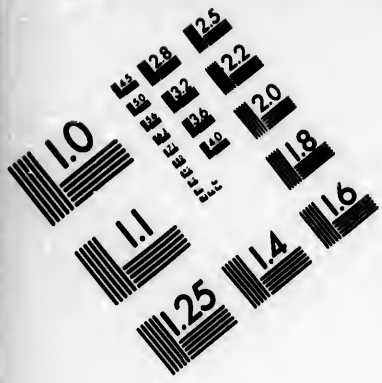


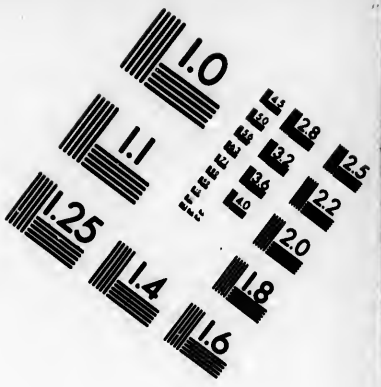
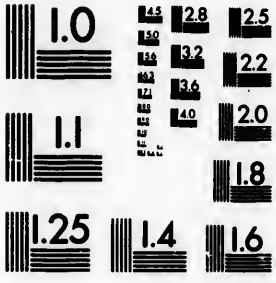
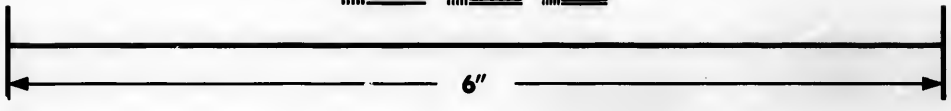


IMAGE EVALUATION TEST TARGET (MT-3)





Photographic Sciences Corporation

23 WEST MAIN STREET
WEBSTER, N.Y. 14580
(716) 872-4503

Technical and Bibliographic Notes/Notes techniques et bibliographiques

The Institute has attempted to obtain the best original copy available for filming. Features of this copy which may be bibliographically unique, which may alter any of the images in the reproduction, or which may significantly change the usual method of filming, are checked below.

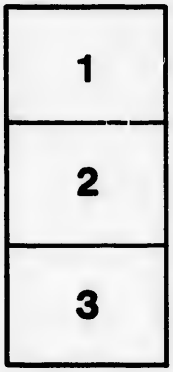
L'Institut a microfilmé le meilleur exemplaire qu'il lui a été possible de se procurer. Les détails de cet exemplaire qui sont peut-être uniques du point de vue bibliographique, qui peuvent modifier une image reproduite, ou qui peuvent exiger une modification dans la méthode normale de filmage sont indiqués ci-dessous.

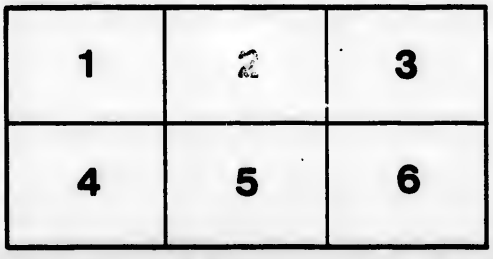
- ☐ Coloured covers/ Couverture de couleur
- ☐ Covers damaged/ Couverture endommagée
- ☐ Covers restored and/or laminated/ Couverture restaurée et/ou pelliculée
- ☐ Cover title missing/ Le titre de couverture manque
- ☐ Coloured maps/ Cartes géographiques en couleur
- ☐ Coloured ink (i.e. other than blue or black)/ Encre de couleur (i.e. autre que bleue ou noire)
- ☐ Coloured plates and/or illustrations/ Planches et/ou illustrations en couleur
- ☑ Bound with other material/ Relié avec d'autres documents
- ☐ Tight binding may cause shadows or distortion along interior margin/ La reliure serrée peut causer de l'ombre ou de la distorsion le long de la marge intérieure
- ☐ Blank leaves added during restoration may appear within the text. Whenever possible, these have been omitted from filming/ Il se peut que certaines pages blanches ajoutées lors d'une restauration apparaissent dans le texte, mais, lorsque cela était possible, ces pages n'ont pas été filmées.
- ☐ Coloured pages/ Pages de couleur
- ☐ Pages damaged/ Pages endommagées
- ☐ Pages restored and/or laminated/ Pages restaurées et/ou pelliculées
- ☑ Pages discoloured, stained or foxed/ Pages décolorées, tachetées ou piquées
- ☐ Pages detached/ Pages détachées
- ☑ Showthrough/ Transparence
- ☐ Quality of print varies/ Qualité inégale de l'impression
- ☐ Includes supplementary material/ Comprend du matériel supplémentaire
- ☐ Only edition available/ Seule édition disponible
- ☐ Pages wholly or partially obscured by errata slips, tissues, etc., have been refilmed to ensure the best possible image/ Les pages totalement ou partiellement obscurcies par un feuillet d'errata, une pelure, etc., ont été filmées à nouveau de façon à obtenir la meilleure image possible.

