Stomaching: a New Concept in Bacteriological Sample Preparation

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An entirely new mixing device, particularly suitable for preparing bacterial suspensions from foods, fabrics, swabs, and other fairly soft materials, has been developed. With this technique the sample and diluent are put into an inexpensive, sterile plastic bag which is vigorously pounded on its outer surfaces by paddles when placed inside the machine. The resulting compression and shearing forces effectively remove even deep-seated bacteria. After samples are taken for analysis the bag and its remaining contents are thrown away. Labor involved in cleaning and sterilizing reusable homogenizer cups or probes is eliminated, and the device is immediately ready for reuse. Running costs are thus drastically reduced, compared with conventional homogenizers. Additional advantages of this device, which is simple and inexpensive to manufacture, are low noise level, negligible temperature rise, and the small storage space required for bags.

Preparation of bacterial suspensions from foods and other materials which cannot immediately be dispersed in water is normally brought about by shaking the sample with diluent in a bottle or homogenizing it in one of a variety of bench-top or hand-held blenders. Considerable labor is then needed to clean or resterilize the homogenizer cup or probe before it can be reused. Such homogenizers frequently possess other disadvantages: for example, high capital outlay for adequate numbers of cups or probes, high noise level, dangerous temperature rises during sampling, and high maintenance costs. To avoid some of these problems, several alternative methods of sampling have been proposed: for example, ultrasound treatment (4, 6), vortex stirring (6), surface scraping (9), water spraying (1, 2), vacuum probe (3), and electrophoresis (8). In certain applications these methods have distinct advantages over homogenization methods, but there is no evidence that they have become popular outside the authors' laboratories. Our experience is that many bacteriologists are suspicious of sampling methods that do not obviously disintegrate the specimen, even though the resulting suspension may, in many cases, be much easier to handle.

Since it is axiomatic that, during mixing, some or all of the mixer surfaces contact the specimen and become contaminated, we looked at ways in which the labor needed for resterilizing those surfaces could be avoided. One way to do this was to construct a mixer which would rapidly and automatically carry out a sterilizing routine using, for example, bactericidal solutions, ultrasound, or microwave or steam heating. An alternative and neater way appeared to be to prepare the sample in a disposable mixer. For routine use in a busy laboratory, however, the only containers likely to be available at reasonable cost were plastic bags, and the possibility of applying adequate mixing forces to the sample through the flexible walls of bags was investigated. Results were so encouraging that a series of devices (which have been given the generic name "Stomacher," after the action employed) were designed and constructed so that the potential of the method could be fully evaluated. The stomaching principle is the subject of patent application (Sharpe and Jackson, British Patent Application no. 41395/71).

Operating principle of Stomachers. All of the devices feature a means of temporarily sealing the sample and diluent inside the bag and of applying forces to the outside of the bag by means of paddles, wheels, or rollers. A particularly simple and successful design, supplied to us by A. J. Seward and Co. Ltd., (P.O. Box 1, 6 Stamford St., London S.E.1), is shown in Fig. 1 and 2. The bag is sealed near the top by being trapped between a rubber pad
A variety of flexible containers have been used, for example, polyethylene, polyester, cellulose-polyethylene-polyvinylidene chloride (PVDC) laminates, and rubber. A standard thin-walled polyethylene bag, preferably base-welded, is entirely adequate, however, and preferable from the point of view of cost, handleability, and freedom from toxic effects.

Removal of bacteria is probably brought about partly by violent shearing forces as the liquid is swept from side to side, and partly by the series of rapid compressions the sample experiences as it is trapped under the paddles. This repeated "sponging" action may be responsible for the effective removal of bacteria present in fissures or crevices, for example, in the veins and capillaries of meat.

Incorrect design can cause rapid bag failure through the production of high hydrodynamic pressures. Failure may then occur at a weld, or in the fabric of the bag, depending on its type. High pressures can be avoided by correct design, however, and catastrophic failure does not occur in the models shown. Sharp objects such as bone splinters may make pinholes through which the suspension will leak, but such damage is infrequent and leaks occur less frequently than with our normal homogenizer cups. The wooden sticks of swabs do not damage bags. Addition of a soft rubber curtain allows even soil samples containing grit and small pebbles to be processed safely.

**MATERIALS AND METHODS**

**Samples.** Food samples were bought locally or were experimental samples obtained from other parts of the laboratory. Frozen foods were thawed, and dried products were resuscitated by soaking for 40 min as 20% suspensions in 0.1% peptone solution before use. Samples were divided and homogenized or stomached separately. Soiled woven fabrics were cut into thin strips and divided. Swabs were added to bags, complete with their sticks.

**Stomaching.** All bacteriological analyses were made using the middle-sized Stomacher, at 230 rev/min. Food (10 g) or fabric samples (4 g) were weighed into sterile polyethylene bags (7 by 12 inches, A. J. Seward and Co. Ltd., or Sterlin Ltd., Richmond, Surrey, England), and 90 ml of 0.1% peptone solution was added. (The procedure was adjusted accordingly where soaking was required.) For swabs, 10 or 50 ml of peptone solution was added. Stomaching times of 30 sec were generally used except where release rates were being studied. With many products (e.g., fruits and comminuted meats) complete dispersal occurred within 5 sec.

