
<tr>
<td>Shaken</td>
<td>0.6</td>
</tr>
<tr>
<td>Blended</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**TABLE 2**

<table>
<thead>
<tr>
<th>Component</th>
<th>No. of Samples Examined</th>
<th>Percentage of Samples Having Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Higher at 32 C</td>
</tr>
<tr>
<td>Vegetable</td>
<td>49</td>
<td>16</td>
</tr>
<tr>
<td>Meat</td>
<td>45</td>
<td>31</td>
</tr>
</tbody>
</table>

1 Dehydrated tellurite glycine agar is now commercially available from Difco, Baltimore Biological and Case Laboratories. Initially, the medium was prepared as described by Zebovitz et al. (1955).
were not influenced by the additional 24 hr of incubation. On the other hand, 11, or 60 per cent, of the meat samples gave higher counts after 72 hr.

Temperature of incubation: 32 versus 35 C. As shown in table 2, results from a total of 94 samples of meats and vegetables indicated that, in general, there was little difference between standard plate counts incubated at 32 or 35 C for 72 hr. A “difference” refers arbitrarily to an average colony count difference of 5 or more.

Isolation of Coagulase-Positive Staphylococci

A comparison between TG agar and PRMS-10 per cent agar was made, using parallel inoculations from the enrichment broth cultures. Of 93 frozen food samples examined in this manner, coagulase-positive staphylococci were recovered from 32 samples on TG agar and from only 23 samples on PRMS-10 per cent agar. All samples from which staphylococci were recovered on PRMS-10 per cent agar were also found positive on TG agar. When enrichment broth cultures were streaked on PRMS-10 per cent agar plates, an overgrowth of sporeforming rods occasionally masked the staphylococci colonies, making it difficult to isolate them for further study. Streaking on TG agar provided more effective inhibition of the sporeformers, permitting the easier isolation of staphylococci.

Comparison of Media for the Detection of Enterococci

Qualitative and quantitative tests for the detection and enumeration of enterococci were made employing four liquid media: namely, S F, B A G G, EP, and AD broths. In one series of qualitative tests for enterococci, 131 samples were examined using S F broth alone. Of 56 samples which were found presumptive positive, 3 failed to give a positive confirmatory test. Conducting a second series of qualitative tests, 100 samples were examined comparing B A G G broth with EP broth. Of these, 89 were positive in B A G G broth with no false positives, while 92 were positive in the EP broth with one false positive.

In the three selective media, S F, B A G G and EP broths, the presence of enterococci is indicated by the formation of acid. However, both the S F and B A G G broths which contain phosphate buffer and bromeresol purple (BCP) indicator often failed to produce a clear-cut acid color change. On the other hand, satisfactorily sharp color changes were regularly observed with the EP medium which omits the phosphate buffer and contains bromthymol blue (BTB) indicator. Therefore, two modifications of the B A G G broth formula were tested, one substituting BTB indicator for the BCP, the second using the same indicator, BTB, without the phosphate buffer. The sharpness of the color change was not improved in either modification.

In a third series, quantitative methods were employed comparing EP broth with AD broth. A total of 57 samples representing 10 commercial lots of frozen foods were examined for most probable numbers of enterococci using both media. EP medium recovered enterococci from 55 items and AD broth indicated that all samples were presumptive positive. EVA confirmations of these presumptive positive samples was obtained for 52 with the EP broth and 56 with the AD medium. When the sum of the presumptive positive tubes in the three significant dilutions was considered, the 57 samples furnished a total of 229 cultures from EP medium with 170 confirmations (74.2 per cent), whereas the corresponding values for AD broth were 301 presumptive positive cultures with 209 confirmations (69.4 per cent).

Typical data, illustrated in table 3, show reliability of presumptive positive tests in the two media. It will be noted that the number of presumptive positive tubes was generally greater in the case of the AD medium.

