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INOCULATING LOOP FOR ANAEROBIC ROLL TUBES,
PRE-REDUCED ANAEROBICALLY STERILIZED (PRAS)
LIQUID MEDIA AND ROUTINE MICROBIOLOGICAL
PROCEDURES¹

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ABSTRACT. An inoculating loop has been designed for anaerobic techniques, rotating plate streaking and routine procedures. It consists of a terminal portion of platinum wire and a basal portion of stainless steel wire. A 60° angle bend 4 mm from the loop tip of the wire with mounting on a 14 cm handle gives a balanced and responsive instrument. A small section of one side of the loop is flattened to a sharp projecting edge which can cut out agar blocks and incise roll-tube agar surfaces enabling underlying colony material to be removed with ease. This inoculator has the advantages of a non-oxidizing platinum tip with the rigidity of the total wire being sufficient for microbiological procedures.

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Mudge (1930) noted that the bacteriological inoculating needle was one laboratory tool that had not been improved in the course of time, and then suggested that platinum and copper wire be used together to form a transfer needle. He showed an instrument the terminal two-fifths of which was platinum wire and the basal three-fifths copper wire. He fused the copper to the platinum with a bunsen flame.

Hastings (1930) reported using platinum-iridium wire for his inoculating needles, but recommended 24 gauge nichrome wire for student work.

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Pure platinum is frequently replaced today, because of its inherent softness with modern alloys such as platinum-15% iridium, platinum-3.5% rhodium, and platinum-5% ruthenium. These alloys increase the rigidity of the wire while retaining the beneficial characteristic of being non-oxidizable.

Tungsten alloys and various stainless steel wires have also been used as transfer wires. Fused loops were an important advancement and are now used as the standard loop in milk testing procedures of the American Public Health Association (1967).

Holders are of two types, the fixed wire of Mudge (1930), Hastings (1930) and also the commercial Ravenel type. The other and most popular type is the removable wire holder such as the chuck types of Kolle, Rosenberger and Greenman and the Jorgensen collet-type with a push-pull collar.

The development of roll-tube anaerobic culture techniques with pre-reduced anaerobically sterilized (PRAS) media continuously maintained in the anaerobic state through inoculation, cultivation, examination and subculture was developed by Hungate (1950). Techniques were further advanced by Bryant and Burkey (1953), Hungate (1966) and Moore (1966).

As these techniques became established, Hungate (1966) and Moore (1966) reported the need for special precautions in transferring fastidious anaerobes. They both found, for example, nichrome wire to be unsuitable and Hungate remarked that nichrome needles oxidized the medium when inserted immediately after flame sterilization. He used a micro spatula made by flattening a platinum-iridium needle for some of his transfer work.

Moore (1966) recommended a loop and needle of stiff 20 gauge stainless steel wire placed in a short handle for picking colonies from roll streaks and roll tubes. He, however, stressed caution in the use of these long needles of stainless steel when working with pathogens because of the spring action of the wire.

Holdeman and Moore (1967) made inoculating loops from Herter's Belgian stainless steel wire and reported that it was satisfactory for transferring cultures of Bacteroides and further suggested that pure platinum or stainless steel wire is preferable for use with all anaerobes since nichrome wire oxidizes the medium.

Since our laboratory uses roll-tube and PRAS liquid media procedures as well as routine microbiological techniques and plate-rotator inoculating, the need to develop an all purpose

inoculating loop was evident. The inoculator had to meet the following specifications: 1) be able to transfer liquid or solid inocula from conventional media, roll-type and PRAS systems; 2) that portion touching the inocula and subsequently inoculated media must not be oxidized in flame sterilization procedures; 3) should be of adequate length to reach the bottom of the longest roll tube (160 mm) and be able to enter the narrowest culture tube (10 mm diameter), the hub not entering the culture system; 4) no overt spring action so as to be safe in handling pathogens as a conventional loop is; 5) should do roll-tube, conventional petri dish and rotator streaking; and 6) should be convenient to use in preparing smears and wet mounts of culture material.