**Homogenization.** The same sample and diluent quantities were homogenized in sterile 200-ml stainless-steel cups on an Ato-Mix blender (MSE Ltd., Spenser Street, London S.W.1., England) for 2 min.
(15 sec at 6,000 rev/min, 90 sec at 12,000 rev/min, and 15 sec at 6,000 rev/min). This device is similar to the Waring Blendor. The program is used by many of our laboratories.

**Count procedure.** Three types of counting method were used during the study. Serial 10-fold dilutions in 0.1% peptone solution were inoculated into plate count agar or violet red bile agar (Oxoid Ltd., London S.E.1, England). Total viable aerobic counts (on plate count agar) were made after 48 hr at 30 C, and coliform counts (on violet red bile agar) after 24 hr at 37 C. On other occasions, counts were made by using a rapid technique employing agar droplets (7). Other total viable aerobic and coliform counts were made by using an automatic diluting, inoculating, and pour plate preparing machine (5). Coliform counts were made on only about 20% of samples, and no distinction has been made between the two types of count in analyzing the data.

**RESULTS**

About 550 comparisons of samples treated by Stomacher and Ato-Mix blender showed an excellent recovery of bacteria by the new method (Table 1). Release rate studies indicated that for most foods 15-sec stomaching is adequate, but for routine work 30 sec is preferred. The beef group contained some rather fatty forequarter samples, and it was noticed that, for these, recovery decreased with increasing fat concentration. For samples containing about 95% fat, the recovery was less than half that of the Ato-Mix samples at 30 sec, but improved with increasing stomaching time. Lower recovery was also noted for short-crust pastry and dairy cream. For high-fat meats etc., therefore, 1 min or more may be preferable. A rough guide is obtained from the appearance of the sample, which is easily visible through the transparent walls of the bag. Recovery efficiencies for coliforms were as good as for total viable aerobic organisms, and it is unlikely that selective retention of any organism would be experienced, except perhaps in high-fat materials. No difference was noted between Stomachers with paddles side by side, or one above the other, and no decrease in count was noted during stomaching times of more than 5 min.

Temperature rise during stomaching was negligible (approximately 0.8 C/min for liquids at ambient temperature). This contrasts markedly with certain mechanical homogenizers, in which a most probable rise of 17 C (i.e., most samples warming to 37 C) during the 2 min has been found. Temperatures up to 59 C have, in fact, been recorded in our laboratories in homogenizers having bearings in poor condition.

**DISCUSSION**

Stomaching appears to have many advantages as a sample preparation method for any bacteriology laboratory dealing with reasonably soft materials, particularly in regard to the elimination of labor for recycling homogenizer cups or probes. Capital outlay is likely to be low, and running costs are negligible. Plastic bags require very little storage space (1,000 bags are less than 15 cm high), and a large reserve can be kept by. They are excellent for taking and transporting samples from factory processing areas. The base-welded bags formed from flat polyethylene tubing are always to be preferred since the method of manufacture virtually precludes the possibility of more than the occasional organism being found within a bag, and these will be suitable for most routine analyses on foods. Bags can, if necessary, be obtained for which sterility is claimed, but it may be worth checking that these have received actual sterilizing treatment before incurring the extra expense over standard items.

Noise level from the Stomacher is low; its dull squelch is, in any case, far less irritating than the whine of blades homogenizers. The
small temperature rise is desirable when sensitive organisms are likely to be encountered. The whole device is light and easily portable, with a supply of bags. In this respect a Stomacher combined with the rapid droplet technique (7) forms an excellent portable testing method. As an additional benefit, two or three samples may be placed in the Stomacher together and processed simultaneously, thereby effecting even greater economies in time.

Only for products of very high fat content is recovery significantly lower than by conventional blending. On no occasion has the difference been greater than 50% after 1 min of stomaching. For these products, slightly warmed diluent is a useful aid, assisting dispersal of fat. This effect seems to indicate that the temperature rise generated by conventional blenders may be a significant factor in their better performance on fatty products.

Bag failures are remarkably infrequent. The machine is not particularly demanding in its requirements of bags. The use of a good quality bag is recommended of course, but flexibility is more important than toughness. For this reason, the thinner grades of polyethylene bag (e.g., 200-gauge) are preferable to heavier or laminated bags, which are usually considered to be more durable. The Stomachers shown in the figures have complete protection for the motor and all other electrical components in the event of a bag bursting catastrophically. This has not occurred with these machines; only a small number of pinholes (<1%) have been obtained with many thousands of samples. Leaks are more likely to occur near a weld than in the fabric of a bag, and, for this reason also, a bag formed from flat polyethylene tubing is preferred since the length of weld is minimal.

If samples containing pathogenic organisms are to be handled it is quite possible to place the sample bag inside another, for example a PVDC laminated or rubber bag, to obtain complete protection should the sample bag begin to leak. If no leakage has occurred the outer bag may be reused.

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