☑ Additional comments:/ Commentaires supplémentaires: Pagination as follows : [19] - 28 p.

This item is filmed at the reduction ratio checked below/
Ce document est filmé au taux de réduction indiqué ci-dessous.

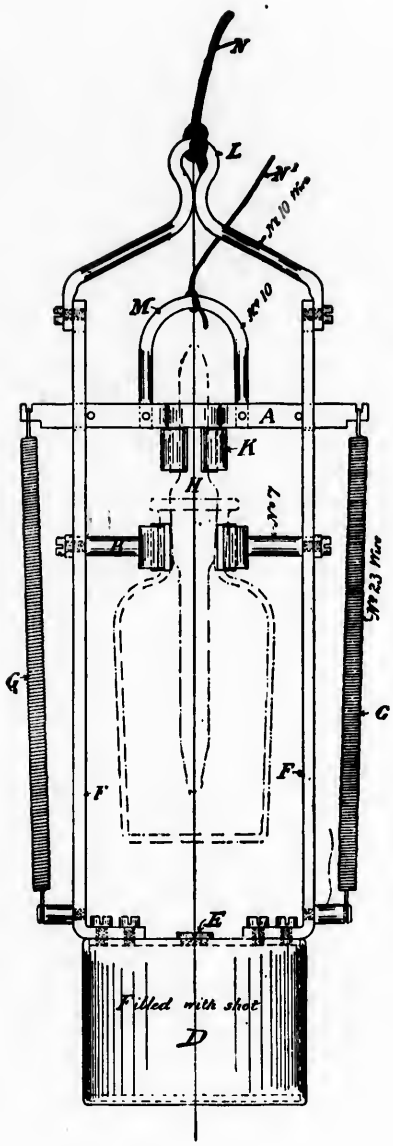
10X	12X	14X	16X	18X	20X	22X	24X	26X	28X	30X	32X
						✓					





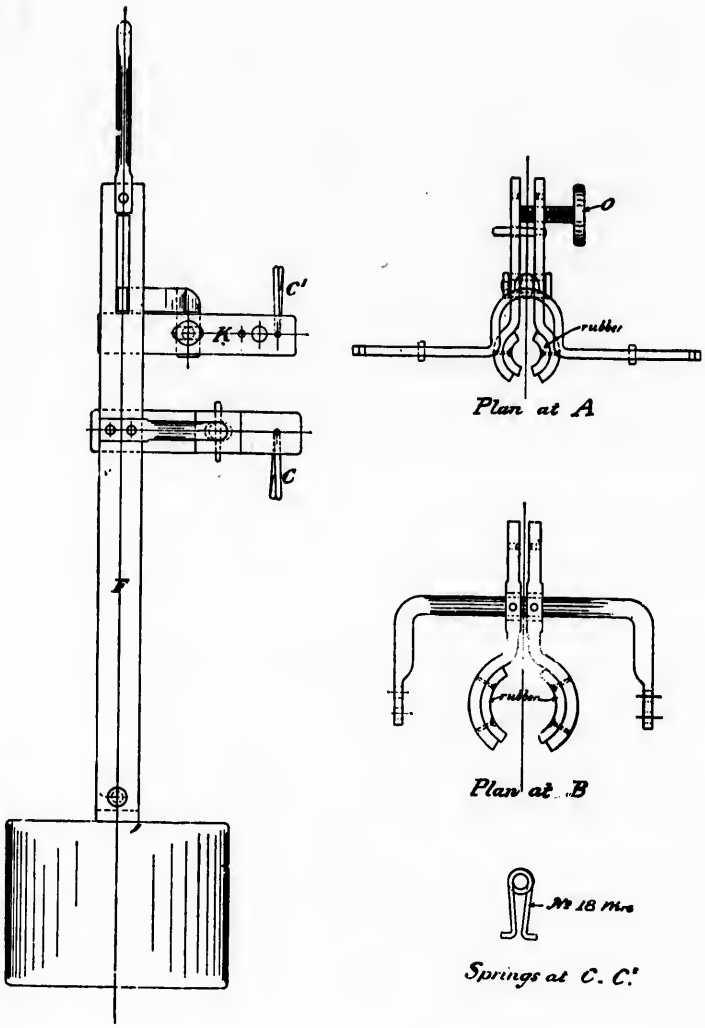
On the Collection of Samples of Water for Bacteriological Analysis.

