

JOURNAL OF THE ROYAL SANITARY INSTITUTE

TESTING THE GERMICIDAL POWER OF VARIOUS PRODUCTS BY THE THREAD METHOD (Dried Cultures Method).

By A. SHERIDAN DELEPINE, M.Sc., M.B.,
Director of the Public Health Laboratory, University of Manchester.
(FELLOW.)

R. KOCH'S THREAD METHOD.

THE thread method which I have used since 1892 differs from that employed in the experiments published by Koch in 1881, the only common features between the two being that threads impregnated with bacteria are used for the purpose of testing disinfectants. Koch used threads loaded with anthrax spores; these were left for various lengths of time in the solution to be tested, washed, and then placed in nutrient gelatine or used for inoculating animals. The germicidal value was estimated by the duration of the exposure necessary to cause the death of the spores.

Owing probably to an impression that only sporing organisms were suitable for the application of this method, it appeared to most observers that its use was very limited. In 1892 I made a series of experiments to ascertain how far non-sporing organisms could be used instead of anthrax spores, and found that with most pathogenic organisms it was possible to prepare threads which could be kept for a considerable time without the bacteria losing their power of growing actively. For certain purposes I used small square pieces of pure filter paper instead of threads, but for general work silk threads are more convenient. This method has been used regularly in my laboratory for some fifteen years, and during that time I have not found it necessary to modify it except as regards minor details in the apparatus used. The use of threads is not essential, and the method might perhaps be better called *dried cultures method*.

2 *Testing Germicidal Power by the Thread Method.*

DRIED CULTURES (OR THREAD) METHOD, USED BY THE WRITER.

In the first instance I will describe the method as carried out when the *Bacillus coli communis* or the *Bacillus typhosus* is used as the test microbe.

PREPARATION OF STANDARD CULTURES.

A sloped agar tube is inseeded with 1 loopful (about 1 milligramme) of an actively growing culture of bacillus giving all the reactions characteristic of the organism. The loopful of material is spread all over the surface of the agar.* The culture is incubated for 48 hours at a temperature of 37° C.

PREPARATION OF THE EMULSION.

At the end of this time the surface of the agar should be covered with an abundant growth; 2 cc. of bouillon* are poured over the culture, and with a sterilised platinum needle the growth is scraped off carefully (without damaging the surface of the medium) and mixed with the bouillon. The tube is then shaken until a homogeneous emulsion is produced. Good cultures should not produce flocculent emulsions.

PREPARATION OF THREADS.

Closely twisted silk thread,† a little less than $\frac{1}{2}$ millimetre in diameter, is cut in 2 cm. lengths. 50 threads (2 cm. long) are placed in a small covered capsule about 3 cm. in diameter.

They are then sterilised by saturated steam at 115° C. for half an hour.

When the capsule is cool again, the 2 cc. of freshly prepared emulsion are poured into it, and the threads are stirred with a sterilised needle until they are all thoroughly soaked. After a contact of a quarter of an

* The nutrient agar is prepared with peptone bouillon made according to the method used in Koch's laboratory. The reaction is + 5 per 1,000. Lately I have also been using + 10, and also + 15 nutrient agar and peptone bouillon. There does not seem to be any serious reason to prefer + 10 to + 5 or *vice versa*, but the + 15 agar does not give as good cultures as the more alkaline media. To simplify matters I would propose to use a + 10 reaction in all cases.

† When certain disinfectants (*e.g.*, strong solutions of chlorinated lime) are tested it may be necessary to use cotton or flax thread, but for general purposes silk is preferable.

At first I employed loosely-twisted surgical silk, but of late years I have found closely-twisted silk more suitable. Lister's super quality No. 12 white button-hole silk is very suitable; the thread is composed of three closely-twisted strands, its diameter is about 0.4 millimetre, and one metre of it thoroughly dried weighs on an average 0.072 gramme.

Originally I submitted the silk to a thorough washing in dilute hydrochloric acid, followed by a second washing in dilute caustic soda, and a final washing in distilled water until all trace of alkalinity had disappeared. Afterwards I treated the silk by a mixture of hot alcohol and ether, after which it was washed in distilled water. Further experience showed me that these precautions were unnecessary. The threads prepared with silk washed in acid and alkaline solutions behaved exactly like those which had been simply sterilized by steam. Those washed with alcohol and ether seemed to be a little more resistant than those not so treated, but the effect was slight and doubtful.

hour's duration the threads are removed *one by one* by means of *fine pointed forceps*, and arranged in parallel rows on the bottom of shallow sterilised glass capsules (a Petri dish is very convenient). The threads must not touch each other, and must be moved about as little as possible.