**Table 3**

<table>
<thead>
<tr>
<th>Product</th>
<th>Enterococcus Presumptive Broth</th>
<th>Dextrose Broth</th>
<th>Aspirate Dextrose Broth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. presumptive positive</td>
<td>No. confirmed positive</td>
<td>No. presumptive positive</td>
</tr>
<tr>
<td>Chicken pot pie</td>
<td>6 3 7 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef pot pie</td>
<td>5 0 5 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roast turkey and dressing</td>
<td>6 6 5 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green peas†</td>
<td>3 3 6 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parsley buttered potato</td>
<td>5 5 8 5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Sum of positive tubes in 3 significant dilutions.  
† Fresh frozen.
The per cent of false reactions appears to vary somewhat from lot to lot, and this range may be gauged from the table.

In Table 4 are shown estimates of enterococcus populations in a few commercially frozen foods as obtained by the two media. As noted before, results obtained with AD broth are generally higher than those with EP. It will be seen that vegetables such as green peas, which are uncooked, may be expected to have high enterococcus counts despite the fact that these products are usually subjected to a mild blanch prior to freezing. Precooked frozen foods, on the other hand, possess lower enterococcus counts dependent on the extent of cooking and the degree of recontamination during packaging.

**Discussion**

Owing to the heterogeneous character of precooked frozen foods, the bacterial content is likely to be unevenly distributed. Thus, Logan et al. (1951) found, for chicken à la king, that the bacterial content of the gravy was quite low, whereas the chicken meat contained 70 per cent of the total count. If, in sampling, the proportions of chicken meat to gravy were not maintained approximately equivalent to those of the processed product, the analytical results were subject to appreciable error. When these foods are prepared for analysis it is important to minimize sampling errors by weighing a relatively large sample and preparing a uniform suspension so that the bacterial composition of any small subsample is representative of the average composition of the whole product. Therefore, in preparing samples for analysis, each component of a meal was taken in its entirety and blended. A meal component, as referred to here, is an individual serving of a meat preparation or potato or other vegetable item. Such portions range in weight from approximately 3 ounces (85 g) to 6 ounces (170 g), depending on the food. Since many of the meat items weighed as much as 6 ounces, the blender method was found most practical for obtaining uniform suspensions using the entire sample.

As described above, a comparison was made of the bacterial counts obtained from samples prepared by blending and by shaking with glass beads. The present findings confirm observations by Jones and Ferguson (1951) that use of the blender method results in slightly higher counts. Coarse suspensions of shaken materials often furnish mechanical difficulties in transferring sample aliquots and lead to pipetting errors. Conventional shaking with glass beads is insufficient to reduce the particle size of some precooked frozen foods and, despite the use of 50-ml volumetric pipettes with cut-off tips, plugging by food particles cannot be avoided. Not only is it easier to pipette the blended sample, but it is also less time-consuming and the frozen pieces of food are not subjected to a mere surface washing as they are in the shaking method. It will be noted that the practice of blending a frozen product in the manner we have described does not permit the temperature of the resulting suspension to rise above 18°C, which is well below the incubation temperature range. Any heat generated during such a 3-min blending is insignificant as it relates to possible heat destruction of bacteria in the sample.

To further minimize sampling errors within production lots, it is important that a sufficient number of individual packages be selected for analysis to represent the average bacterial content throughout the lot. Variations in counts which occur during the processing and packaging of precooked frozen foods are dictated by the degree of sanitary control in the different plants, and this, in turn, influences the number of units needed for a representative analysis of a lot. Wherever possible in this study, at least five complete meals from a single lot were examined with the objective of obtaining fairly

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**TABLE 4**

*Estimation of enterococci* in precooked frozen foods. *A comparison of two media*

<table>
<thead>
<tr>
<th>Product</th>
<th>Most Probable Number per g</th>
<th>Enterococcus presumptive broth</th>
<th>Aside dextrose broth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken pot pie</td>
<td></td>
<td>25</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
<td>40</td>
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<tr>
<td></td>
<td></td>
<td>75</td>
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<td>75</td>
</tr>
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<td></td>
<td></td>
<td>45</td>
<td>250</td>
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<tr>
<td>Beef pot pie</td>
<td></td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
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<td>4</td>
<td>9</td>
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<tr>
<td></td>
<td></td>
<td>25</td>
<td>1100</td>
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<tr>
<td>Green peas†</td>
<td></td>
<td>1100</td>
<td>750</td>
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<tr>
<td></td>
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<td>250</td>
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<td></td>
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<td>1500</td>
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<tr>
<td>Parsley buttered potato</td>
<td></td>
<td>25</td>
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<td>45</td>
<td>95</td>
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<td></td>
<td></td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

* Confirmed.
† Fresh frozen.
good representation. The present military specification provides for a minimum of three meals of each day's production of 5000 units or less and one additional meal for each additional 2000 units per lot.

