

## New Instruments and Suggestions

### A MICROSCOPIC TEST FOR PASTEURIZED MILK (PRELIMINARY PAPER)

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#### METHODS FOR DETECTING HEATED MILK

Numerous methods have been proposed to test quickly and simply whether or not a given sample of milk has been heated. The methods suggested fall into three main groups:

1. Those based on the changes which the protein undergoes when milk is heated. These have not proved applicable in practice.

2. Those which depend on the presence of oxidizing enzymes in milk. Of these, Storch's test is generally regarded as most satisfactory. This, however, as is well known, can be used only on milk heated from 78 to 80 C. (172.4 to 176 F.). It cannot, therefore, be applied to milk heated to the temperatures employed for pasteurization in this country.

3. A microscopic test devised by myself and described in a paper by Frost and Ravenel in 1911 (*A Microscopic Test for Heated Milk, Proc. Am. Assn. Med. Milk Commissions*, May, 1911, p. 127).

Two features of this method interfered with its usefulness. One was the difficult technic and the other was the fact that safranin clotted the milk unless added with the greatest care. So far it has been possible to avoid this danger only by diluting the stain, and in this case the action of the dye is largely neutralized.

#### PRINCIPLES INVOLVED

The original idea that I had in mind was that by means of an *inter vitam* stain it would be possible to color the living leukocytes, as I assumed them to be in raw milk, in one way, and the dead cells, if such they are, in the heated milk, in another way. The technic was suggested by that employed in making opsonic index determinations. That it is possible, with proper experience, to differentiate pasteurized from raw milk by this means, is still my firm conviction.

Further study, however, has shown that it is possible greatly to simplify the technic, and at the same time emphasize the differential characters. It has also appeared that the principle of *inter vitam* staining need not be considered. The attempt can rather be made to stain the dead cells deeply and characteristically in the heated, and contrast these with the unstained or at most slightly tinged cells in the raw milk. It thus appears that the method here described may as well be considered a new method as a modification of the former or previously described method.

#### METHOD OF PERFORMING THE TEST

One part of a saturated aqueous solution of methylene blue is added to 5 parts of the milk to be tested. The dye should be added to the milk slowly to prevent the possibility of the stain coagulating the milk, for when this coagulation occurs the test is rendered unreliable. The method of adding the stain, which I have usually followed, is to put the milk in a small flask or beaker and add the dye slowly from a small pointed pipet while the milk is kept in motion by a rotary movement of the vessel.

The stain should be left in contact with the milk for from fifteen to thirty minutes, and then the sediment of the stained milk is precipitated in a centrifuge and spread on a glass slide. When the smear is dry it is ready for examination.

Probably the most convenient way of collecting the sediment is by means of the Stewart-Slack tubes, although it can be done quite readily in the tubes of a centrifuge such as are ordinarily used for collecting urinary sediments. In this case from 5 to 15 c.c. of the milk can be used, and this might be desirable in separated milk and cream. The 2 c.c. which the Stewart-Slack tubes hold, however, will ordinarily be found quite sufficient with whole milk.

The sediment is, perhaps, best spread on the glass slide by means of a glass spreader such as is frequently used in making blood smears. It is also quite possible to make the smear in the same way that is suggested by Slack for the direct enumeration of bacteria, that is, by means of rubbing the stopper over a definite area, such as 4 or 6 square centimeters.

#### THE STAIN

The methylene blue stain referred to above is made by adding about 7 gm. of Gruebler's dry dye to 100 c.c. of distilled water, allowing it to stand several hours with frequent shaking, and then filtering. Similar results can be obtained with saturated, aqueous solutions of safranin O soluble in water and Coget's Thionine and other stains, but so far the methylene blue has proved most satisfactory.

#### INTERPRETATION OF RESULTS

When the stains prepared by the method described above are examined under the microscope, the following pictures are seen:

1. *Raw Milk*.—The entire microscopic field is stained a light blue, the depth of the stain depending on the thickness of the film. In this blue background appear numerous clear areas, when viewed either with a low power (100 $\times$ ) or under an oil immersion objective. The smaller ones of these are fat globules, and the larger ones may be large fat globules, or clusters of the same, or more likely they are the leukocytes of the milk. These smears of the raw milk are quite uniformly stained, and the background is quite constantly more highly colored than any of the smaller objects in the fields. Under the high powers, oil immersion objective, the leukocytes are practically all colorless; but with some experience it is not difficult to learn to recognize them, and then it will be noted that the cells of the polymorphonuclear variety are irregular in outline and about 12 microns in diameter. The nuclear material, if differentiated at all, is not well defined. The mononuclear cells are always well stained, and are not considered in this test, and all descriptions of leukocytes apply only to the polymorphonuclear ones.