The threads are then dried rapidly at a temperature of 37° C. The drying should be completed in less than three hours. The capsule must remain covered, but the lid should be raised slightly so as to allow evaporation.

When the threads are dry the capsule is closed again, and transferred to a dark cupboard at the ordinary temperature of a living room. During all the previous operations exposure to direct sunlight, and even to strong diffused sunlight, must be avoided.*

EXPOSURE OF THE THREADS.

10 cc. of each of the dilutions† of the disinfectant to be tested are placed in glass capsules 4 to 4½ cm. in diameter and of about 20 cc. capacity. The depth of the fluid should be as constant as possible. Two impregnated threads are then placed in each dilution. A series of dilutions can thus be infected successively at intervals of one-third or half a minute. Each thread is picked (from the capsule in which it has been dried) by means of sterilised fine pointed forceps,‡ and waved three or four times in the disinfectant before being allowed to sink in it.

* The threads so prepared retain a fairly constant amount of culture; the following weighings taken during the course of one year show the amount of variation:—

Bacillus coli communis (2 days old culture).....	Maximum weight of culture retained	0.00032 gramme.
	Minimum " " "	0.00020 "
Bacillus typhosus (2 days old culture)	Maximum " " "	0.00033 "
	Minimum " " "	0.00025 "
Bacillus anthracis sporing (7 days old culture)		0.00038 "
Bacillus pestis (3 days old culture)		0.00043 "

These threads offer the maximum degree of resistance to disinfection during the first two or three days after drying. Their resistance diminishes very slowly and gradually during the following months. The bacillus typhosus becomes less resistant more rapidly than the bacillus coli communis.

† The preparation of the dilutions is in this, as in all other methods, a matter of the utmost importance. The original material used in the preparation of the dilutions is weighed and not measured by volume (unless its solutions can be accurately titrated). A first dilution of the strength of 1 in 50 or 1 in 100 being accurately prepared by weight, the higher dilutions may be prepared from it by volume without material error. In estimating the relative cost of various disinfectants the weights may afterwards be reduced to volumes when necessary. The chances of serious error in the preparation of the solutions are greatly diminished when fairly large quantities are made.

‡ The flame used for sterilising the forceps must be placed sufficiently far from the row of capsules to prevent any of the capsules being heated. At a distance of 8 or 10 inches a Bunsen burner may cause a material rise of temperature (up to 5° C.) in 20 minutes.

4 *Testing Germicidal Power by the Thread Method.*

After infected threads have been placed in all the disinfecting solutions, the glass capsules containing them should be slightly shaken two or three times; this is specially important in the case of emulsions which have a tendency to produce a sediment.

DURATION OF THE EXPOSURE.

According to the test microbe employed the standard time of exposure differs.

If phenol is used as standard of comparison, and the strongest solution of phenol used is of the strength of 1 in 50, the following times are the shortest that can be conveniently adopted:—

Five minutes for the *Bacillus pestis*; ten minutes for the *Bacillus typhosus*; twenty minutes for the *Bacillus coli communis* and the *staphylococcus pyogenes aureus*.

Sporing bacteria cannot be conveniently used when phenol is taken as standard.*

TEMPERATURE.

Experiments should be conducted at temperatures ranging between 15.5° C. and 18° C. (60° F. to 65° F.), which are easily obtainable in living rooms. The effects of a marked rise of temperature are greater in the case of some disinfectants than in that of others, and comparative experiments carried at various temperatures do not give comparable results.

ARREST OF THE ACTION OF THE DISINFECTANT AND WASHING OF THREADS.

To arrest the action of the disinfectant as rapidly as possible and at the same time wash the threads, a number of covered glass capsules similar to those containing the disinfectant solution are used, in each of which 10 ccs. of sterilised water have been measured. At the end of the exposure each thread is taken out of the disinfectant separately by means of fine pointed forceps, and transferred to the corresponding capsule of distilled water; the threads are waved two or three times in the water before being deposited. The threads are left for ten minutes in the water, during which time the capsule is shaken from time to time.

