A NOTE ON UHLENHUTHS METHOD FOR SPUTUM EXAMINATION, FOR TUBERCLE BACILLI.

J. J. KINYOUN, M. D., PH. D.,
Bacteriologist, Health Department, D. C.

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THE writer, in 1909, made a preliminary verbal report to the Laboratory Section of the Public Health Association, on Uhlenhuths method for detecting tubercle bacilli in sputum. Since that time until now this method with some important modifications has been employed in the daily routine examination of sputum sent to the laboratory. Now since it has been so satisfactory for rapid and accurate results, and in view of the fact that this method has not been adopted, to the extent I believe it should, it is deemed now of sufficient importance to make a further report somewhat in detail, how it is best employed. Before doing this I wish to disavow any claim for originality of this method, for all credit belongs to Uhlenhuth and his co-workers, and the so-called "Kinyoun method," as it has been referred to by some of my friends, is a misnomer. The only thing which I can lay claim to is to make the method practical for the routine examinations.

The original method consisted of dissolving the sputum in an alkaline solution of hypochlorites of soda (this requiring several hours) and the centrifugalizing the specimen to throw down the bacilli, after which the fluid was poured off, sediment examined. Later, ligroin was added to the digested sputum, which was shaken to mix the ligroin, then the sputum was placed in a water bath and raised to 60°C. By this means the bacilli will rise to the surface together with a layer of detritus, in which the bacilli will be found.

These procedures required several hours, and therefore would handicap a laboratory in getting a prompt report to the physician, which is important. Notwithstanding these drawbacks, the method had such good points, that it was too good to abandon, so I began to experiment with it with a view of overcoming the objections, and soon found how this could be done. In the first place, the digestion of the sputum by the alkaline hypochlorites can be done rapidly, provided the sputum be shaken vigorously for a few minutes. This is best accomplished in a Ricard's Sputum Shaker. The other point is that ligroin can be added to the sputum together with the alkaline hypochlorites without interfering in anyway with the digestion.

The sputum is sent to the laboratory of the Health Department in a wide mouth, square shouldered, bottle of 5 cc. capacity, which takes a No. 10 cork. Each bottle, when sent out, contains 2 cc. of a 1 per cent. solution of compound Cresol (or Lysol) which is placed therein to prevent decomposi-

tion of the sputum before it reaches the laboratory.

The routine which is followed is: On the receipt of the sputum, one cc. of ligroin of a specific gravity of 0.715 is added and sufficient amount of the alkaline solution of the hypochlorites to fill the bottle to the shoulder; this may vary according to the content of the bottle. In case the bottle is full, the same must be poured out, usually about one-third, and then filled as above indicated.

It is then placed in the sputum shaker and vigorously shaken from five to ten minutes, then placed over in the centrifuge which has buckets made to receive the bottles, and centrifuged for ten minutes at moderate speed. The centrifugalization brings the ligroin to the top and a soap-like layer of detritus just under this. The tubercle bacilli will be found in this layer. A platinum loop of the soapy material is spread on a slide and dried fixed by heat or by methyl alcohol and stained for the tubercle bacillus. The ligroin should have a specific gravity from 0.715 to 0.720, this gives much better results than any below or above these points. The action of the ligroin seems to be two-fold; it combines in some way with the waxy envelope of the bacillus, thereby making it lighter, and lubricating it. Another advantage is that there is a tendency for the bacilli to clump, on centrifugalization; where the bacilli are few this is a decided advantage.

Efficiency: I have found that with this method that there is an increase of 19 per cent. of the positive specimens, others have found from 14 to 16 per cent. increase, these latter were based on a larger number than mine.

It is not only more accurate, but is a great time-saver, the number of specimens which one can examine by this method as compared with the old, is at least ten times as many, and with little or no eye strain. The time required to examine a specimen under the microscope is not more than one minute. If after a search of one minute you do not find the bacilli, it is useless to search longer.

Tissues and other substances suspected of being tuberculous, such as pus, blood and feces, can also be digested and examined for the bacilli, but the solvent action is somewhat slower than for sputum. Tissues should be ground with sand or powdered glass to a moderately fine pulp, then the alkaline hypochlorites and ligroin is added to the mass, shaken and centrifugalized. Pus and blood are to be treated in the same manner as sputum.

Feces should be treated in the following manner. If solid, a small amount of water is added and shaken to break up the lumps, then a small quantity of this is placed in a container, to which the alkaline hypochlorites and ligroin are added. This is again shaken and then centrifugalized. Feces, as a rule, contains but very little albuminous matter, unless blood or pus be present, therefore, it does not adhere to the slide, so in order to overcome this, it is best to fix the specimen to the slide, by methyl alcohol, or a small amount of egg white, or horse serum, as the case may be.

