IN THE LABORATORY

A Method for Determining the Volume of Small Solid Objects[1]

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Frequently it is difficult to determine with accuracy the volume of small objects of unknown density, especially when the object must be kept moist. In this laboratory we found it necessary to determine the volume of a large number of teeth which could not be permitted to dry. The most logical solution to this problem appeared to be the measurement of fluid displacement. The illustration shows the plan by which the fluid displaced by the object (F) is measured in a small-bore pipette. The potential accuracy of the instrument is primarily dependent on the ratio of the volume displaced to the bore of the pipette.

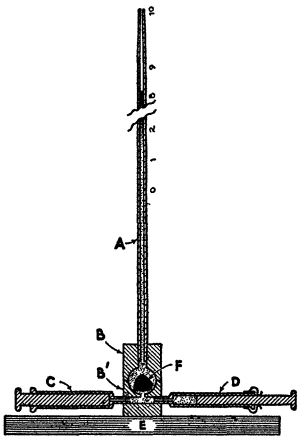

FIG. 1

The equipment consists of an ordinary graduated pipette (A), accurately faced methyl methacrylate blocks (B and B'), a large glass syringe (C), a smaller syringe (D), and a metal base (E). The syringes and the pipette are fitted into the plastic blocks through ground joints, making it possible to use various syringe combinations as well as pipettes of various sizes, provided that they are uniformly tapered. For our work, a 1-cc. pipette, a 2-cc. and a 1-cc. syringe were adequate. Stopcock grease should be used between the plastic blocks in order to prevent leakage. Volume determinations are made by filling both syringes and a portion of the lower chamber with distilled water. The upper section is then put in position and the plunger of the larger syringe inserted completely. The height of the water in the pipette is then adjusted to the zero mark by means of the smaller syringe. The plunger of the adjusting syringe should have a tension clamp to prevent free movement. Then, by withdrawing the plunger in the larger syringe, the water is removed from the upper unit and partially removed from the lower chamber. The blocks then are separated, and the object whose volume is to be determined is placed in the lower chamber. The upper unit is put in position as before, and the plunger of the large syringe is inserted completely. Since the same volume of fluid has been replaced in the system, the difference between the original height of the column and the present height is equal to the volume of the object in the chamber. The reading may be taken directly from the calibrated pipette. After each determination, the water column must be adjusted for the initial reading as described above. This method is rapid and, we believe, quite accurate. The size of the apparatus is dependent on the volume of the objects to be studied. Any fluid compatible with the materials in the apparatus may be used.

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A Contact Culture Method for Detecting Molds on Surfaces

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A number of methods for the detection of molds on surfaces have been devised. In one of these (*2*) melted agar is placed on the surface to be tested, while in others (*1*, *3*) solidified agar is brought into contact with the surface. The method described below is of the latter type. It differs from other methods of this type in that a culture area equivalent to that in a standard-sized Petri dish is employed. The materials necessary for its preparation are readily available in the laboratory. The method has been used for culturing molds from contaminated surfaces in food plants. With the acid types of media employed yeasts also appeared on the plates, and by the choice of appropriate media the method could also be used for bacteria.

From a coarse type of filter paper discs approximately 8.5 cm. in diameter are cut, a small tab approximately 5 x 15 mm. being left on one side of each disc. The discs are soaked in a 0.5 per cent water solution of methylene blue until thoroughly colored and then rinsed in water to remove excess stain. When the rinse water is relatively free of stain, each disc is placed in a Petri dish and sterilized in the usual way by dry heat. Three ml. of melted sterile agar is added to hold the paper flat to the bottom of the plate. After cooling, 7 ml. of sterile agar, of appropriate composition to make a thin coating over the paper, is added. When the agar hardens, it is ready for use.

To test exposed surfaces in food plants for contamination with molds and yeasts, the agar-coated paper disc is removed

aseptically by grasping the tab with sterile forceps and placed carefully, face down, upon the area under test. It is then removed and replaced in the Petri dish in its original condition.

