A SIMPLIFIED LABORATORY METHOD FOR THE DETERMINATION OF THE PHENOL COEFFICIENT OF DISINFECTANTS.*

BY ALBERT SCHNEIDER.

The need of a simple and practical method for determining the germ destroying power of the disinfectants has become apparent to all laboratory workers familiar with the various attempts at the standardization of these products. The older methods which have been employed from time to time are entirely too cumbersome and too expensive for practical purposes. There are in fact very few laboratories that make any pretense at the determination of the phenol coefficient or of the toxicity coefficient. The silk thread method (Robert Koch) and the garnet method (Koenig and Paul) were for a time extensively employed in Germany; the Rideal-Walker and Lancet methods were popular in England; and modifications of these methods, as the Anderson-McClintic (U. S. Public Health Service) method and the Ohno-Hamilton (Parke, Davis and Company) method, have been used in the United States. These several methods call for special equipment, special technical skill and the difference in result is great. Two workers of apparently equal ability may get results varying from five to thirty percent, a difference entirely too great to make the method practical or to entitle it to the distinction of standard.

Without entering into a discussion of the special uses and special properties of disinfectants, an outline of a method is given which is simple, easy, and which gives results fully as valuable and as reliable as those by the older methods and having furthermore a much smaller error factor. The method is outlined as follows:

1. *The Test Organism.*[1]—The test organism used is an approximately pure culture of *Paramecium caudatum*. The stock culture may be readily maintained in a gallon or half gallon museum jar, kept about two-thirds full of equal parts of filtered horse manure extract and well or tap water. Paramecia are essentially vegetarian in habit and can readily be maintained in such a solution, merely adding a small amount of fish meal ("Welesco" or other brand or Zwiback or hard, dry rolls may be substituted for the fish meal) from time to time. The jar should be thoroughly cleansed once a month and a new culture started. It takes about two weeks to get a

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[1] *Paramecium caudatum* is the most common infusorium in stagnant water. It is comparatively large, measuring from 150 μ to 325 μ in length. The cell is spindling in form with a deep oral groove on one side which takes a spiral course. The entire surface of the cell is covered by cilia, those at the anterior end and about the oral groove showing the greater activity, while those at the posterior end of the cell are longer but less active. Each cell contains two contractile vesicles with radiating canaliculi and Vorhöfe, which invariably contract alternately. The vesicles are located one nearer each end and nearer the dorsal surface. Near the center of the cell is a large but not very distinct nucleus. The cell contains numerous so-called food vacuoles and granular matter. The spiral groove enters the cell and ends as a blind sac at the lower three-fourths.

The paramecium feeds upon bacteria and other organic matter found in stagnant water. It is therefore a scavenger organism and is useful rather than harmful. Types of paramecia are found in the intestinal tract of aquatic animals, as fish, frogs, eels, salamanders and water snakes, but whether these associates are harmful or beneficial to the host is not definitely determined. Paramecia multiply by transverse septation as a rule. They may also septate in the long axis of the cell. Conjugation appears to be isogamous.

This organism may be obtained from stagnant water, from ponds and ditches, from long standing pools, from aquaria, and from the cultures in the biological laboratories of high schools and universities.

culture well started. From this stock culture make the 48-hour transfer or sub cultures, in 25 Cc. beakers, by pouring in 15 Cc. of the mixture of horse manure extract and tap water, with one teaspoonful of the stock culture, adding about 1 Gm. of broken fish meal or hard bread crust, and set aside at the room temperature. In 48 hours the sub culture will be ready for use.

The stock culture should be examined from time to time. If abundant dead paramecia are found, it indicates that conditions are not suitable. The trouble should be located and corrected. The occasional emptying and cleansing of the stock culture jar is necessary to prevent the excessive invasion by bacteria and infusoria. Associated organisms, such as amebas, rotifera, other species of paramecia, bacteria, etc., do not interfere with the tests. The cultures should, however, be comparatively pure and should be kept so.

2. *Making the Mixing Loop.*—Use 1 mm. wire (platinum or alloy). Bend one end into a circular loop having an internal diameter of just 3 mm. Fasten the other end into a glass rod or other convenient holder. The wire should be about 6 cm. long, and the loop must be closed or very nearly so. If the loop is left fairly open it will not take up and hold the drop. Silver wire may also be used, as it is not necessary to expose it to high temperature. Copper wire may not be used, because of the fact that this metal becomes colloidally diffused very readily.