To meet the above criteria an instrument was made from wires of two different materials (Figure 1). The base wire consists of 21 gauge (.034 in.) stainless steel wire. The end wire can be platinum or a platinum alloy, depending upon the rigidity requirement. We used a 26 gauge (.019 in.) pure platinum wire. The two are fastened together by first making a shallow U loop on one end of an 8 cm length of stainless steel wire and then winding the softer 9 cm length of platinum wire 3 times around that portion of the U continuous with the wire. The stainless steel wire U is then pinched together so that the two wires are held by friction. A loop of 2.0 mm inside diameter is then made at the end of the platinum wire.

A small section of the terminal side of the loop is flattened to a sharp projecting edge by tapping gently with a small blunt instrument. Only the outside edge should be flattened, the inner edge should remain continuous with the contour of the loop. As the platinum is soft the outside edge can be drawn out to a protruding knife edge (Figure 2, D).

The platinum wire is then bent at an angle of 60°, 4 mm from the loop tip.

We used a Kolle handle modified by slipping off the outer insulation, cutting off 13 cm of the handle and then replacing the insulation which was trimmed to provide a 1 mm overhang. This resulted in a handle 14 cm long which gives good balance and a more delicate and responsive instrument.

This type of inoculator has been used for 2 years and performs well, fulfilling the requirements as set forth above. By using wires of two different metals, desired total stiffness can be obtained yet marked spring action is not present. The bend at the tip is necessary for roll-tube streaking and facilitates plate rotator streaking. The position of the loop

for plate streaking and roll-tube streaking is shown in Figure 2, B and C. For roll-tube streaking the tube spinner is turned off while the inoculator is placed to the bottom of the tube; it is then turned on and the loop edge gently placed against the agar and slowly drawn straight up the side of the revolving tube.

For hand streaking of a petri-dish the edge of the loop contacts the agar and then is moved back and forth with light pressure on the plate surface while the loop is slightly rotated.

For rotator streaking, the edge of the loop tip is placed at the periphery of the plate as the plate is rotated. With light pressure the loop is slowly pulled across the agar surface in a straight line to the center of the revolving plate and then lifted from the surface.

For smears and wet mounts, the loop is more convenient than the conventional loop. The hand is not close to the laboratory bench but above as the handle is held in a pen grasp with good control. The procedure allows good visualization. The loop contacts the slide as shown in Figure 2, A.

The knife edge made at one side of the loop (Figure 2, D) has been of great value in removing minute or adherent colonies on agar plates by going under the agar and shaving off some of the agar with the colony. Also, this edge can be used to cut out a block of agar containing the colony by making four line slices around the colony to the dish bottom and then using the loop tip to spear the block so that it can be removed for subculture or a contact impression. The knife edge has been very useful when subculturing roll-tube colonies growing under the agar surface. An incision can be made in the agar over the colony and the loop inserted with a minimum of pressure so the platinum wire will not bend. As the knife edge is on one side of the loop and not near the tip it does not cut into the agar during streaking.

The instrument works well with isolated and average size or larger colonies in roll tubes. However, it is not useful for transferring minute colonies or in removing one colony closely adjacent to another. For this use, a manipulator developed for use under the microscope has been developed (to be published).

This inoculator represents a departure from the long established standard instrument. It has functioned well as a replacement for the standard loop and, in addition, can perform procedures in which the regular loop cannot be used.

Figure 1. Inoculating loop with terminal segment of platinum wire and stainless steel basal portion.

Figure 2. Use of inoculating loop.

- A. Smear preparation
- B. Plate streaking
- C. Roll-tube streaking
- D. Loop showing knife edge

REFERENCES

- American Public Health Association. 1967. Standard methods for the examination of dairy products. 12th ed. American Public Health Association, New York, N. Y.
- Bryant, M. P. and L. A. Burkey. 1953. Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. *J. Dairy Sci.* 36:205-217.
- Hastings, E. G. 1930. Inoculating needles. *Am. J. Pub. Health* 20:538.
- Holdeman, L. V. and W. E. C. Moore. 1967. In: Report of technical views and reviews. The loop. 1(2):2.
- Hungate, R. E. 1950. The anaerobic mesophilic cellulolytic bacteria. *Bact. Rev.* 14:1-49.
- _____. 1966. The rumen and its microbes. Academic Press, New York, N. Y.
- Moore, W. E. C. 1966. Techniques for routine cultures of fastidious anaerobes. *Internatl. J. Syst. Bact.* 16(2): 173-190.
- Mudge, C. S. 1930. Convenient platinum needle. *Am. J. Pub. Health* 20:195.