Incubation factors which influence the standard plate count appear to depend partly on the nature of the food and its accompanying microflora. Thus such foods as meat, containing large numbers of organisms producing pin-point colonies, showed increased bacterial counts when incubated longer than 48 hr. However, counts for vegetables remained substantially the same after 48 or 72 hr. For this reason it appears unnecessary to incubate plates longer than 3 days. Desiccation of agar and interference from spreading colonies are also thus minimized. The 72-hr incubation period allowing pin-point colonies to develop enough to be more easily differentiated from the background debris may account for the higher bacterial count found in 60 per cent of these meat samples. Most vegetables, on the other hand, contained large sporeforming rods that produced large, easily recognized colonies within 48 hr. Bacterial counts of meats and vegetables were not significantly influenced by varying the incubation temperature between 32 and 35 C.

Since the number of coagulase-positive staphylococci present in precooked frozen foods is usually very low in proportion to the total number of organisms, direct streaking of low dilutions on a selective medium such as PRMS agar and TG agar was not satisfactory. However, if an enrichment medium such as tryptase soy broth with 10 per cent NaCl added is employed, the isolation of these organisms becomes easier. In preliminary studies, the inoculated enrichment broth that had been incubated at 32 C for 48 hr was streaked onto PRMS-10 per cent agar plates. Because cocci, other than the coagulase-positive staphylococci, and gram positive sporeforming rods also grew well on these plates and produced acid, colony differentiation alone could not be relied upon. When TG agar was used, gram positive rods were easily differentiated from the staphylococci on these plates. The colonies produced by sporeformers were small and colorless or grey in contrast to the larger, entirely black, buttery colonies of the coagulase-positive staphylococci. The latter were confirmed by staining and by the coagulase test. Confirming the observations of Zebovitz et al. (1955) it was found inadvisable to hold the TG plates much longer than 24 hr because of the delayed appearance of coagulase-negative staphylococci which also produce black colonies. Since colonies of coagulase-negative staphylococci occasionally appear within 24 hr, a test for coagulase production is essential.

Of the several media employed for the isolation of enterococci, EP broth gave the most clear-cut results. The change from blue to bright yellow on acid production made the reading of a presumptive positive test much easier. Although AD broth seemed to be a more sensitive medium, as seen by the greater number of confirmed positive tests, the larger number of subcultures that had to be made was more time-consuming. Since EP broth gives good recovery of enterococci and the number of false positive reactions are few, it can be used satisfactorily on a qualitative basis for control work. Azide dextrose broth, on the other hand, is more applicable where greater accuracy for the quantitative estimation of enterococci is needed.

It is desirable that the EP broth be preheated at 45 C before inoculation to prevent the growth of streptococci other than those of Group D, since growth at 45 C is one of the differentiating characteristics of the enterococci. Although at 45 C this medium is highly selective for enterococci, confirmatory tests are required.

In his studies, Burton (1949) found that, as an indication of contamination, the coliform organisms appeared to be superior to the enterococci in unstored or briefly stored frozen foods, while the enterococci were a more reliable index in foods which have been held in freezer storage. The majority of the military meals used for the present analytical studies had been stored about 4 months; however, the storage history of the commercial foods was not always known. Coliform organisms in the stored precooked frozen foods should have been absent in most cases, since they are easily destroyed by heating. The results of these studies substantiate Burton's observations. Coliform organisms were recovered from very few of the precooked frozen items except where the original bacterial count was relatively high whereas enterococci were found much more frequently.

Presence of coliform organisms would indicate either that the foods had been cooked insufficiently or that they had been recontaminated later in the processing. Presence of enterococci would also indicate undercooking and possible recontamination during handling.