3. *Making the Loop Mixtures.*—Take up one loopful of the solution to be tested and deposit it upon the middle of a clean slide. See that the loop is completely emptied. (There must be no film remaining across the mesh of the loop.) It may be necessary to touch the loop upon the slide several times to empty it. Rinse the loop in water and heat to dryness in Bunsen flame. When cool take up one loopful of the paramecial culture and add it to the loopful of the solution already upon the slide and mix the two loopfuls. The loopful of the culture should contain from three to six active test organisms. No cover glass is used.

Examining the Loop Mixture.—At once place the slide containing the loop mixture on the stage of the microscope and examine under the low power (90 diameters) and note results.

The tests are to be made at the room temperature at about 20° C., and away from air currents and from direct sunlight. Trial dilutions may also be made by means of the standard loop. Thus two, or even more, loopfuls of the trial solution of a disinfectant to one loopful of the culture, or conversely one loopful of the disinfectant to two, or even more, of the paramecial culture will indicate roughly what the next trial dilution (of disinfectant which is being tested) shall be. These trial loop dilutions and concentrations in the hands of a skilled worker are great time savers, making it possible to arrive at conclusive results quickly. We will suppose that the analyst has made the usual loop mixtures of a 1 : 500 dilution of some disinfectant (or 0.2 percent) and the paramecial culture and finds that the test organisms are promptly killed within one minute. He may next try one loopful of the disinfectant and two loopfuls of the paramecial culture which would roughly give the same results as though he had used a trial dilution (of the disinfectant) of 1 : 1000. The results may show that the test organisms are retarded in their activities but not killed within three minutes. This would suggest a dilution of 1 : 800 for the next trial, which would probably be fairly near the actual killing dilution.

It is self-evident, that according to the manner of making the loop mixtures of the disinfectant and the culture, the dilutions and also the percentages as given in the table are not the actual killing dilutions and percentages for the paramecium. One loopful of the disinfectant and one of the paramecial culture, dilutes the former one-half. Thus the actual killing dilution of phenol is about 1 : 1000, instead of 1 : 500 as given.

4. *Minimal Killing Dose.*—That percentage solution of the disinfectant tested which will kill all of the paramecia present within three minutes of time in three successive mounts, but not within one minute of time, shall be designated as the minimal killing dose (M. K. D.).

The Trial Dilution of the Disinfectant to be Tested.—All dilutions are to be made with distilled water.[2] (*a*) *If a Liquid.*—Make a trial loop mixture of the undiluted substance. If death is instantaneous, make a 1–1,000 dilution. If death is very prompt or practically instantaneous, make a 1–100,000 mixture and test as before. Let us suppose that with the 1–100,000 dilution all paramecia die within one minute, a few having died immediately. Then

[2] For making the percentage dilutions, carefully graduated 1, 2 and 5 Cc. pipettes and graduated mixing cylinders are required. Small amounts of the mixtures to be tested are poured into watch crystals from which the loopfuls are taken. All dilution mixtures must be thoroughly shaken before using.

next try the 1–125,000 dilution, and it may be found that the 1–125,000 will kill within three minutes, but not within one minute.

(*b*) *If a Soluble Solid.*—Make 1:10 or 1:100 dilution in distilled water and proceed as for liquid disinfectants.

5. *The Phenol Standard.*—Make a 1 percent solution of pure phenol (crystals) solution in distilled water, and determine the M. K. D., in the manner described. This M. K. D. shall be the standard of comparison, or the phenol standard, given as 1. The phenol coefficient of the compared disinfectants is obtained by dividing its killing dilution by the killing dilution of pure phenol. Thus, we get as the phenol coefficient for lysol 800 ÷ 500 = 1.60, and for borax 110 ÷ 500 = 0.22. (See table.)

6. *Determining the Death of the Test Organism.*—The behavior of the test organism toward the test solutions may be briefly stated as follows:

(*a*) Excitation—temporary. Increased rate of motion, followed by

(*b*) Retardation of motion, slowing and even complete inhibition of the vesicular pulse, then,

(*c*) Slow progressive motion with axial rotation; spinning top motion.

(*d*) Cessation of motion, and,

(*e*) Projection of peritrichia, with

(*f*) Protrusion of vacuoles, which invariably means death.