* When high dilutions are tested, the time of exposure must be increased. No investigation can be considered complete unless the time which dilutions in practical use take to kill the test microbe is ascertained, and to obtain the relative value of these dilutions it is necessary to find which dilution of phenol would produce the same effect in the same time. For practical purposes dilutions which are not germicidal in a fairly short time are of little value. Thus it is unlikely that dilutions which do not kill non-sporing microbes in less than 12 hours, or even 6 hours, are of any practical value. Some disinfectants are much more affected by dilution than others.

At the end of ten minutes the threads are transferred from the water to covered tubes containing 5 or 6 cc. of peptone bouillon + 5.*

INCUBATION.

The loaded tubes of bouillon are then incubated at 37° C. They are examined at the end of twenty-four hours, and again at the end of forty-eight hours, and any evidence of growth is noted each time.

CONTROLS.

Each experiment is controlled by means of threads belonging to the same batch as that used for testing. These threads are left in 10 cc. of sterilised water for a period of time equal to that of the exposure plus that of the washing, before they are transferred to the bouillon. Other threads of the same batch are submitted to the action of dilutions of the disinfectant used as standard for comparison. All the exposures must be made at the same time and under the same conditions.

EVIDENCE INDICATING DEFECTS OF TECHNIQUE.

When in a series of six or twelve tubes corresponding to dilutions of gradually increasing strength one finds among the sterile tubes a stray tube showing some growth, or *vice versa*, among the non-sterile tubes some showing no evidence of growth, some error must have been committed.

In a series of several hundred sets of experiments, I have not observed such accidents in more than two or three sets out of each hundred sets.

With reasonable care it is easy to conduct a very large number of experiments without accidental contamination, provided covered vessels are invariably used.

APPARATUS.

Ordinary culture tubes and capsules are quite sufficient for the purposes of such experiments; but to avoid waste of time and confusion leading to accidents, I have found it convenient to use capsules and test tubes covered with glass caps. Culture tubes plugged with cotton are very inconvenient. Stands upon which particulars can be entered opposite each test tube also save much time. All the vessels used for making dilutions, containing sterilised water, or culture media, should be of good glass, as insoluble as possible. Jena or Resistance glass is the most suitable for the purpose.†

* The reaction + 5 has been used in my laboratory, but I would see no objection to + 10 being adopted (*see note under "Preparation of Standard Cultures"*).

† Some ten or twelve years ago a large amount of cheap test tubes, flasks, etc., were put on the market, and on trying to discover the cause of the abnormal appearance of some cultures, I found that the culture media had become highly alkaline. This was due to part of the glass having been dissolved. It is obviously useless to pay attention to the

6 *Testing Germicidal Power by the Thread Method.*

INFLUENCE OF VARIOUS PRODUCTS ON THE ACTION OF DISINFECTANTS.

So far I have supposed that the object in view was to test the relative value of various disinfectants diluted with water, but this seldom gives an accurate idea of the way in which the disinfectant would act in practice.

The action of each disinfectant should be tested in presence of such products as are likely to require disinfection; the following are fairly representative :—

Blood.	Milk.	Sputum.
Urine.	Fæces.	Soil.*

In laboratory investigations simpler compounds may also be used, *e.g.* :—

Acids.	Various salts.	Various carbohydrates.
Alkalis.	Various proteids.	Urea, etc., etc.

When testing the effects of these normal or morbid products upon disinfection, I place the threads in 5 cc. of the material, and after one minute I add 5 cc. of a solution of the disinfectant, the strength of the dilution being twice that which one desires to test.

The mixture is then stirred thoroughly several times during the exposure. Fæces and sputa are the most useful products for practical purposes.

In testing the action of sputa, it is desirable to mix some five, six, or more sputa of different types, some being thin purulent, and others fairly thick and mucous. The mixture should be sterilised in the autoclave, and then thoroughly shaken; in this way a manageable fluid is obtained, and the mixture secures a fairly average composition.

Typhoid or diarrhoea stools are prepared in the same way.

Blood should be first defibrinated, and if properly collected need not be sterilised.

Blood serum collected aseptically may be used without previous sterilisation.

Milk must be sterilised, it is seldom needed.

One part of soil must be mixed with ten parts of water, and then sterilised by steam at 115° C. for half an hour at least.

To find again the threads in these mixtures, it is generally sufficient to pour the contents of the capsule into its lid.

reaction of the media, if care is not also taken to avoid using glass of this kind. To test whether the glass is of good quality, the test tubes or flasks are rinsed with cold water, then after being partly filled with distilled water they are placed in the autoclave and kept at a temperature of 115° C. for half an hour; if the glass is bad the water becomes strongly alkaline.