2. *Heated Milk*.—Smears made from heated milk do not have the background as deeply stained as the smears from raw milk, but in thick portions may be quite blue. The leukocytes are always more deeply stained than the background, and under the low powers of the microscope may be readily seen as dark blue areas in a lighter blue field. The background immediately surrounding these leukocytes frequently takes the stain deeply and shades off into the color of the background, thus forming a halo about the leukocytes. Under the oil immersion lens the leukocytes are readily visible; they are less irregular in outline than are those in the raw milk, that is, they are rounded up, or shrunken, and are only about 7 microns in diameter. The nuclei are distinctly stained. The amount of shrinking and the depth of stain vary somewhat with the degree of heat applied.

Even a cursory examination of the two smears shows a distinct and readily recognized difference between the smear from the heated milk and that from the raw milk.

The effect of heat on the leukocytes, so far as this test is concerned, is twofold. It alters the shape and size of the cells, and changes their staining reactions. The shape of the cell is probably gradually changed as the degree of heat increases, and the shrinking begins to appear at a lower temperature than that used for pasteurization; but the fixing of the nuclear material, which makes possible the absorption of the stain, seems to take place definitely at practically the same temperature as that fixed on as suitable for the pasteurization of milk, namely, at from 60 to 63 C. (140 to 154 F.).

#### REQUIREMENTS OF PASTEURIZED MILK

Properly pasteurized milks then, when examined by this method, should show the polymorphonuclear leukocytes more deeply stained than the background, rounded up and less than half the usual size of such cells, and should especially show the nuclei definitely stained. Controls of raw milk should probably always be run.

## RESULTS OF USE

This method has been tested on many samples experimentally pasteurized in the laboratory and on several samples of commercially pasteurized milks. In a considerable number of cases, myself and two others, one with considerable practice and the other with practically none, have found it readily possible to differentiate the properly pasteurized from the raw samples. I feel sure that any one who will take the necessary time to familiarize himself with the test will be able to separate milk heated to 60 C. and above from raw milk, with the same ease and certainty that the bacteriologist now makes a laboratory diagnosis of diphtheria or rabies, and in little more time than is required to stain and examine for tubercle bacilli in sputum.

Part of the work done on this test was performed while I was working in the Laboratories of Preventive Medicine and Hygiene at the Harvard Medical School, and I desire to thank Dr. M. J. Rosenau for many courtesies extended. I also wish to thank my assistant, Miss V. A. Armstrong, for constant help.

## THE VACUUM TUBE OF KEIDEL, AS APPLIED TO BLOOD-CULTURE WORK\*

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While blood-culture has long occupied an important position as a diagnostic method in all modern hospital laboratories, it has almost completely failed to popularize itself among general practitioners. The reasons for this are self evident. Very few general practitioners probably have private laboratory facilities at their disposal which would enable them to carry to the sick-bed all the paraphernalia which blood-culture, as hitherto practiced, demands. Moreover, a single negative result is rarely of value, while repeated examinations would frequently not be tolerated by the patient, to whom a vein puncture with a large needle is by no means an indifferent procedure. To meet these objections to an otherwise most valuable method of investigation, it occurred to us that the entire procedure might be very much simplified by the use of large vacuum tubes analogous to those which were introduced by Keidel for the collection of blood for Wassermann work, *the culture medium being placed in the tubes*. Preliminary experiments which Dr. G. H. White carried out during the fall of 1912 and the following winter showed conclusively that the method is thoroughly practical and trustworthy, and since then all blood-culture work both at the laboratory of the College of Physicians and Surgeons and in Dr. Simon's private laboratory have been conducted on this basis.

