At the 1951 Reciprocal Meat Conference our committee presented a report on methods currently in use for microbiological phases of meat research. That report was based on a survey of methods in use by many of the leading laboratories of the country and pointed out areas of substantial agreement as well as others marked by considerable confusion. The worker in search of a prospective method might find our report helpful for certain procedures but quite the opposite for others. It therefore seems logical that as an extension of last year's report we ought now to present for your consideration tentative methods for the microbiological examination of meat and meat products. These methods are based on a combination of the survey report presented last year, on published methods and on our own experience and opinions. They are by no means final nor do they embrace the whole field of meat microbiology. They are, however, the methods that we consider to be entirely adequate for use as standard technique.

**Sampling**

All samples should be as truly representative as possible of the meat or meat products being studied. A sample composed of small portions from different locations will often help in attaining this. Methods of sampling large pieces of fresh and cured meat are reviewed by Jensen (3) and Tanner (6). In sampling trimmings or comminuted meats the method of pooling many separate samples is invaluable. All sampling should be done with sterile forceps, scalpels, and other necessary instruments and samples should be placed in sterile containers for rapid transportation to the laboratory. Curing brines may be sampled by using a large sterile pipette made from glass tubing and collected in a sterile flask.

**Preparation of Sample**

Fifth gram samples of meat should be weighed into a tared, screw capped sterile, Waring Blender jar and 150 ml. of sterile diluent added. This mixture should be blended for 30 seconds to one minute (see discussion of Waring Blender below) and four grams of the resulting 1:4 mixture collected by weighing into a sterile tared dilution bottle and 96 ml. of diluent added. This procedure gives a 1:100 dilution of the original sample which can then be diluted further as required in the usual manner. In cases where it is not feasible to collect 50 grams of original sample, 10 gram samples and 90 ml. of diluent can be blended and the resulting 1:10 mixture further diluted by pipetting. The latter alternative seems simpler in that it eliminates the necessity of weighing out the blended material but experience at Beltsville has shown that 50 grams of sample gives much more reliable counts than 10 grams.
Diluent

For fresh and frozen meats and meat products containing less than 3 percent sodium chloride physiological saline solution is the diluent of choice. For cured meats containing more than 3 percent sodium chloride, curing brines and similar substances, five percent sodium chloride solution should be used.

Plating Medium

Either veal infusion agar made by adding 1.5% Bacto agar to Difco Veal infusion medium or Tryptone Glucose Extract Agar (Difco) should be used for plating. In the case of samples containing more than three percent salt, five percent sodium chloride should be added to the agar. Our own experience indicates that veal infusion agar reveals more differences in colonial form and color than TGE agar and we prefer it for that reason. Comparative counts using both agars in experiments at Beltsville have not shown any difference in total number of organisms recovered.

Incubation

Since meat and most meat products are normally kept in refrigerators, 20°C. is the best incubation temperature because it encourages growth of a more representative meat flora than higher temperatures. Plates incubated at 20°C. should be held for 72 to 96 hours before counting. Where specific groups of microorganisms are sought, an incubation temperature close to the optimum temperature of the organisms should be chosen.

Reporting Results

Counts should be recorded and averaged logarithmically as described in the A P H A standard method (1) and reported as organisms per gram of meat.

Techniques for Special Groups of Microorganisms

Yeasts and molds should be counted by plating the prepared sample (see above) on acidified dextrose agar. This can best be accomplished by adding one ml. of a sterile solution containing 50 percent dextrose and five percent tartaric acid to each plate just prior to pouring the agar.

Coliform organisms can be determined by adding portions of the appropriate dilutions to tubes of brilliant green lactose broth and estimating the coliform count by the most probable technique of Hoskins (2).

Lipase forming microorganisms can be estimated by the Jensen-Turner (3) method or by the modification of that method described by Sulzbacher (5).

Oxidase forming microorganisms can be estimated by flooding plates with a one percent solution of p-aminodimethylamino oxalate (Eastman). Colonies of oxidase positive bacteria will turn a bright bluish purple color in a brief time.

Routine methods are available for certain other groups of interest to meat bacteriologists. Tanner (6) describes or quotes references for many of these and both Difco and Baltimore Biological Laboratories publish information on the use of special media for isolating specific organisms. Enterococci and staphylococci are mentioned as examples of such groups of organisms. Although
routine methods exist for their isolation and enumeration we do not consider them sufficiently well established for inclusion in these proposals at this time.