It has no advantage over other

methods for detecting tubercle bacilli in urine. This method has its advantages, and disadvantages, it is not at all satisfactory for milk, it does not digest the butter-fat, at all, and acts very slowly on the casein. Even were it satisfactory, I question its value because there are frequently found in milk acid-fast bacilli, which are not tubercle. The specimen does not adhere strongly to the slide as is the case with untreated sputum, so frequently there is a tendency for some of the film to become detached, sometimes, nearly all, when not properly fixed, and will be washed off. So it is necessary to prepare one specimen to a slide rather than several on a single slide, as is sometimes done. Fixation of the specimen is best done by methyl alcohol, but heat is very satisfactory provided it is a little longer than for the untreated sputum.

The method which I have found to be satisfactory for preparing and staining a specimen of sputum is as follows: Specimens are prepared on individual slides. Two lines are drawn across the slide with a wax pencil, allowing a space of about $\frac{3}{4}$ inch between them. A loop of the soapy layer is spread on the slide between the wax lines, and allowed to dry, then fixed by heat or methyl alcohol. Carbol-fuchsin is added until the space between the wax lines is covered. These wax lines confine the carbol-fuchsin to the space and prevents it from running over the slide, moreover, there are occasionally specimens, which are refractory to the stain, which will run off unless so confined. The staining is made at ordinary temperature and in from one to three minutes, heat is not necessary although is not contra indicated.

Decolorization is best accomplished by a 3 per cent. solution of hydrochloric acid in 95 per cent. alcohol, and stained with Löefflers methylene blue for contrast. Gabbetts decolorizing solution does not work well particularly when the film is thick or unevenly spread. The carbol-fuchsin solution which is employed for cold staining is one which I have been using for the past 20 years, and differs somewhat from the Zeihl-Neilsen. It is:

Fuchsin basic	4 grams.
Acid carbolic (Cryst. Merck)	8 grams.
Alcohol 95 per cent.	20 cc.
Water	100 cc.
Filter.	

The solution of the alkaline hypochlorites is practically the Liquor Sodæ Chlorinæ, U. S. P., of a double strength to which is added $7\frac{1}{2}$ grams of caustic soda to each 100 cc. The amount of chlorine is important and should be from $5\frac{1}{2}$ to 6 per cent. Any percentage less than this is not so effective, and below 3 per cent. is of no value.

The solvent action depends on the amount of available chlorine, and of the percentage given. A larger amount of available chlorine does not seem to hasten the digestion, nor an increase of the free alkali, but on the other hand interferes with the staining quality of the bacilli. This solution is fairly stable, it lasts from 2 to 3 months without much deterioration.

The commercial article known as "Antiformin" has not given as satisfactory results as could be desired, several lots were tried out, and all ap-

peared about the same strength. The amount of available chlorine was much below 6 per cent. So on comparing a home-made article with this, the trouble was found to be in the chlorine content. Since then all the alkaline hypochlorites have been prepared in the laboratory. It is rather tedious to prepare, although not difficult.

The prerequisite is a good fresh chlorinated lime which has at least 30 per cent. available chlorine. Ten pounds or 4,500 grams of lime are placed in a covered stone or glass jar and sufficient water is added until it is a thick mush. Three thousand two hundred and fifty grams of washing soda is put in a stew pan and sufficient water, about 2,500 cc. is added to bring the soda in solution, when boiled. While boiling add this to the lime and stir it well when adding. Allow this to stand for 4 to 6 days, stirring it well each day. Then filter and estimate the amount of available chlorine. If above 6 per cent. add sufficient water to dilute it to this amount. Then add 7½ grams of caustic soda to each 100 cc. and filter.

If the chlorine content is high, a liter of water can be added to the lime residue and filtered off. Sometimes 2 to 3 exhaustions can be made, before the chlorine content falls below 6 per cent.

The hypochlorite solution can also be made by passing chlorine gas through a 50 per cent. solution of caustic soda, than after ascertaining the amount of chlorine, add 7½ grams of caustic soda. Unless large quantities are required, this method is not available.

The alkaline hypobromates also digest sputum, but its action is somewhat slower, but it does not interfere with the staining quality of the tubercle bacillus.

A method for preparing it, which I have found to be good, is to take an Erlenmeyer flask of 1,000, to 1,500 cc. capacity, and pour into it about 100 cc. of water, then pour in from 5 to 10 cc. of bromine. The water will prevent escape of the gas and the flask will confine almost all the gas set free in the pouring. Small quantities of a stock solution of 50 per cent. caustic soda are added until all the bromine is conbined, and a bright greenish yellow fluid results. This can now be tested for the amount of available bromine and diluted to 7½ per cent. when 5 grams of chloride of lime (not the hypochlorite) or chalk are added, to each 100 cc., together with 15 cc. of the 5 per cent. solution of caustic soda, then it is filtered and ready for use.

The addition of a lime salt is essential, for unless lime be present, the unsoluble soapy layer will not form, and although the bacilli are brought to the top, with the ligroin, the preparations are fixed with difficulty and are more liable to wash off during the process of staining.

The same may also be said with regard to making the hypochlorites from chlorine gas, here the addition of a lime salt is necessary just as for the hypobromites.