If death is very sudden, there is generally no plasmic contraction and the outline of the organism remaining about normal. If death is gradual, there may be plasmic contraction and more or less cell distortion.

7. *Adjusting the Bacillus Typhosus Phenol Standard and the Paramecium Caudatum Phenol Standard.*—The phenol killing dilution for the *Bacillus typhosus* is 1 : 80, while the comparative phenol killing dilution or percentage of phenol for paramecium must be increased nearly six times in order that it may prove efficacious against *Bacillus typhosus*, or fully six times that amount if a little allowance is made for good measure. Such adjustments are not required in practice since the essential fact to be determined is the relative killing powers of the disinfectants in comparison with pure phenol. As is known the *B. typhosus* phenol killing dilution, namely 1 : 80 (1.25 percent) is not the strength solution employed in practice, rather double that strength, or even more is used. As a surgical dressing and for the purpose of active sterilization, a 2.5 or a 5 percent phenol solution is generally employed.

Pure phenol carefully applied to an infected wound or tissue through its albumin coagulating properties, sears the surface of the tissue covered and the phenol is held there and then slowly osmoses into the deeper tissues at the rate of about a 5 percent strength. This somewhat theoretical deduction has given apparent justification to the, in some instances, reckless use of pure phenol for the purpose of tissue disinfection. Numerous cases of phenol intoxication have followed this use of pure phenol, especially when applied to mucous membranes from which absorption is rapid. However, these special behaviors of disinfectants have nothing to do with the methods for determining the relative germ destroying powers of the several disinfectants.

On comparing the M. K. D. by the method outlined it will be found that the dilutions are universally higher than the dilutions which will kill the typhoid organism used in the methods of the U. S. P. H. Service and of Rideal-Walker, but the comparative results are nevertheless closely similar. It is not intended to imply that the killing dilution for paramecia is also the killing dilution for pathogenic bacteria, but the comparative results are apparently closely similar and the results obtained are as useful as far as giving indication of the comparative germ destroying power of the various disinfectants is concerned, as are those by the more tedious and difficult older methods. Paramecia appear to be especially susceptible to the action of the solutions of the heavy mineral salts, as may be seen from the

results with mercuric chloride, silver nitrate and copper sulphate. It would appear from the tests made that the difference in the behavior toward solutions of disinfectants on the part of *Paramecium caudatum* and *Bacillus typhosus* is one of time rather than of greater or lesser resistance to the antiseptics. For example, while a 1 : 500 dilution of phenol kills *P. caudatum* within three minutes, this dilution will also as surely kill the typhoid germ within one hour of time. It will also be seen from the table that Paramecium is remarkably susceptible to the action of quinine sulphate.

The following are a few disinfectants and toxic agents which have been tested according to the proposed method. No attempts at accuracy were made, the solutions being made hurriedly and the reactions not very carefully checked, the work being done by students in the College of Pharmacy of the University of Nebraska, under the direction and supervision of the writer. A working laboratory method, in order to be practically usable, must be sufficiently simple to be carried out by investigators of average ability. The uniformity in the results by the different members of the entire class was striking. More carefully conducted tests are now under way with a view to determining the more exact killing dilutions and phenol coefficients.

TABLE OF M. K. D. AND PHENOL COEFFICIENT OF SOME DISINFECTANTS AND TOXIC SUBSTANCES.

Name of substance.	Dilution.	Percent.	Phenol coefficient.
Acetic acid	1:350	0.28	0.70
Alcohol	1:8	12.50	0.0016
Antiseptic powder N. F	1:1000	0.10	2.00
Argyrol	1:400	0.25	0.8
Boric acid	1:110	1.10	0.22
Borax	1:110	1.10	0.22
Chloroform	1:200	0.50	0.40
Coffee	1:4	25.00	0.008
Copper sulphate	1:160,000	0.0006	320.00
Ether	1:37	2.70	0.074
Formalin	1:2,500	0.04	5.00
Hydrochloric acid	1:1,200	0.12	2.40
Lysol	1:800	0.29	1.60
Mercuric chloride	1:800,000	0.00012	1,600.00
Nicotine	1:200	0.50	0.40
Nitric acid	1:1,500	0.066	3.00
Phenol	*1:500*	*0.20*	*1.00*
Phenol sheep dip	1:1,400	0.07	2.80
Quinine sulphate	1:1,600	0.06	3.20
Silver nitrate	1:100,000	0.001	200.00
Soap (castile)	1:150	0.66	0.30
Sodium chloride	1:100	1.00	0.20
Sulphuric acid	1:4,000	0.025	8.00
Strophanthus	1:10	10.00	0.02
Strychnine nitrate	1:75	1.33	0.15

COMPARISON OF THE OLD AND THE NEW METHOD.