**Waring Blendor Sampling**

In the past 10 years more and more laboratory workers have turned to the Waring Blendor as a tool for preparing mixtures of solids and liquids. Bacteriologists have been particularly pleased with the ease with which mixtures could be prepared for plating by this handy device. Workers in meat laboratories have been almost unanimous in adopting the Waring Blendor because meat presents many sampling difficulties that are easily overcome by its use. I was very much interested, therefore, in a paper by Jones and Ferguson (4) pointing out certain disadvantages to the bacteriologist of Waring Blendor sampling. Nor was I the only worker so impressed. In fact, during the past several months the question of the proper use of the Waring Blendor has occasioned considerable discussion among food bacteriologists everywhere. It seems only fair then, since we are recommending the Waring Blendor for the comminution of meat samples for plate counts, that some account be presented to this group of the problems involved and our conclusions regarding their solution. Briefly, Jones and Ferguson observed that when dehydrated vegetables were sampled in a Waring Blendor considerably lower counts were obtained than when a shaking machine was used; that with a miscellaneous group of food samples some higher and some lower counts resulted when the Waring Blendor was used; and that with pure cultures of microorganisms lower counts were obtained with the Blendor than with a shaking machine, except for one culture of *Sarcina* which produced clumps. The advantages to meat work of using the Blendor are so great and because of the difficulty of obtaining a homogeneous meat suspension by other means, I am disposed to continue its use unless much stronger evidence against it is brought forward. However, we are now in the process, at Beltsville, of re-analyzing our counting technique in the light of Jones' and Ferguson's findings. Table 1 presents the results of a preliminary experiment showing the effect of length of blending time and sample size on bacterial counts. All samples were ground meat and samples C-1 through C-4 were all subsamples of the same lot of meat. In general, the data in table 1 seemed to indicate less variation in count due to differences in blending time than the differences imposed by taking successive samples of the same batch of meat. Before definite conclusions can be drawn we must repeat this work with several carefully prepared uniform samples. These preliminary data do serve, however, to emphasize certain elementary precautions that should be observed in the use of the Waring Blendor. These are:

1. Blendor jars must be kept in good mechanical condition. The largest heat rise we have noted is 10°C in 3 minutes. This is not excessive but it would be much greater if the blending mechanism were permitted to corrode and tighten.

2. Mix for as short a time as possible to obtain good homogeneous mixtures. Ground meat or sausage can be adequately suspended in 20 seconds or less. Chunks of fresh meat may take as long as 45 to 60 seconds and frozen meat even longer. It is almost never necessary to mix a meat sample as long as 3 minutes to get a good suspension.
Table 1. - Counts (org./gm.) of Sample Portions Withdrawn After Various Blending Times

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time in Blender</th>
<th>Sample Number Size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 sec.</td>
<td>30 sec.</td>
</tr>
<tr>
<td>A</td>
<td>10</td>
<td>13,000</td>
</tr>
<tr>
<td>B</td>
<td>50</td>
<td>83,500</td>
</tr>
<tr>
<td>C-1</td>
<td>10</td>
<td>169 x 10^6</td>
</tr>
<tr>
<td>C-2</td>
<td>10</td>
<td>129 x 10^6</td>
</tr>
<tr>
<td>C-3</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>C-4</td>
<td>50</td>
<td>199 x 10^6</td>
</tr>
</tbody>
</table>

Dr. Kastelic and myself, the members of the Microbiological Subcommittee, sincerely hope that the foregoing proposed methods for microbial meat analyses will prove helpful in your research work and that this Conference will serve as a starting point from which generally accepted standard methods for meat research in all its phases can eventually be established.

References

2. Hoskins, J. K. Most probably numbers for the evaluation of coli-aerogenes tests by fermentation tube method. Public Health Reports 49, 393 (1934)
3. Jensen, L. B. Microbiology of Meats 2d Ed. 1945
MR. HANKINS: Thank you, Mr. Sulzbacher.

Dr. Kastelic, have you something to say?

DR. KASTELIC: No, I don't have much to say. I would just like to say one thing as far as I am concerned personally. I have a feeling that we should be very much more critical of methods, procedures, and so on. I think if we are going to call ourselves scientists or research workers we must be. That does not mean to say that we should tear everything apart, but I certainly think that we should look to the validity of the procedures or the statements that may be offered concerning procedures or methods of measurements.

