For reading, the syringe is detached from the vial and the scale read by sighting across the flat end of the plunger and estimating to the nearest fifth or quarter of a division, again using a lens.

References

1. KROGH, A., and KEYS, A. B. *J. chem. Soc.*, 1931, Part II, 2436.
2. SCHOLANDER, P. F., EDWARDS, G. A., and IRVING, LAURENCE. *J. biol. Chem.*, 1943, **148**, 495.

Improved Technique for Enumeration of *Escherichia coli* on Black Walnut Meats[1]

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The enumeration of microorganisms found on solid materials is, in the main, based on their removal by washing a known weight of the solids with a known volume of washing fluid and then making serial dilutions from this fluid prior to the use of one of the common modes for determining numbers.

In the course of an investigation on the effectiveness of pasteurization for killing *Escherichia coli* on inoculated black walnut meats, it was found that washing the meats to remove the bacteria was not satisfactory when the Halvorson-Ziegler (*1*) method of determining the "most probable number" (MPN) of bacteria was used. The number of positive tubes found in the 10 replicate tubes of each of the three lowest dilutions gave codes which, when applied to the probability table, were equivalent to such "MPN" values as to make it evident that the seeding of the solid itself was needed if more accurate codes were to be secured.

Accordingly, an attempt was made to use the meats themselves in the replicate plantings, so that one member of the resultant code would be of no dilution. This was accomplished

TABLE 1

Test	MPN/gram	
	Washed meats	Blendor-treated meats
1	0	0.7
2	0.9	275.0
3	0	240.0
4	0	1.6
5	0	7.9
6	0	16.9
7	3.2	130.0

by cutting a known weight of the meats into small particles in a sterile Waring blendor container. After a homogeneous mass was secured and 10 replicate 1-gram samples had been removed for planting into lactose broth fermentation tubes, a known volume of sterile water was added to the container and the mixture further agitated until a creamy mass was secured. This material could be pipetted easily in making the 10 replicate plantings from this dilution, and it could also serve as the starting point for making subsequent dilutions.

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The results of typical tests made on inoculated meats which had received various heat treatments are shown in Table 1. This table contrasts the "MPN" of *E. coli* per gram of meats when washing was used for the removal of these organisms with the "MPN" gained by the use of the Waring blendor procedure. Meats from the same batch were used when comparing the procedures.

TABLE 2

Test	MPN/gram	
	Washed meats	Blendor-treated meats
1	6,220	11,600
2	24,400	39,900
3	2,900	4,930
4	3,990	9,200
5	11,400	32,900
6	1,960	4,840
7	7,420	34,900

The superiority of the Waring blendor procedure is shown also in Table 2. Inoculated nut meats which had not been heat treated served as source material for contrasting the two modes of removal. Meats from the same batch were used when comparing the procedures.

The results of these tests suggest that, where the solid lends itself to subdivision, the use of the Waring blendor for this process permits the seeding of homogeneous, undiluted solid material into the tubes of broth. This procedure, together with the actual "carrying over" of the solid into subsequent dilutions, makes for a greater recovery of the organisms than that secured by washing the solid materials.

Reference

1. HALVORSON, H. O., and ZIEGLER, N. R. *J. Bact.*, 1933, **26**, 559-567.

Cultivation of Microorganisms With the Aid of Cellophane Membranes

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This brief report describes the use of thin sheets of cellophane for the cultivation of bacteria and fungi. We first used cellophane for this purpose during the German occupation, when Petri dishes were unobtainable.

A sheet of cellophane was rolled around a rod and sterilized by autoclaving. It was then unrolled on a table, and melted agar medium was poured out over the sheet. The surface was inoculated by spraying, and the agar immediately covered by a second cellophane sheet. To save space, the whole culture was finally rolled up again loosely around the rod and incubated.

Recently we have taken up the problem from another side, using the cellophane sheets not only as a substitute for glass but as a dialysing membrane. These sheets are impermeable to bacteria and viruses, but they allow the passage of water and solutes (except those of very large molecular weight). Thus, colonies of bacteria can be grown on one side of the cellophane,