The Old Method.	The New Method.
Technic.	
Complex and difficult and much experience required. Plate cultures required.	Extremely simple. No special skill or experience required. No plate cultures made.

Equipment.	
Special equipment required. Cultures of the test organisms must be maintained at cost and with care.	Cultures of test organism are easily maintained. The only special device is the platinum loop.
Reliability of the Experimental Results.	
Results variable by the different analysts. Error ranging between 5 and 20 percent.	Results very uniform. Error ranging from 1 to 2 percent.
Time Factor.	
From 2 to 3 days required in which to complete the test.	Time required, 1/2 to 1 hour.
Cost Per Test.	
To test one disinfectant will cost from $50 to $150.	A charge of from $2.50 to $5.00 per disinfectant tested would be a fair remuneration for time and skill.

The method is believed to be fully as valuable as an indicator of the relative germ destroying power of disinfectants as are any of the older methods which are now largely rejected or considered so highly technical as to be practically inoperative, excepting in a few of the larger laboratories, and is at the same time so simple that a laboratory worker of average ability can test out any of the disinfectants the germ destroying power of which he may care to ascertain. The method as herein crudely outlined requires further careful working out as to the exact details. It has, however, been sufficiently tried out to justify this report. A number of details require careful consideration. For example, the evaporation of water and disinfectant from the loop mixtures on the slide requires further consideration. Thus far it has appeared that because of the brief period of exposure, namely one to three minutes, this factor may be ignored. Naturally, in the case of highly volatile substance as ether and chloroform (in water), the solutions become weaker and weaker with the period of exposure, whereas in the case of salt solutions, as mercuric chloride, copper salts, etc., the evaporation will result in an increase in the concentration. This possible source of error can be corrected by making vaseline sealed hanging drop mounts of the loop mixtures.

There is a very marked difference in the reaction of different species of Paramecium to the same percentage dilution of a given disinfectant, closely comparable to the difference in the reaction of different species of the group bacteria to the same disinfectant. A very minute species of Paramecium, a common associate of *P. caudatum* in the older stock culture, showed an unusual resistance to disinfectants. Strength solutions which killed *B. caudatum* within two minutes produced no appreciable lessening in the activities of the dwarf associate.

No tests were made to indicate the change in the reaction to disinfectants in the presence of albuminous matter, nor are such tests of any special significance, as it is generally known that the presence of albuminous matter interferes to a very marked degree with the action of disinfectants. This interference is, therefore, proportional to the amount of albuminous matter present and the albumin coagulating power of the disinfectant itself. A method for determining the albumin coagulating power of disinfectants has been given in full elsewhere. (Bacteriological Methods in Food and Drug Laboratories. P. Blakiston's Son & Co. 1915.)

The test organism used in the proposed method is as valuable as is the *Bacillus typhosus*, as an indicator of the germ destroying efficiency of disinfectants in gen-