By WYATT JOHNSTON.



Dr. Wyatt Johnston's apparatus for collecting Sampl

Reduced one-half (li



tus for collecting Samples of Water for Bacteriological Analysis.

Reduced one-half (linear).

"*Reprinted from the Canadian Record of Science, January, 1892.*"

On the Collection of Samples of Water for Bacteriological Analysis.

By Wyatt Johnston, M.D., Montreal.

(*One Plate.*)

I have been prompted to describe my method of collecting samples of water for bacteriological examination, in the hope of its being of service to those who are anxious to do field work in this department of bacteriology.

Certain principles govern this work which cannet be neglected without introducing serious sources of error. First, the bottles in which the samples are to be taken must be sterilized by a dry heat of 150° C. and afterwards kept out of the reach of contamination from outside sources (especially from dust) until the moment when the water is collected. To this end the mouths of the bottles must be kept from contact with the fingers, and the stopper is only to be removed in the water. Second, the manipulations must be rapid enough to permit of a large number of separate samples being collected, and finally, these should be taken from such points as will ensure their affording a fair index of the body of water under examination, as the number of bacteria in samples taken at different places from the same water often varies considerably.

The method usually adopted, that of immersing the bottle at arm's length and removing the stopper under water, though fraught with much personal discomfort in cold weather, is tolerably secure from contamination at the mouth of the bottle, but it has the disadvantage of only giving

the number of bacteria at the surface of the water. In the case of a rapidly flowing stream this is of little moment as the water is sure to be thoroughly mixed and the bacteria pretty evenly distributed. In standing bodies of water, such as lakes, ponds, reservoirs and wells, the bacteria for the most part sink to the bottom, so that the number of bacteria found at the surface affords no indication of their number in the deeper part, from which usually supply pipes are fed in the case of drinking waters.

In the course of a recent biological examination of the waters of the Ottawa and St. Lawrence rivers it was found necessary to take samples at some distance beneath the surface. In winter, when samples were obtained through a hole cut in ice, often from one to two feet in thickness, the water which welled up into the hole was found to be contaminated by the instruments used in cutting it. On one occasion the water in the ice hole yielded 8,000 colonies per c. cm., while a sample obtained from the running stream beneath the ice only gave 30. Lying beneath the solid ice running water there is often found a stratum of "*frazil*" ice. This consists of a dense mass of small, sharp ice fragments which have at one time been in contact with the bed of the stream and have then become contaminated from the soil. That water obtained from the midst of a bed of "*frazil*" ice is unsuitable for bacteriological examination was shown by one examination of St. Lawrence water made in mid-winter, when two samples from a bed of *frazil* yielded respectively 473 and 480 colonies per c. cm., while clear water from an adjacent spot gave only 77 and 39.

In endeavoring to obtain some apparatus suitable for obtaining deeper samples, I was surprised to find no mention of anything of the kind in any dealers' catalogues; their poverty in this particular contrasting strangely with the wealth of appliances available for other purposes.

It thus became necessary to procure some simple form of apparatus, secure from sources of extraneous contamination

and rapid enough in its working to enable me to obtain a large number of individual samples.