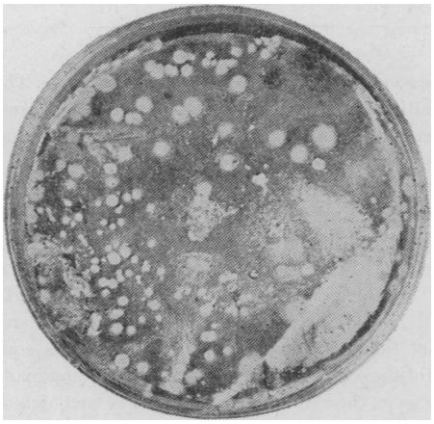

FIG. 1. Appearance of typical colonies on filter paper culture.

Mold and yeast growths may be noted in 24 hours with the Greenough microscope. Colonies usually become distinct within 48 hours. The blue paper is used merely to facilitate counting of the colonies. A typical culture is shown in Fig. 1.

References

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The Color Reactions of Sulfonamides With β-Naphthoquinone-4-Sulfonate

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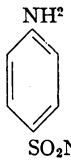
The reaction of β-naphthoquinone-4-sulfonate with amino groups to yield colored substituted naphthoquinones may be utilized as a qualitative method of differentiation for the various sulfonamides now used in antibacterial therapy.

For the purpose of detection and identification of the pure compounds or of the compounds in pure systems, the varying colors produced by the surface reaction of β-naphthoquinone-4-sulfonate solution with an aqueous suspension of the various sulfonamides, as well as the speed of the reaction or color formation, may be a useful device.

Reactions of the naphthoquinone were noted with p-aminobenzene sulfonamide (Sulfanilamide), 2-sulfanilamido thiazole (Sulfathiazole), 2-sulfanilamido pyrimidine (Sulfadiazine), 2-sulfanilamido-4-methylpyrimidine (Sulfamerazine), 2-(N^4-succinylsulfanilamido) thiazole (Sulfasuxidine), 2-sulfanilamido guanidine (Sulfaguanidine), and 2-sulfanilamido pyridine (Sulfapyridine).

All of these compounds with the exception of Sulfasuxidine contain a primary aromatic amino group and all except Sulfaguanidine have a portion of their structure in common:



Sulfaguanidine contains no hydrogen on the sulfonyl nitrogen attached to the guanidine carbon by a double bond.

The R moiety of the molecule varies in each case and this apparently influences the color of the compound formed and the speed of the reaction.

The test is carried out by suspending approximately 100 mg. of the sulfonamide in 5 cc. of distilled water in a test tube and adding 1.0 cc. of a 0.5 per cent solution of β-naphthoquinone-4-sulfonate as the sodium salt.[1] The color formation and color change are observed during the first minute and again after 5 minutes. The addition of 5 cc. of 10 per cent ammonium hydroxide provides a confirmatory test in some instances.

TABLE 1

Compound	Color after 1 min.	Color after 5 min.	Color after addition of NH_4OH
Sulfanilamide: *stable* bright orange suspension formed within 30 seconds	Bright orange	Bright orange	
Sulfathiazole: *rapid change* from yellow through orange to a brick red in 2–3 minutes, then to a reddish brown, and finally to a *stable* purple brown	Dark orange	Purple brown	
Sulfadiazine: *very slow change* from lemon yellow	Deep yellow	Orange yellow	Clear, deep brown changing to emerald green (differentiation from Sulfamerazine); *stable* for several hours
Sulfamerazine: *rapid change* from lemon yellow to orange yellow to orange (not as dark as Sulfanilamide and changes much more slowly)	Orange	Orange	Dark orange changing to clear, dark brown (differentiation from Sulfadiazine); *stable* for several hours
Sulfasuxidine: *stable* lemon yellow	Yellow	Yellow	
Sulfaguanidine: *rapid change* from yellow to a *stable* bright crimson within 30 seconds	Crimson	Crimson	Clear brown solution (differentiation from Sulfapyridine)
Sulfapyridine: *slow change* from yellow to orange (differentiation from Sulfaguanidine)	Orange	Crimson	Brown solution rapidly changing to emerald green (differentiation from Sulfaguanidine)

Results of the test using the compounds listed are shown in Table 1. It is thus possible to identify or to distinguish between various sulfonamide compounds now commercially available for therapeutic use.

[1] Sodium β-naphthoquinone-4-sulfonate, Eastman Kodak Company.