The tubes which we are now using are manufactured by the Steele Glass Company of Philadelphia. They are illustrated in the accompanying diagram. Their capacity is approximately 50 c.c. From 15 to 25 c.c. of glucose bouillon containing 1.5 per cent. of sodium citrate (to prevent coagulation) are placed in each tube. This is most conveniently accomplished by drawing out the neck at the mark *a* to a comparatively fine caliber, without seriously weakening the glass, when each tube is connected with a suction pump and a vacuum established. The connecting rubber tube which leads to the pump is clamped, slipped off and plunged into the culture medium, when this is again connected with the suction pump, the desired degree of vacuum established and the neck sealed at *a* where it was previously drawn out. The tubes are then provided with a stout rubber cuff, armed with a No. 24 needle which, in turn, is supplied with a stylet. Thin tubing is unsuitable, as it will collapse when suction is established. The needle which we use is a

trifle larger than that going with the Keidel tube, but still sufficiently small as not to cause any inconvenience to the patient. Needle and cuff are guarded with an outer glass tube, as usual, and the entire apparatus is sterilized—best by heating in the autoclave for five minutes after the pressure has reached 20 pounds. Thus prepared, the tubes may be stored, and are then ready for use at any time.

When a blood-culture is to be made, the patient's arm at the bend of the elbow is cleansed with soap and water, and alcohol and ether in the usual manner; a constricting band is placed around the upper arm, and any prominent vein punctured that may present itself. After this has been done, the sealed end within the rubber cuff is broken off, when approximately 5 c.c. of blood are allowed to enter. The constricting band around the arm is removed as soon as the blood begins to flow. The needle is then quickly withdrawn, the guard tube replaced, and the reassembled outfit carried to the laboratory. If this is at a distance, necessitating the mailing of the tube, the rubber cuff is removed under aseptic precautions, and the broken end of the collecting tube sealed in a Bunsen or alcohol flame. Otherwise this is not necessary and the entire apparatus may be placed in the incubator, as it is.

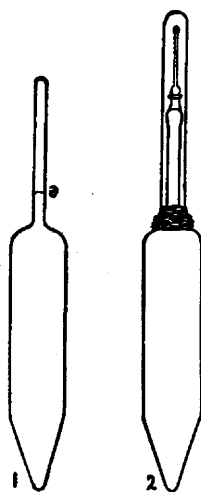
After twenty-four hours the corpuscles will have settled to the bottom, while the supernatant fluid becomes perfectly clear and remains so, if no growth has occurred. If positive, a turbidity results which develops either within twenty-four hours, or later, and varies in appearance with the character of the organism. Then, and not before, are the contents of the tube examined in the smear, and, if desired, by subcultures, though the latter are not always necessary.

The results which we have obtained with this procedure have been most gratifying. The isolation of all the common organisms which may be found in the blood (including the various types of streptococci and pneumococci) is effected without difficulty, and we feel that blood-culture with this method is now a sufficiently simple matter to be within the reach of any physician who is provided with even very modest laboratory facilities. The inconvenience caused the patient is, moreover, so slight that no objection is likely to be raised to even frequent repetitions of the puncture.

While the procedure, as above described, is one that will usually be adopted for the isolation of aerobic organisms, the same method may be employed if anaerobes are to be cultured. In that event, a vacuum is again established after inoculation of the culture medium, and the tube sealed off as in the beginning. Or, as an extra precaution, the medium may be layered with liquid paraffin before sterilization.

We may mention that we are using corresponding tubes for the collection of blood for various other purposes. For coagulation-time determinations, according to Howell's method, a smaller model is employed, charged with oxalate solution and graduated accordingly. Plain collecting tubes of large caliber are used for the purpose of obtaining blood for the Abderhalden and Fauser reactions, for the preparation of salvarsanized serum, for the collection of antisera in animals, etc.

The underlying principle in all, of course, is the vacuum method of Keidel, to whom we cheerfully give all credit for its extension to other purposes also than the collection of blood for the Wassermann reaction.



Tube for blood-culture work; one quarter natural size: 1, open tube; 2, parts assembled; *a*, point at which the tube is sealed after exhaustion.

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**Observation and Theory.**—It is not true that observations compel any one theory. In fact, as Poincaré has shown, there is an infinite number of explanations of any finite set of facts. From among this enormous totality we must select the explanation which is most satisfying for us from considerations of convenience or from the demands of the esthetic sense. This is actually what we always do. It should be done consciously.—R. D. Carmichael, *Science*.