My first attempt was made with the assistance of Dr. R. F. Ruttan. We prepared a set of wide-mouthed bottles, fitted with perforated corks in which two open glass tubes were fitted. To one of the tubes a long rubber tube was attached, the end being guarded by a stop-cock. The bottles, with their glass tubes attached, were sterilized by dry heat and the rubber tubing was steamed separately for several hours. After sinking the bottle to the required depth by attaching a weight, upon opening the stopcock the water displaced the air in the bottle. The method seemed to give accurate results, and in each case a bit of the tubing reserved for the control test of washing it out with sterilized water yielded no bacteria colonies. The sterilization seemed to be perfect, but the method was abandoned as it was found too troublesome to sterilize a separate length of tubing for each sample that was to be taken.

I had obtained by this time some collecting bottles from Messrs. Eimer & Amend of New York. These were made of very heavy glass and held about a pint, the stoppers consisting of a rubber ring fitted round to a glass rod which lay within the bottle, and was so arranged that the stopper could be pulled up against the lower part of the neck from within by means of a wire attached to the glass rod.

In using this bottle one line was attached to the neck of the bottle and one to the wire fastened to the rod shaped stopper. The bottles were lowered by this second line, thus holding the stopper tightly against the neck of the bottles and so preventing the water from entering, until, at the desired depth the strain was taken off the stopper by pulling in the line attached to the neck of the bottle allowing it to fill, the stopper being heavy enough to fall from its own weight. This was open to the obvious objection that the neck of the bottle above the rod shaped stopper filled with water from the surface, most of which was afterwards naturally washed into the bottle. Besides

this the precautions necessary to guard against contamination of the wire while attaching the string, and the necessity of having a separate bottle for each sample collected, rendered them inconvenient for field use.

The method of using sealed tubes or flasks with a tapering end bent at right angles to be broken off under the water, has been recommended by Escherisch of Munich. This is much more free from technical sources of error than the apparatus last mentioned, but the trouble of preparing such flasks is considerable, as one has to be manufactured for every sample to be taken.

In the last edition of Rohrbeck's catalogue I find an apparatus figured for collecting bacteriological samples at different depths. From the impression conveyed by the illustration it seems too complicated to be easily handled, and the entire apparatus evidently requires to be re-sterilized before a second sample can be taken.

At this stage my attention was directed to a most ingenious apparatus invented by Prof. Ellis of Toronto University, which differed from all the others in principle. This was a device by which sterile glass stoppered bottles could be placed in a weighted frame and lowered to the required depth. By pulling a string the stopper could then be raised sufficiently to allow the water to enter. By releasing this end the stopper was instantly replaced by means of a spring. Any number of samples could be taken, as the bottles could be placed in the frame one after another with very little loss of time. The advantages of this as compared with the plans described above are very great. There is absolute certainty that no water is obtained from any except the required depth. There is no limit to the number of samples which can be taken, and all the preparation necessary is limited to storilizing the bottles. It is also far more economical, as a single sinking frame contains in itself the attachments for opening and closing the bottles.

The instrument I am about to describe is a modification of that devised by Prof. Ellis, and I can claim no originality whatever with regard to the principle of opening

and closing glass stoppered bottles under water. My apparatus, though a modification of Prof. Ellis', contains improvements of my own which render it specially adapted to taking large numbers of samples by making it simpler in construction and more rapid and accurate in action. All who have worked at water analysis know the great importance of making a very large number of separate observations before drawing conclusions.

My outfit consists of one collecting frame, shown (reduced to one-half its linear dimensions) in Plate III., into which the bottles can be successively fitted. It was made under my direction by Mr. O. Wendell, of 170 Coursol Street, Montreal, and cost about eight dollars. It may be briefly described as a sinking frame, to which the bottle is attached by a fixed clamp, while a movable clamp is used to raise and lower the stopper.

The frame is made of brass and has for its base a hollow cylindrical box D, 2½ inches deep and 2 inches in diameter. The box contains two pounds of shot and can be filled at a small hole E, which is closed by a screw. Attached to the top of this box are two flat brass bars F F, in the upper part of which a slot is cut allowing the movable cross bar A sufficient vertical play (1 inch) to admit of the bottle being opened beneath the water.

