The wire having been heated in a flame and allowed to cool, without being touched or laid down, a minute portion of a single colony is taken up on its point; the test-tube, containing nutrient solidified in a slanting direction, is held in the left hand, the plug removed between the backs of the third and fourth fingers of the right hand, taking great care that the part of the plug which enters the tube shall not come into contact with any other object, the wire is then passed into the tube without touching the sides, and gently drawn across the medium without injuring the surface. It is now again plugged, and placed in the incubator—if gelatine, a temperature of 20° C.; if agar-agar, or potatoes that of 37° C.—is usually employed. These cultivations show certain peculiarities of growth whereby further differentiation of species may be obtained.

For puncture, the nutrient should be solidified horizontally, and the tube being held mouth downwards the infected wire is pushed upwards through the medium, right to the bottom of the tube. Here, again, differences soon become apparent: the bacteria may grow along the whole track of the needle, or only at or near surface, or at bottom only; the growth may be a fine cord or a thick column, with or without radiating processes; the colony may spread over surface or be confined near the puncture; the gelatine may be liquefied in a funnel-shaped or other depression from surface, or equally over whole surface, etc., etc. Some species produce bubbles of gas, whereas others do not.

Having in this way ascertained that several species are present, small portions of each culture may be examined microscopically, when it is possible that further differences may be observed, e.g., two colonies, otherwise very similar, may be found to be a bacillus or micrococcus respectively. The growth of a colony may be observed by cultivation in the hanging drop; that is, by inoculating with a very minute speck of a colony, a small drop of gelatine or agar-agar on a cover-glass, inverted over a glass slide having a depression in centre, the cover-glass being kept in position by a minute portion of vaseline at one corner.

By these means and other special cultures, when necessary, the number of species may be ascertained. Migula states that no good drinking water contains more than 10 different species.

3. THE SPECIAL EXAMINATION OF POLLUTED WATER FOR INDIVIDUAL SPECIES OF BACILLI.

Pathogenic bacteria frequently find access to water used for drinking purposes, mainly through sewage pollution, and, unfortunately, some of these species are capable of living in water for considerable periods of time, thus giving every opportunity for spreading the disease.

It has been demonstrated that *Bacillus typhosus* is capable of existing in a living condition in sterilized water for some months, but in ordinary water its duration is more restricted; this is probably due to "crowding out" by other and more numerous water bacilli.

The cholera spirillum is rapidly destroyed when introduced into sterilized distilled water, but the addition of small quantities of nitrates or chlorides greatly increases its vitality. Most shallow wells or streams of a polluted character contain these salts in considerable traces, hence the conditions are favorable for the conservation of this bacillus, should it obtain access. The experiments hitherto made upon the vitality of cholera spirillum in ordinary potable water are not very satisfactory, but there is no doubt that it is capable of living for a considerable time. Moreover, the experience of Hamburg and Altona, already quoted, would seem to show this.

The particular bacteria which have usually to be sought are those of typhoid fever and cholera, although others, such as those of anthrax, septicæmia, or tetanus, have occasionally been found. I shall confine my remarks to the detection of the bacilli of typhoid fever and cholera.

The Typhoid Bacillus.

The difficulties surrounding the detection of this bacillus are very great, partly because it is commonly accompanied by far greater numbers of other bacilli derived from sewage, and partly because it is a disease not adapted for physiological test upon the lower animals.

On this account, an ordinary plate cultivation can scarcely ever be successful in giving a culture of the specific organism unaccompanied by other species, particularly the *Bacillus coli. communis*, constantly present in human fæces. Under these circumstances, special methods must be adopted to destroy the other species, after which tests are applied to distinguish between the *B typhosus* and *B coli. communis*, or any other species which may occasionally be met with. The water is first introduced into phenol-broth, or the sediment obtained by filtering a large quantity of the water through a Berkefeld or Chamberland-Pasteur filter, and is cultivated in the same medium. This medium is prepared as follows:—

Some beef-broth is prepared exactly as described for gelatine-peptone, but omitting the gelatine, and making neutral instead of slightly alkaline. A number of test-tubes each receive 10 cc. of the liquid, and in addition three, six, or nine drops of the following solution:—

Pure phenol	5 grms.
" hydrochloric acid	4 "
Distilled water	100 "

These tubes are kept in the incubator at 37° C. for twenty-four hours, whereby any microbes will be destroyed. To these sterile tubes one to ten drops of the water are added, and, after admixture, replaced in the incubator. If the sediment be used, a larger quantity of phenol-broth should be employed. At the expiration of twenty-four hours, and again at forty-eight and seventy-two hours, any of the tubes which appear turbid are to be submitted to plate cultivation, and the resulting colonies carefully examined for resemblance to those of the typhoid bacillus, and if any be present these are tested by (*a*) cultivation on potatoes, (*b*) inoculation into gelatine tubes, (*c*) cultivation in milk, (*d*) indol test, and (*e*) general microscopical characters.

The plate-cultures of typhoid bacillus develop colonies of two forms. Some spread themselves out upon the surface, forming a translucent, almost transparent, film with uneven edges; radiating lines may be seen like medullary rays, and in addition are lines similar to the annular zones of wood. These colonies may become as large as one-third inch in diameter. Other colonies do not grow upon the surface, and are quite small, opaque and yellowish-gray in color, and somewhat lemon-shaped in form.

(*a*) Cultivated upon potatoes at 37° C. these interior colonies produce an almost invisible grayish-white growth after two days, but on touching the surface with a needle, it is found to be covered with a felt-like pellicle. This remarkable appearance is not always shown, depending upon the acidity, or otherwise, of the potatoes.

(*b*) Introduced into gelatine tubes by puncture, it grows chiefly on the surface, producing a thin, grayish-white surface colony. If, however, the gelatine be melted and the bacilli then added, carefully mixed, and allowed to solidify, then cultivated at 20° C. no air-bubbles appear in the mass; this is a very important test, because *B. coli communis* always produces gas-bubbles.

(*c*) Milk is sterilized by heating to 58°-65° C. for an hour or two on five to eight successive days; into this medium some of the bacilli are introduced, and placed in incubator at blood heat; after twenty-four to forty-eight hours the milk is faintly acid and not coagulated, whereas the *B. coli communis* renders it strongly acid with coagulation.

(*d*) The indol test is made, as suggested, by Kitasato. To 10 cc. of the culture in ordinary peptone broth, grown for twenty-four hours, 1 cc. of a solution of sodium or potassium nitrite (.02 grm. in 100 cc.) is added, and then a little strong sulphuric acid; the *B. coli communis* produces indol, yielding a rose or deep red coloration, a reaction not obtained from cultures of the typhoid bacillus.

(*e*) Microscopical Characters.—The typhoid bacillus is about three times as long as broad, with rounded ends, and mostly occurs singly. It is very motile, and has numerous long flagella. The *B. coli communis* is broader in proportion, and is provided with one to six flagella. For examination, it is necessary to stain the bacilli, which is carried out in the following way. A small quantity of one of the colonies having been mixed with a little water on a glass side, a minute drop