* For general routine work, fæces, sputa and blood are sufficiently representative.

TO TEST THE ACTION OF DISINFECTANTS WHEN APPLIED TO A SURFACE.

For this purpose I use the threads* in two ways:

(A.) The threads are dipped in the disinfectant for one minute, after which they are laid in a large sterile capsule, where they are allowed to dry slowly. They are then taken up at intervals of one hour for six hours, then, at the twelfth and twenty-fourth hour, washed for ten minutes in sterilised water, and then placed in tubes of nutrient bouillon as previously explained.

(B.) The threads are placed on a piece of sterilised paper or cardboard and exposed to the action of the sprayed disinfectant, or the disinfectant is brushed over them with a soft brush. After this they are treated as explained above.

TO TEST THE ACTION OF GASEOUS DISINFECTANTS.

The threads are placed in small covered capsules which are distributed in various parts of a room of known capacity and then uncovered. A known quantity of the gas is generated, and at the end of 6, 12, or 24 hours the room is opened. To make comparable experiments, a special room, at least 1,000 cubic feet in capacity, with properly guarded openings for observations, is devoted to this work in my laboratory.

TO TEST THE PENETRATING POWER AND DIFFUSIBILITY OF GASEOUS DISINFECTANTS.

To test the penetrating power of disinfectants I use a board, against which a metal ring can be tightly clamped. Under the ring several layers of filter or other paper are placed, so that when the ring is clamped the spaces between the sheets of paper are tightly closed at the periphery. Before clamping the ring a few threads are placed between each two layers of paper. At the end of the exposure the ring is unclamped, and the threads exposed after each layer of paper has been removed are treated as previously stated. The diffusibility of gaseous disinfectants is tested by using glass tubes a quarter of an inch in diameter, and varying in length from 3 inches to 12 inches; the threads are placed at the bottom of the tubes, which are distributed in various parts of the room.

TO TEST METHODS USED FOR THE DISINFECTION OF TUBERCULOUS SPUTA.

As it is difficult to load threads with sputum, I prefer to dry tuberculous sputa on pieces of sterilised pure filter paper $\frac{1}{2}$ cm. square. When the sputum is quite dry the paper is treated in the same way as the threads, but instead of using the culture method to find out whether the tubercle bacilli have been killed it is necessary to inoculate guinea-pigs, which are

* or infected pieces of paper.

8 *Testing Germicidal Power by the Thread Method.*

kept under observation and killed at the end of one and two months respectively. A careful post mortem examination is then made. This method is used for testing methods of surface disinfection. For testing the action of disinfectants on fresh sputa, the disinfectant is mixed with the sputa, and the action tested by the inoculation of guinea-pigs with $\frac{1}{4}$ to $\frac{1}{2}$ cc. of the mixture. Several guinea-pigs are inoculated with various parts of the mixture.

GENERAL REMARKS AND CONCLUSIONS.

This account of the thread method, though incomplete, is sufficient to show that nearly all the problems of disinfection can be studied by means of this method. By the exercise of reasonable care it has in my hands yielded very constant results, and has not led me to form an exaggerated opinion of the practical value of any of the disinfectants I have tested. It is certainly more complicated than the old fluid methods, and does not bring out such marked contrasts as those obtained when naked bacteria are used; but considering the fact that one has never to deal with naked bacteria, and that the chief object of testing disinfectants is to find out how far they effect their purpose, I feel justified in recommending the thread method as a general method, whenever *the relative practical value of various disinfectants* is under inquiry. I believe also that before expressing an opinion the bacteriologist is bound to test the action of the disinfectant under the various conditions which may affect materially the power of these products.

Any method, such as the drop method, by which the action of various chemical agents upon bacteria suspended in distilled water is ascertained, may be useful to chemists engaged in the manufacture of disinfectants, or in researches bearing upon the relative activity of various derivatives of one group of substances. The indications obtained by this method show the directions in which good results may be expected; they may also be used to test the strength of a given product. But I do not believe that the action of disinfectants upon naked bacteria can be taken as the sole basis for estimating for practical purposes the relative value of various disinfectants, some of which are soluble, while others are insoluble and used in the form of emulsions. To express the relative power of disinfectants by a carbolic acid coefficient obtained by such a method is misleading, unless the results so obtained are controlled or modified by a statement of the results obtained by a method, such as the thread method, by which it is possible to place the bacteria under conditions resembling those likely to occur in practice. It is obvious, however, that the standardising of each method is a matter of importance.