The neck of the bottle is grasped at B by a brass clamp, the jaws of which are lined with soft rubber, fastened on by rivets. These jaws work on pivots and are attached to the upright bars F. F. by means of a brass rod bent outward so as to bring the neck of the bottle into the line of traction. The pivots allow some lateral play. The clamp is kept closed upon the neck of the bottle by a brass spring C made of No. 18 wire.

The stopper H of the bottle is in the form of a tapering glass rod which is grasped by another clamp K and kept closed by the brass spring C. This clamp is secured to the sliding cross bar A by a horizontal pin working in slots which allow of sufficient backward and forward play to permit the stopper to adjust itself to the bottle. At the point

where the pin is fastened the cross bar is bent outwards to bring the jaws of the clamps into the line of traction. The shoulder thus formed bears the entire strain in opening and closing the bottle as both ends of the clamp are balanced beneath it. It will be seen that these attachments are not rigid, thus preventing any straining or jamming of the stopper.

A loop of heavy brass wire L connects the two side bars F. F. above and another loop M is attached to the cross bar A. To these loops strings are attached enabling apparatus to be worked under water.

A pair of spiral springs G. G. made of No. 23 wire are hooked over the ends of the cross bar A above and fastened to the foot of the upright bar below. They close the bottle when it has been opened and keep it closed at other times. To place a bottle in the frame the ends of the clamps C and C are compressed between the thumb and forefinger sufficiently to open the jaws. The frame, with bottle in position, is then lowered by means of a heavy string N attached to the loop L, when the desired depth is reached the stopper is raised by pulling a lighter string N, attached to the cross bar loop O. On releasing this again the springs close the bottle. The movement of raising the stopper can easily be felt at a depth of 15 or 20 feet.

The bottle fills in about 20 to 30 seconds and the bubbles of displaced air can usually be seen. It is better not to fill the bottles quite full, but to leave some space for subsequent shaking.

In very swift currents or when the sample is to be taken at a greater depth than 30 feet an additional weight in the form of a small bag of shot may be tied to the lower part of the frame.

To prevent any tendency of this frame to rotate while being lowered in a current, and thereby tangle the strings, I allow one string to glide in each side of my forefinger or else hold one in each hand.

Before placing a bottle in the frame it is well to ascertain

that the stopper is not jammed in the neck of the bottle from unequal expansion in the hot air sterilizer.

In working at considerable depths I have found it convenient to use a screw at O. This increased pressure upon the wings of the clamps holds the stopper more firmly. At other times the screw is not needed. By substituting a wire for the string attached to the cross bar, the opening and shutting can be readily controlled at very great depth.

A bottle is removed by simply compressing the wings of the clamps and lifting it out from the jaws. The ease and rapidity with which the apparatus works will be understood from the fact that I am able to collect 10 separate samples of water at a depth of 20 feet in from 10 to 15 minutes.

The bottles made use of are those dropping bottles fitted with ground glass pipettes now in common use for holding histological reagents. Both ends of the pipettes are sealed up in a gas flame, thus converting them practically into glass rods. As these bottles are kept in stock in the laboratory, one can always be replaced if it happens to be broken. The ones I employ hold 50 c. cm., but I would have preferred 100 c. cm. bottles had they been obtainable. The method of clasping the bottle by the neck admits of various sizes being employed in the same frame as there is space to spare between the cross bars.

The differences between the model here described and the original form introduced by Dr. Ellis are that the bottle is grasped by the neck instead of being forced into a socket from above. The use of spring clamps to hold the bottle, enables bottle and stopper to be brought into position by a single act instead of taking them apart and putting them in separately. The chief advantage of using the dropping bottles described lies in its giving a long tapering stopper, the lower end of which remains in the neck of the bottle when open, and guides it back into position, and it seemed preferable to use a bottle readily obtainable rather than to order a special form, which could not be replaced if broken.

The little sinking frame I have just described was ori-

ginally designed to enable a sample to be collected at any required depth with the same safety and precision as at the surface, but as it also fulfils all the precautions for collecting samples in general and saves one the necessity of repeatedly plunging one's arm into the water, I employ it whenever a sample is to be collected from an open body of water. In securing samples by hand from a stream I was previously under the necessity of either securing the services of a boat or else taking the sample from off the bank, with the great chance that in the latter case the shallow water near the shore might not be typical of the general body of the stream. But from this apparatus, which can be lowered into the water from a bridge, or by a rod, much more uniform results are obtained.