I want to make only one more comment. It is concerning the use of the Waring blender. I concur with Dr. Sulzbacher about the heating of the blender if it is not in a good state of repair. We particularly were concerned with it because we felt that homogenizing a tissue in the Waring blender might result in subsequent degeneration because of the heat rise, and we used ice cold solutions, ice cold blender jars and cold everything, and we found that in three minutes you can get an appreciable temperature rise. However, in looking in the literature, we know that some Canadian workers, Snow particularly at the Atlantic Fisheries, have described a baffle that you can drop inside the jar, made out of plastic or perhaps of some other material, of the general outline of the inside of the jar. You drop it in and it breaks up the vortex. We find that it very considerably decreases foaming. There is much less air beaten into the material that is being homogenized. However, it greatly adds to the load of the motor driving the blades and you will notice a susceptible slowing up of the motor. That may be of some interest to you. The details are given in this article of Snow's in one of the fishery publications. If you are interested, I can get it for you.

MR. HANKINS: Are there any comments or questions?

MR. HENRICKSON: I just want to say that we, too, are interested in this problem of the use of the Waring blender, and we find that in working with various types of products, that we need to blend the products for a longer period of time. In some preliminary work over a period of one minute through eight minutes we found that the time required to blend and to get it through the pipette varied with the type of sausage product we might be working with, and we found that two minutes on some of them and three minutes on others was the most desirable blending time.

I just wonder, too, why we specify the screw cap on the blender.

MR. SULZBACHER: Maybe I should explain something further. Skill in pipetting is very important to the bacteriologist using the Waring blender. For handling 50-gram samples we use a straight-side tube pipette not constricted, and draw out four grams. We draw up a quantity and weigh it in a square jar. The reason is that you just cannot pipette a heavy 1:4 mix with a volumetric pipette. For this particular sample the blender was just a whipped mass of ham and moisture. It is still better than blending with sand and water.
The screw cap is used because we sterilize these jars well in advance of when we need them. We have about 20 jars that we keep sterilized all the time in the cupboard. These are smaller jars than the jars with the loose bakelite caps and they are heat-resistant caps. They are generally more convenient. They fill on the balanced tray better. The screw cap stays on. It is more watertight, and I think they probably stand up better with continued use because of being able to use this flask.

DR. KASTELIC: Do you use any anti-foams?

MR. SULZBACHER: We have not. In the case of sausage and frozen meat, if you let the sample set for about a half minute, the foam subsides enough so that you can get material underneath with very little air. In the case of some of the ham samples, the fat has been sufficiently hydrolyzed that it will foam badly and even have a whipped cream effect, but that is very unusual. You don't often find it.

DR. HALL: That reminds me of some other whimsies that the Waring blender jars are subject to sometimes -- new ones that we inspect. I have seen a new blender jar in which the blades were set to run counter to the direction in which the motor turned them so that you cut with the back side of the blade instead of the sharp side.

MR. SULZBACHER: I have found that in about half the jars.

DR. HALL: And I have found jars in which the blades were crossed. One cut one way and the other cut the other way. So it was bound to be right half the time.

Another point is that the blades are seldom sharp. If you want to get the best efficiency out of it, it is a good idea to disassemble it and take the blades out and sharpen them before you use it.

MR. HANKINS: Is there anyone else? Do I hear a motion to adopt?

MR. RUST: I so move.

MR. HENRICKSON: Second.

MR. HANKINS: It has been moved and seconded that this report be adopted. All in favor say "aye"; opposed. It is adopted.

Now we will go to the discussion of organoleptic procedures. Dr. Johnson.

DR. JOHNSON: Dr. Deatherage has handed me this report and I am sure it is going to be short. I won't say anything about how sweet. It reads as follows:

"The subcommittee on organoleptic methods last year gave a survey of the methods in use in meats research. This subcommittee was directed by the conference to follow up on seven institutions which sent in questionnaires in 1951, yet did not indicate the degree of reproducibility of
tenderness determinations by taste panels. This has been done. Only two of the seven institutions replied but these replies gave no additional information. Consequently the 1951 report of this subcommittee will remain unchanged."

(The following paper was also submitted by Mr. Deatherage for inclusion in the proceedings)

# # #