THE MILLIPORE FILTER TECHNIQUE IN THE DAIRY INDUSTRY

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The Millipore Filter technique is a new method for the concentration, removal, and enumeration of bacteria in liquids and air. Its application to the evaluation of the sanitary condition of pipe lines cleaned in place is discussed, together with other possible applications to water, milk, and food bacteriology. This procedure will be a valuable tool in quality control and bacteriological research.

INTRODUCTION

The Millipore Filter Technique is a comparatively new method for the concentration, removal, and enumeration of microorganisms from liquids or the air. The terminology used when referring to this technique varies inasmuch as it is known as the "Molecular Filter Membrane," "Membrane Filter," the "Molecular Filter," or the "Millipore Filter." The term "Millipore Filter" (MF) is the name used by the Lovell Chemical Company of Watertown, Massachusetts, which manufactures and distributes the filters and accessories.

Originally the technique was developed as a method for testing the sanitation of small-scale water supplies in the field in Germany during and after World War II. The first report in the United States was prepared by Dr. Alexander Goetz in 1947 as Report 1312 of the Joint Intelligenee Activities Agency, Washington, D.C., a report of the Department of Commerce of the U.S. Government. The first public report on this technique appeared during the summer of 1951. A write-up in Life on biological warfare describes the technique and discusses its application to the examination of water supplies as a method of defense against biological warfare. This report followed the Public Health Report release on the "Membrane Filter in Bacteriology" in July 1951. The technique was not released for commercial application until early 1952 and at the present time details of the methods of preparation of the membranes are still under military classification. However, the membranes are now available commercially and their application is unrestricted.

The important contributions in this field have been made during the last five years primarily through the efforts of Dr. Goetz and his collaborators at the California Institute of Technology and the investigators at the Environmental Health Center. These workers have studied methods for the manufacture of the membranes and the development of suitable media for the growth of bacteria collected on the membranes. Now the technique can be visualized as having many applications in the field of bacteriology.

DESCRIPTION OF EQUIPMENT

Briefly the filter membrane is a small (2 inches in diameter) circular paper-thin disc containing about 500 million pore openings. Although the membrane looks like paper, it has no fibrous structure and no binding agent. This membrane will filter out and collect on its surfaces all the bacteria present in any liquid which is forced through it, usually with the aid of suction. After the bacteria have been collected, the membrane is aseptically removed from the filter mount and placed on an absorbent pad to which has been added a small amount of double-strength nutrient broth. An indicator is usually added to the broth which aids in the identification of the colonies. In some diagnostic studies a series of nutrient materials are utilized and the membrane is transferred from one to another after varying periods of incubation. The membrane and pad are incubated in a highly humid atmosphere at suitable temperatures. Within four to eight hours colonies usually develop which can be observed with a low magnification microscope. After a 12 to 15 hour incubation period the colonies develop to such a size that they can be readily distinguished by the naked eye.

The filter membranes may be sterilized by autoclaving at 15 pounds pressure for 15 minutes if they are packed with absorbent pads between each filter disc and the discs and pads wrapped in kraft paper. They also can be sterilized by exposure to ethylene oxide. The filter mount, which consists of any suitable funnel arrangement capable of supporting the membrane filter under vacuum, also can be sterilized by autoclaving or by exposure to vapors resulting from the burning of methyl alcohol. This last method of sterilization makes the equipment adaptable to field work where laboratory facilities are not available. (Figures 1-4).

APPLICATIONS

The applications of this technique reported to date have been concerned primarily with water bacteriology. In these studies the investigators have been interested in the recovery of certain types of microorganisms from water supplies, and the development of suitable differential media for the classification of these organisms. Reports indicate that coliform organisms are easily detected and that by proper differential media the
typhoid organisms also can be recovered from polluted water samples.

One investigator has shown that the method can be applied to marine microbiology. He states that results would indicate that higher numbers of bacteria capable of multiplying on sea water broth may be determined than by the usual plating methods. Other investigators have been concerned with the use of this technique in the recovery of organisms from aerosols or various air supplies.

During the past 18 months, studies have been made on the application of the Millipore Filter technique to problems of the dairy industry by investigators at National Dairy Research Laboratories. Preliminary studies were concerned with the recovery of *Escherichia coli* from artificially infected water supplies. Low concentrations of this organism were added to sterile water and the water passed through the Millipore Filter apparatus. Control counts were made by standard plating procedures. These studies indicated that when low numbers of coliform organisms were present in a water supply, the Millipore Filter results were higher than those obtained by standard plating procedures. Further studies using the public water supply available at the Laboratories resulted in higher counts when the Millipore Filter technique was employed. These results indicated that when only a few organisms were present in a large volume of liquid the Millipore Filter technique was a more reliable procedure to use in their enumeration than standard plating techniques.

The evaluation of the sanitary condition of cleaned and sanitized dairy equipment has always been a problem. Theoretically there should be no organisms present on equipment which has been properly cleaned and sanitized. However, it is realized that frequently small numbers of bacteria may be present. Rinse techniques and swab techniques have been applicable when the equipment is disassembled for the cleaning and sanitizing operations. However, since in-place cleaning is becoming an acceptable method for cleaning and sanitizing dairy equipment, it was realized that the swab technique, at least, could be applied only with difficulty. With a rinse technique
the large volumes of water necessary to rinse lines cleaned in-place made standard plating procedures impractical.

