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Today ~~koli~~-titer is determined by the ~~fermenting~~ fermentation method or by the method of determining the ~~koli~~-index by filtering the water through 35 millimeter diameter membrane ultrafilters. As is known, the ~~fermenting~~ fermentation method is cumbersome, labor-consuming, and only relatively precise. Testing with the aid of membrane ultrafilters, on the other hand, is a method that would have to be considered the most perfect in principle, if only the work did not become complicated by too small a diameter in the ultrafilters (35 millimeters) and single-stage filtration.

~~We use~~ The small diameter of ~~the~~ ultrafilters is suitable only for minimum amounts of water, for example, 10 milliliters, with a ~~negligible~~ insignificant microbe content. If 100-200 milliliters of water or more have to be filtered, this quantity it must be divided into many small ~~amounts~~ portions and filtered 25-50 milliliters at a time. After being filtered through ultrafilters of small diameter, ~~they~~ the filters are placed on a culture medium, four on each dish. ~~If necessary,~~ it is possible to count the colonies growing on the small ultrafilters and if necessary, to separate them out only when the water or other liquid being tested contains a ~~negligible~~ insignificant quantity of bacteria and suspended particles. ~~On the other hand~~ Otherwise, even as a result of filtering only 25-50 milliliters, such an enormous amount of colonies grow on the surface of the ultrafilter that they can neither be counted nor separated out, especially if a large deposit of suspended particles forms on the surface of the ultrafilter, hiding the colonies of bacteria.

In order to eliminate the defects described above, we suggested ultrafilters with a large diameter, 87 millimeters, while for testing liquids with a considerable amount of suspended articles, we suggested first filtering through coarse-pored filters. /

When the 87-millimeter ultrafilter is placed on a standard-size 100 millimeter Petri dish (with a dense culture), it actually covers the whole working area of the Petri dish, leaving an open space of about six millimeters around the circumference. This space is necessary for correctly placing the ultrafilter on the surface of the dense culture (without the formation of air bubbles), and for freely removing the ultrafilter growing to the surface of the culture.

The 87-millimeter diameter increases the area of the ultrafilter six times in comparison with the area of the 35 millimeter ultrafilter being used now. Consequently, we do a kind of simultaneous filtration through six small filters. In this way, if we can put a maximum of four small filters on one Petri dish, then, if we put one large ultrafilter on the Petri dish we have a growth of colonies not on four but on six ultrafilters, i. e., 50 percent more. In addition, it is much easier to count and separate out the colonies on the large ultrafilter. The large amount of colonies heaped together and fused on the small ultrafilter lack such advantages.

The second defect of the method in use now is single-stage filtration. This is the formation of a deposit of suspended

particles on the surface of the ultrafilter which hinders distinguishing, separating out, and counting the colonies growing on the surface of the ultrafilter. Such a deposit usually is formed when unpurified waters from natural reservoirs and such non-alcoholic drinks as kvass, cranberry juice, etc., containing suspended particles, are filtered. To eliminate this defect we suggested (in 1936) simultaneous double-stage filtration and we first made the coarse-meshed filter in 1936, calling it the "preliminary" or "prefilter" in such a way that bacteria of the typhoid-intestinal group passed through it while the suspended particles were held back. Water going through the profilter and becoming purified of suspended particles filters much more easily through basic filter No 3 on which the bacteria, planted there as a result of ultrafiltration, are growing and forming pure cultures free of the incrustation of suspended particles.

Figure 1. Double Membrane Ultrafiltration Apparatus

1. Bunker funnel; 2. Cover of funnel; 3. Tapered passage; 4. Irregularly-shaped nut; 5. Funnel-shaped muff; 6. Bottom funnel; 7. Fremework with mesh; 8. Cylinder-ring; 9. Inner tube (for water); 10. Outer tube (vacuum); 11. Vacuum cock; 12. Collector; 13. Observation window; 14. Irregularly-shaped nut; 15. Drain cock.

The factory which makes membrane ultrafilters today began to manufacture coarse-meshed membrane filters but they were for intercepting plankton and the eggs of intestinal worms. We tried to use the so-called plankton filter instead of our prefilters, but unfortunately many of the experiments conducted gave unsatisfactory

results because the plankton filters have extremely uneven pores. Because of this defect, such a filter, when used as a prefilter, holds back not only large portions of suspended particles but also bacteria which should have passed through and settled on the surface of the bottom dense ultrafilter No 3. Therefore, the corresponding portions of the lower ultrafilter appear sterile. This purely technical defect in the production of ultrafilters can not censure the method of double filtration in the bacteriological testing of water. The necessity for and the experience of such a method should be a stimulus to the directors of the factory for membrane ultrafilters to master the production of prefilters which satisfy the corresponding technical requirements.

Figure 2. Stage of the bottom funnel with supporting mesh

Figure 3. Upper flat cylinder-ring with supporting mesh

The method which we proposed, of double membrane ultrafiltration through large-diameter ultrafilters, can be accomplished with the help of an apparatus of new design. (See our dissertation of 1946 "Methods of Bateriological Testing of Drinking and Sewage Waters for the Presence of the Typhoid-Paratyphoid Group." (Central Medical Library of the Ministry of Public Health). From Figure 1 it is evident that our newly designed apparatus consists of the following parts: bunker funnel 1 with cover 2, which is fastened by the tapered passage 3 and the irregularly-shaped nut 4 to the funnel-shaped muff 5 of the filtering part of the apparatus -- the middle unit.