As the apparatus left little to be desired, as far as regards the rapidity and safety with which the act of collecting is performed, it only remained necessary to ensure the necks of the bottles against contamination previous to using them. Instead of using sterilized rubber caps for each bottle, a constant source of trouble and annoyance, I had a tin box made which holds forty bottles at once, each kept in position by cross partitions of tin. The bottles are numbered serially, before sterilizing, by writing in pencil upon the ground glass of the stopper, and by noting where each bottle is used the use of labels is unnecessary. Instead of a simple lid, the cover of the box is a tray four inches deep, in which a lump of ice is placed in warm weather. A small tube at one of the corners of this tray conducts the water away as fast as the ice melts. I find this keeps the temperature within the box below 8° C., even in the hottest weather. A handle across the tray serves to carry the box, and a small padlock in front guards it against an ever too inquisitive public.

Though I have not yet had cause to use it for this purpose, I think that my box, with its lump of ice on top, would form a better means of sending samples of water by express than any I have seen recommended. The temperature is kept down to a point where no increase of the

bacteria can go on, and the ice could be replenished by the officials from time to time, while the padlock or a seal would prevent its being tampered with. The space occupied by this box (18″×11″×8″) admits of its being placed in a large hot-air sterilizer and heated together with its contents to 150° C. A small piece of fine string placed in the neck of each bottle permitted the escape of any moisture, so that it is unnecessary to dry the bottles thoroughly before heating them. As the box is quite dust-tight the necks of the bottles remain sterile until the time comes to use them, doing away entirely with the employment of rubber caps.

In some cases when it seemed of interest to examine great stretches of water I took my samples from off the bow of a passenger steamboat in a very simple manner. By using a stout fishing rod and about twelve feet of line a sample can be secured well outside the "wash" of the boat, even at a speed of 12 to 15 miles per hour. To ensure the bottle sinking I wrapped a piece of sheet lead round it. By making the cast well ahead the bottle usually sank 6 to 8 feet.

As these examinations were always made in duplicate the accidental encounter of any extraneous source of pollution would infallibly have been shown by an abnomal excess of growth in one of the two samples. A striking proof of the delicacy of the method is that the duplicate samples always gave practically the same number of colonies. This "fishing" was often found a convenient method of obtaining a sample from the banks of a stream.

To ensure the accuracy of the result in estimating the number of bacteria in a water it is of great importance to curtail to a minimum the time which elapses between the collection of the sample and the plating of the cultures, to guard against a possible increase of bacteria in the interval. It is also advisable to make the cultures in some flat vessel which permits of their being counted from time to time without exposing the gelatine to the danger of receiving

additional bacteria from the air. Both of these objects are met by the flattened glass flasks designed by Petruschky.

These flasks contain the nutrient gelatine ready for use, so that it is only necessary to warm them gently and so melt the gelatine, drop in the proper amount of water and after shaking them gently to lay them on their side till the gelatine stiffens.

As these flasks are expensive and not always easy to obtain, it may be of interest to those who work under conditions which make it difficult to obtain apparatus to know that I have found ordinary flat sided, common, white glass vials, obtainable anywhere, answered the purpose admirably. Owing to the small size of the bottle necks I find it best to plug them by wrapping the cotton wool about the end of a wooden toothpick, which is then broken off short. By doing this the plug can be readily inserted and removed. The colonies are readily counted with a lens, and to facilitate this I rule with a writing diamond a couple of parallel scratches on the flat side of the bottle in the axis. Cross lines are not usually necessary. Any of the colonies can be fished out with as much ease as from a Petruschky flask. The only respect in which these bottles are not satisfactory is that, being made of rather thick glass, when using a low power microscope, the object appears somewhat blurred. This also could probably be obviated by using a correcting lens. They possess, however, a distinct advantage over the Petruschky flasks in being much stronger. They also pack closer, owing to their flat sides, and having flattened bottoms they can be stood up.

For summer field work I was able to pack 160 of the bottles in a small double walled tin chest or portable refrigerator, measuring 20″ × 16″ × 18″, and this included a space of 8″ × 8″ × 18″ for the ice chamber.