A comparison of the recovery of coliform organisms and enterococci is shown in table 5, where results of analyses on 414 samples were evaluated. These samples included military and commercial precooked frozen meats, vegetables, and pot pies, as well as commercial fresh frozen vegetables. From a total of 234 items prepared under military specifications, coliform organisms were found only in 2.5 per cent of the samples, and enterococci in 41 per cent of the samples. Of 180 commercially prepared items, 42.7 per cent not only contained coliform organisms, but they were present in much greater numbers than in the military meals. Enterococci were present in 92.7 per cent of the commercial samples. In the 42 samples of commercial fresh frozen vegetables the coliform count was less than 10 per g in 16.6 per cent; however, 78.5 per cent were positive for enterococci.

That coliform organisms and enterococci had been found less frequently in precooked frozen foods produced under military specifications may imply the need
in commercial production for better cooking standards, better plant sanitation, and better quality of raw materials. These data point up the fact that, when continuous supervision and inspection are exercised, a high quality standard for precooked frozen foods can be attained. Qualitative tests described above for detecting the presence of staphylococci and enterococci are useful aids in maintaining quality control. Further work is planned to investigate quantitative population levels of these organisms.

Incorporating the optimal features of the present investigation, the authors suggest the following prescribed procedure for the microbiological examination of precooked frozen foods:

Sampling. Take 1 package from each of 5 shipping cases of the same production lot in question. (Adequacy of this sampling will vary in relation to the size of the lot and, if there is any significant variability in the lot, this fact will become evident in the majority of cases through individual tests on the 5 samples.) Keep samples frozen with Dry Ice (solid CO₂) or other suitable refrigerant if analysis is to be delayed or sampling point is at some distance from the laboratory.

Preparation of sample. (a) Casserole meals—including pot pies. Remove aluminum foil cover. Using aseptic precautions, with a sterile knife cut or chop wedge-shaped sections of the frozen meal (including the crust, if present) into approximately 1-in. blocks. Weigh 100 g into a tared, sterile, screw-cap aluminum Waring (or similar type) Blender jar. Measure into a sterile, graduated cylinder a volume of sterile distilled water necessary to make a 1:5 suspension. Add aseptically to the chopped material in the blender jar approximately ½ to ⅔ of the required amount of water. Blend 1 min. Add the remaining water and blend for 2 additional min. Prepare a 1:10 suspension by pipetting 50 ml of the 1:5 suspension into a sterile, 50-ml buffered water dilution blank containing a teaspoonful of glass beads. (To facilitate this transfer, the tip of a 50-ml volumetric pipette should be cut off before sterilization to provide a wide bore.) Shake the diluted sample rapidly at least 50 times through an arc of 1 ft in order to insure homogeneity. Prepare consecutive decimal dilutions at the 1:100, 1:1000, and higher levels (if necessary) using sterile buffered water blanks.

(b) Multi-compartment tray meals. The contents of each compartment of a tray meal are analyzed as separate entities. (Thus, for example, a frozen block consisting of meat, gravy and dressing is considered an individual sample.) Remove sufficient aluminum foil cover to expose the compartment desired for analysis. Using aseptic precautions, cut or chop the entire frozen block of the product (50 to 175 g) into approximately 1-in. blocks with a sterile knife. Transfer all these pieces aseptically to a tared, sterile screw-cap blender jar and proceed as directed above for casserole meals.

Methods of analysis. (a) Standard plate count. Prepare duplicate plates from each dilution using 1 ml of inoculum per plate. Mix thoroughly with about 15 ml of melted milk-protein hydrolysate glucose agar and incubate at 32 to 35 C for 72 hr.

(b) Coliform plate count. Prepare duplicate plates from appropriate dilutions using 1 ml of inoculum per plate. Mix thoroughly with melted desoxycholate lactose agar and incubate at 37 C for 18 to 24 hr.

(c) Coagulase-positive staphylococci. Incubate duplicate tubes of trypticase soy broth containing 10 per cent NaCl with 1 ml amounts of appropriate dilution of sample. Incubate at 32 C for 48 hr and from each tube of the trypticase soy salt broth streak a loopful onto a tellurite glycine agar plate. After incubation at 37 C for 24 hr, pick typical black colonies (glossy and "buttery", 1 to 2 mm diam) to brain heart infusion broth. Incubate at 37 C for 18 to 20 hr. Prepare smears from these tubes to verify staphylococcal morphology and test for coagulase. (Directions for test are provided with ampules of the desiccated coagulase plasma.)