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The middle unit consists of the following principal parts: the bottom funnel into which the supporting frame with the mesh is inserted (Figure 1, 6, and Figure 2). When work is being done, double membrane ultrafilter No 3 is placed on this mesh. A double ring in the shape of a flat cylinder, the upper part of which has the frame with the supporting mesh for the prefilter Figure 1, 8 and Figure 3 inserted in it, is placed on the bottom funnel with the supporting frame and mesh. The entire middle unit is covered with a one-piece funnel-shaped muff (Figure 1, 5) which is connected, as is shown above, by its upper part to the bunker funnel (Figure 1,1) and is bolted hermetically on the bottom by butterfly bolts to the bottom funnel (Figure 1, 6 and Figure 2). The latter ends in a double tube. The inner tube (Figure 1, 9) is the one through which the filtered liquid flows. The outer tube (Figure 1, 10) is a vacuum and is used to create a vacuum through the vacuum cock (Figure 11) in the collector (Figure 1, 12). The vacuum cock makes it possible not to lose energy through continuous pumping out of the air during the entire filtration. When the vacuum has been created, it seals itself and one can stop pumping out the air because the vacuum created in the collector will be preserved.

For technical reasons, at that time we performed only orientation experiments on the ultrafiltration of water contaminated with a mixture of bacteria, consisting of typhoid, paratyphoid, ~~intestinal~~ and coli as well as bacilli, ~~and~~ proteus.

The ultrafiltration was done in an apparatus of our own design through ultrafilter No 3. The ratio between the cultures was as follows: into 250 milliliters of sterile tap water we put approximately 15 microbe cells each of the causal organism of typhoid and

and paratyphoid B and 1000 microbe cells each of ~~intestinal~~ Coli bacilli and Proteus vulgaris (according to the optic standard). After this artificial contamination of the water through membrane ultrafilters No 3, the latter were placed on bismuth-sulphite agar in Minkevich's modification. After 36 hours of incubation at 37 degrees, five colonies of typhoid bacilli were separated. In addition to this, a negligible amount of yellowish-green and brownish-green colonies of saprophytic microflora (~~intestinal~~ Coli bacilli and Proteus vulgaris) grew on the culture. After 48 hours the amount and sizes of all the colonies increased considerably. A secondary inspection of the dishes gave us the chance to separate out three more colonies of the bacteria of typhoid and eight colonies of the bacteria of paratyphoid B. The experiments were made in ultrafilters 72 millimeters in diameter.

Figure 4. Bottom insertion piece with small supporting mesh

Figure 5. Upper supporting ring with small detachable supporting mesh

In the next models the diameter of the ultrafilters was increased to 82 millimeters, while the final variant had a maximum diameter of 87 millimeters with respect to the existing standard Petri dish, which has a diameter of 100 millimeters.

There can be cases when there is no need to use the large filtration areas which we proposed, and then one can confine onself to

small filters with a 35 millimeter diameter.

For such cases special insertion pieces are included in our apparatus. The bottom mesh is taken out and in its place a plate is inserted, the center of which has a mesh piece 30 millimeters in diameter (Figure 4). For double filtration the middle cylinder with the large diameter mesh is replaced with another cylinder with a small, 30 millimeter diameter mesh (Figure 5).

All the parts of our apparatus are made of stainless stell or brass, and in the latter case are chrome-plated both inside and out. Thanks to this, the entire apparatus lends itself to an extremely simple method of sterilization -- passing all the parts through a flame [flambirovaniye].

The given apparatus is portable. It fits into a case which also holds all the apparatus needed for making tests outside the laboratory, at the place from which the specimen is taken. This apparatus consists of the following: a Shints vacuum pump (velocipede type), an apparatus for boiled ultrafilters (a round, metal sterilizer with a cover and an alcohol lamp with a collapsible tripod), forceps, and a box of ultrafilters.

Figure 6. Sterilizer

Just a few words about the sterilizer we suggest.

It is a round aluminum bath with a diameter of 120 millimeters and a height of 90 millimeters, with a cover with a wooden knob (Figure 6). Inside the bath, two-thirds up, its height a

disk, with a large number of round openings (85-90) 5 millimeters in diameter, is placed. The disk has a collapsible handle by which it is placed in and removed from the bath. The bath is intended for simultaneous sterilization of ultrafilters and supporting meshes by boiling, since the supporting mesh cannot be passed through a flame because of the danger of its melting.

The Petri dishes with the corresponding dense cultures are placed in a special box, which is in another case, along with sterile vials for the specimens of the tested liquid and a collapsible bathometer of Dianova and Voroshilova design. (The bathometer of Ye. V. Dianova and A. A. Voroshilova design is used in several institutes. Its description was published in the Informatsionniye materialy (Information) of the Institute imeni Erisman in 1950).

The described apparatus and method also make it possible to conduct the required tests even on expeditions. It makes analyses considerably easier and improves their quality, since time lapses between the moment the specimen is taken and the time it is tested are eliminated.

## CONCLUSIONS

1. The use of the proposed apparatus considerably eases the technique for the bacteriological testing of large quantities of water. It should increase the ~~sifting~~ seeding out of pathogenic bacteria of the ~~typhoid~~ intestinal group in the bacteriological testing of water and make the bacteriological testing of drinks with large quantities of suspended particles easier and more accurate.

2. The proposed method for determining the coli-index is, in a certain sense, a combination of the method of concentration of bacteria on membrane ultrafilters and Marman's method, but it is a far more simple and effective modification.

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