eral. As is known to all bacteriologists, there is a wide range in the susceptibility of one and the same disease germ of the bacteria group to different germicides, and on the other hand the different disease germs respond differently to one and the same disinfectant. Thus for *Bacillus dysenteriae* the phenol coefficient with pyxol (a crude phenol) is 18.00, 20.00 for *B. typhosus*, 30.00 for *B. diphtheriae*, 41.5 for *B. pestis*, 56.00 for the Koch bacillus of Asiatic cholera and 57.5 for the Meningococcus. Just because a given strength solution of a disinfectant kills the *B. typhosus* within a specified period of time, does not prove that the same solution will also kill the pathogenic streptos or the pus formers. It must, however, be admitted that the tests made according to the methods of Rideal-Walker and of Anderson-McClintic have indicated and proven to the satisfaction of all scientists, that certain substances are efficient, in the comparative sense, for the destruction of microörganisms, pathogenic as well as non-pathogenic, whereas others are comparatively inert. This very important information may be ascertained by the proposed method with equal certainty and reliability and in a greatly simplified manner and at a greatly reduced cost per test. The proposed test does not give any indication as to the special value of the disinfectants tested for the purpose of wound sterilization, nor as to the effect upon phagocytosis, or upon the formation of granulation tissue, nor does it indicate the relative toxicity of the various disinfectants; but neither do the other methods mentioned. It may here be stated that the Paramecium was originally selected with the hope that it might serve the purpose of measuring the relative toxicity of the disinfectants, on the supposition that the comparative complexity (morphological as well as physiological) of this single-celled organism might impel this organism to respond to toxic agents in a manner indicating parallelism with the effects of toxic agents upon the higher organisms, as mice, guinea-pigs and rabbits, which are the animals now employed to some extent for such determinations, as in the Worth Hale method. The tests with such highly toxic substances as strophanthus, nicotine and strychnine, demonstrated at once that Paramecium was no more susceptible to such agents than are the group of bacteria. There are indications that the test organism will give much valuable information as to the manner in which the different disinfectants kill, as may possibly be observed from the extremely variable reacting behavior toward disinfectants. This phase of the experiments will not be touched upon in this report. The writer is furthermore not sufficiently familiar with the morphology and the physiology of the organisms to even interpret correctly the many varying responses which were noted in the limited tests made. Thus the heavy mineral salts kill without any plasmic contraction, whereas alcohol causes marked plasmic contraction. Some disinfectants cause marked temporary stimulation as is indicated by the increase in motion and in the pulse rate of the contractile vesicle, just prior to the changes which soon end in death. There is also indication that Paramecium does react toward toxic agents of the non-metallic kind, such as the alkaloids and glucosides in proportion to the relative toxicity of such substances, as may be seen from the table. Further carefully conducted tests along this line are necessary.

Paramecium may also be employed experimentally in making tests with plasmic poisons, leucocytic poisons and cell poisons generally. The method herein outlined also promises to be of great value in testing and standardizing the value

and efficiency of amebacides. An endless series of interesting experiments suggest themselves. It is certainly reasonable to suppose that a skilled and experienced investigator can derive vastly more information from a series of tests and observations made upon a comparatively complex organism of which the multitudinous and highly complex reaction behaviors are constantly before the eye, than from a series of tests which reveals nothing more than an indication of either death or no death, as in the older methods for the determination of the phenol coefficient. Undoubtedly the proposed method will in time give us more accurate information regarding the manner in which the different germicides and disinfectants cause death. A few observations made upon Paramecium in the presence of toxic agents under the ultramicroscope would indicate that this method will disclose many interesting facts regarding the behavior of toxic and germicidal substances *via* the realms of colloidal chemistry, in which the beginnings have already been made. It is for example known that bacteria suspended in liquids behave as negatively charged colloids and are, therefore, driven to the anode end of the electric current. Bacteria are precipitated by the ions of the heavy metals and they take up certain of the disinfectants according to the law of adsorption. Disinfectants may kill by virtue of forming chemical compounds within the bacterial cell, or they may kill because of the precipitating effects upon the plasmic proteins, or through the adsorption and subsequent absorption, osmosis and chemical decomposition of the chemically or physically (colloidally) active ions.

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ABSTRACT OF DISCUSSIONS.

The foregoing paper was discussed by Drs. Bernard Fantus, Horatio C. Wood, Jr., and H. C. Hamilton. It was the consensus of opinion that the weak point in the present methods is that they have no practical application. The effectiveness of a given disinfectant varies to a very great degree with the organism upon which it is allowed to act. A disinfectant might be very effective in killing streptococci but almost useless against *Bacillus typhosus* and *vice versa*. One of the objections to the method of testing disinfectants as proposed by the Hygienic Laboratory of the U. S. Public Health Service is the high cost of conducting the test. The need for a more satisfactory and practical test was generally conceded.

SOME OBSERVATIONS RELATIVE TO TRAINING IN DRUG ANALYSIS.*

BY C. O. EWING.

When your chairman suggested the preparation of a paper dealing with the teaching of drug assaying and analysis, it seemed to me that the request would more properly have been addressed to one who had had more collegiate teaching experience. Upon further consideration, however, it occurred to me that it might not be amiss to point out certain phases of the subject that some years of Federal and commercial experience have accentuated.

With regard to preliminary training, drug analysis, even in a semi-routine control laboratory, is an occupation requiring a broad general training. It is

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