(d) Enterococci, qualitative tests. Incubate duplicate tubes of azide glucose broth (10 ml per tube) with 1 ml amounts of the appropriate dilution of sample, and incubate at 37 C for 48 hr. A positive presumptive test, indicated by visible growth, requires confirmation for enterococci by subculturing 0.1 ml into ethyl violet azide broth, with incubation at 37 C for 48 hr. A confirmed test is demonstrated by turbidity and the formation of a purple "button" at the bottom of the tube.

As an alternate medium for azide glucose broth, enterococcus presumptive broth may be employed. This medium should be preheated to 45 C before inoculation and incubated at this same temperature. Although a positive presumptive test, indicated by acid production,

<p>| TABLE 5 |
|------------------|------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th><strong>Recovery of coliform organisms and enterococci from military and commercial precooked and fresh frozen foods</strong></th>
<th>Sample</th>
<th>No. Samples Examined</th>
<th>No. Positive for Coliform*</th>
<th>No. Positive for Enterococci*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Commercial</td>
<td>Military</td>
<td>Commercial</td>
<td>Military</td>
</tr>
<tr>
<td>Precooked frozen</td>
<td>26</td>
<td>95</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>meats</td>
<td>30</td>
<td>129</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Precooked frozen</td>
<td>45</td>
<td>10</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>vegetables</td>
<td>37</td>
<td>0</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Chicken pot pie</td>
<td>42</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Beef pot pie</td>
<td>77</td>
<td>6</td>
<td>167</td>
<td>96</td>
</tr>
<tr>
<td>Fresh frozen ve-</td>
<td>180</td>
<td>234</td>
<td></td>
<td></td>
</tr>
<tr>
<td>getables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* For convenience, samples may be weighed a day in advance, kept in freezer, and blended on the following day. In this procedure, the chopped pieces of food are weighed directly into a tared, sterile wide-mouth bottle, placed in the freezer, and transferred aseptically to the blender jar at time of analysis.

* Present in 0.1 g test portion.
may be observed after 24 hr of incubation, it is advisable to keep the cultures at least 72 hr before discarding them as negative. Confirmation in ethyl violet azide broth, as described above, is required.

Quantitative tests. If quantitative determinations are desired, a most probable number (MPN) method may be employed using a series of consecutive decimal dilutions to growth extinction. For this purpose inoculate a suitable number of replicate tubes of azide glucose broth with 1 ml amounts, and, from the significant number of confirmed tubes in each of the appropriate three highest dilutions, calculate the numerical value using MPN tables (Buchanan and Fulmer, 1928).

(e) Salmonella. Inoculate 50 ml of a 1:5 suspension of product (equivalent to 10 g of food) into 200 ml of selenite F broth containing cystine. After 18 to 20 hr incubation, streak the cultures onto brilliant green and bismuth sulfite agar plates and examine as described by Byrne et al. (1955).

**Summary**

Suitable bacteriological methods for the examination of precooked frozen foods are discussed. Tests include standard plate count, coliform plate count, detection of coagulase-positive staphylococci and of enterococci.

For convenience, the use of mechanical blenders to prepare samples for analysis is recommended as a standard procedure. For frozen meals, the entire frozen block of meat or vegetable is blended to insure a representative sample.

Isolation of coagulase-positive staphylococci is facilitated by an enrichment in tryptcase soy broth containing 10 per cent sodium chloride, followed by streaking on tellurite glycine agar.

A comparison of several liquid media for the detection and enumeration of enterococci is described. Of the media tested, enterococcus presumptive broth and azide dextrose broth, when followed by a confirmatory test, are considered suitable for use in the examination of frozen foods.

**REFERENCES**


Burton, M. O. 1949 Comparison of coliform and enterococcus organisms as indices of pollution in frozen foods. Food Research, 14, 434-436.


Litsky, W., Mallmann, W. L., and Fifield, C. W. 1960 Comparison of most probable numbers of *Escherichia coli* and enterococci in river water. Am. J. Public Health, 45, 1049-1053.