The Millipore Filter technique was applied to the evaluation of pipe lines cleaned and sanitized in-place. Preliminary studies were conducted at the Research Laboratories and also in a plant where in-place cleaning was practiced. After the line had been sterilized (either with chlorine or hot water) and drained, water from the public supply was circulated through the line and samples taken for bacteriological examination after 5, 10, or 15 minutes of circulation. Using the Millipore Filter, it was possible to enumerate the bacteria in the circulated water in volumes up to 1000 ml whereas with standard plating procedures an accurate count would not be possible at all. By comparing the counts on the water before and after circulation, the number of bacteria contributed by the pipe line could then be estimated. Ideally a sterile water rinse would be more desirable since all the organisms recovered would then come from the equipment. However the volumes of water needed for circulation through the equipment made the use of sterile water impractical. Work is now in progress to establish standards for this procedure.

**OTHER APPLICATIONS**

At the present time it would appear that the Millipore Filter technique can be applied in any situation where the diluting fluid is low in organic matter. For example in addition to the bacteriological examination of water supplies and the sanitary condition of cleaned and sanitized pipe lines it is possible to determine the bacteriological content of such containers as milk bottles, beverage bottles, ice cream containers, glassware, etc. In the ice cream industry the technique could be used to determine the bacteria content of flavoring materials, bacteria present on fruits and nuts, the numbers of organisms in sugar solution, etc. Here it may be necessary to develop special culturing media which would favor the types of organisms which might be present on these products.

It has been noted that the applications of the Millipore Filter are limited by the amount of
suspended matter present in a solution which ultimately may clog the membrane. Under such conditions the difficulty may be overcome by using membranes of larger sizes. If this can be done, the technique will have applications in the determination of the sterility of various products. This phase of the work is being investigated at the present time but as yet the proper combination of increased filter size and dilution of the product with sterile water have not been thoroughly studied. It is hoped that it will be possible to adapt this technique to testing various high-temperature pasteurization procedures, and to the enumeration of special types of organisms present in dairy products such as psychrophilic or thermophilic organisms. Such applications will require considerable further investigation.

There is the possibility that this technique might be used in germicidal testing where a one hundred percent end point is desired. In this instance, of course, a suitable neutralizing agent would be necessary to inactivate the germicidal solution.

Our investigations have been concerned with the Hydrosol filters. However, there are available Aerosol filters which can be used for the recovery of microorganisms from gaseous materials. Using the Aerosol-type filter, checks can be made on the bacteriological condition of air in enclosed areas. For example, the possibility of air contamination of cottage cheese can be determined by placing an aerosol unit near the cheese vat and collecting samples of the air which coalesces in contact with the vat. The unit also might be used to check the sterility of an air supply by placing an Aerosol filter in an air line.

It has been the purpose of this paper to bring the Millipore Filter to the attention of milk and food sanitarians. Since it has not been possible to review all the literature available on the subject in this short paper, a selected bibliography has been prepared and is presented here. The technique is being evaluated quite extensively in the field of water bacteriology, but it is visualized that there are many other applications of the technique. As more investigators become familiar with this procedure, new and improved applications will become available. There is little doubt that the Millipore Filter technique will eventually become a valuable tool in quality control and research in water, milk, and food bacteriology.

Bibliography

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"DOCTOR JONES" SAYS:"

Paul B. Brooks, M. D.

When I was a boy, on my uncle's farm, we naturally used to have turkey occasionally—Thanksgiving or some other special occasion. We "picked" the turkey, afterward, and as there was anything left on the carcass, the nearest thing they had to refrigeration was a cool cellar. None of us ever got sick from our turkey picking. If we had we'd, maybe have laid it to something else: a change in the moon or something. But the turkeys didn't come frozen. They came fresh from some neighbor's farm.

But, here of late, we've been picking on turkey's from a different point of view. That is: as a cause of outbreaks of food poisoning and infections. We've here's one that looked like the "irony of fate." It was an outbreak of food infection from turkey "a la king" at the annual convention of the International Association of Milk and Food Sanitarians. A very complete and scientific story about it was published, recently, in the association's journal. With the evidence all in it was irony—not of fate but of failure. It was due to failure of hotel food handlers to stick to certain recognized and standard rules of food hygiene.

There was one unusual feature. A thorough investigation showed a heavy contamination of the turkey dish with paracolon bacilli—the apparent cause. They're germs that don't ordinarily cause sickness. But good authorities say they've been known to cause outbreaks, under certain conditions, when the food was loaded with em.

Here's something more recent. Psittacosis ("parrot fever") has recently been found in turkeys for the first time. During the past few years it's been found in pigeons and in ducks and other poultry. Down in Texas there were 63 cases of the disease among workers in poultry dressing plants. Investigators from the University of California traced the source to turkeys and found the virus. The workers apparently caught it from handling em.

All this turkey business—it at least goes to show one thing. Whether it's poultry or people, the more we know about 'em the better we know what to look out for.