The following is an informal translation of Razumov, AS. 1932. The direct method of calculation of bacteria in water: comparison with the Koch method. Mikrobiologiia. 1: 131-146. It was translated by Tatiana Vishnivetskaya to Karen Lloyd, both of whom are at the University of Tennessee. It is certainly imperfect, and we share it publicly in case someone else will find it to be useful for their research, as we have found it to be for our own. The paper refers to “the Winogradsky method” vs. “the Koch method”. These are direct cell counts under a microscope vs. plating of cells and counting colony forming units. Comments from Vishnivetskaya and Lloyd are in red.

**Title of paper:** This is a direct method of counting bacteria in water and comparing to the Koch method.

Page 1

Abstract

This work explains different attempts in using the direct method for quantifying microflora in water. This type of work has been done for a long time, but most of the attempts did not get wide use. Because of that we offer modifications.

Main text:

Winogradsky used the direct counting method for studying soil microflora and this increased interest in these studies. Now microbiologists widely use the “Winogradsky method”. In some microbiology studies, for example during the study of microflora of milk and other milk products, the direct method is widely used and everybody believes them. And this method became more trustable when used in milk enumerations than Koch’s. And it has also been used in water microbiology– list papers that use this method in water (refs 4, 5, 6). When the amount of bacteria in water were high (waste water or fecal material) the direct method did not have any methodological difficulties because, for this type of water, it’s enough to take certain volume of water for example very accurate measurements of a drop of water (Tatiana explains they used drops of water to get small volumes out of pipette). Next one places the drop on a fixed square on a microscope slide, then fix, dry, stain, and count under microscope. However, for other natural environments the main difficulty is the need to concentrate all the bacteria in a small volume to have enough bacteria for direct counting.

Because of that, the authors listed above describe direct counting in similar words to this. However, Kuznetsov proposed to concentrate water bacteria by evaporation. He suggested to take 50 cm3 of clear lake water and evaporate to small volume using negative pressure (15-20 mmHg), and 30-50°C.

Another author (who we don’t know) concentrated the water by filtering water through membrane filter using equipment with a name we don’t know. (we think vacuum)

Page 2

Because the country is developing and building new cities, for all this infrastructure it’s important to look for new methods to identify bacteria in water because it’s important for drinking water for cities.

The main detection needed to follow sanitary rules, is that we need to be able to know the characteristics of water. One way is the Koch method and fecal count titration using different methods. This is method 7. But these methods require a long time (48 hours) for total counting or 5 days for detection of coliform titers. And to conduct these methods, scientists need specially equipped laboratory space. However this is difficult to provide in small villages.

Based on this, the institute that manages the water purity wants to find new methods that can be easily conducted in different ways. So, our first aim is to improve the method for direct counting of bacteria, and this method gives the first characteristics of level of contamination of drinking water reservoirs. First, preliminary characteristics of water quality.

In the beginning, we thought the direct method would be easy to conduct under different conditions and during expeditions. We wanted to find any correlation between the direct method and the quality of the water. We wanted to know if it’s possible to calculate the cut-offs between direct counts that will become a problem for coliform titres.

Methods

These 2 methods (Kuznetsov and Haloudni) were difficult to conduct in the field. Because of that we wanted to find some very fast and suitable method. We used Haloudni as the basis for methods development because we think this method is more convenient – small volume and more concentrated. We just took water of some fixed volume and filtered through membrane filter and used these filters to stain and count under the microscope.

Page 3

For water which contains low bacteria 50-100 cm3 or 50-100 ml. For lake , 25-50 cm3, for rivers from clean regions we used 10-20 cm3, for waste water we diluted it with distilled water and used filtration as for the others. For waste water, e think that this dilution and filtering is better than just using a drop of waste water directly on a microscopic slide because microbes on the filter spread more evenly. And also filtering excludes the method for preparation of microscopic glasses.

Then they describe how they prepared the membrane filters. <<We didn’t translate this because we just buy ours now.>>

We vacuum filter and dry the membrane filter with bacteria. If we filter in the field, we store the filters to transport back into lab. The side of the filter with bacteria is protected.

Next we describe how we stain: 1% solution of apitrazine, 5% of something water for 20-30 min. Dry and then look under microscope.

Page 4

Describe how to count. The membrane filter, during all these procedures became transparent and you can see microbes. The microscope was ocular 4, objective 1/12’’, and 20-40 squares were counted. Then they give equations for how they work up the counts.

Results

1. Because this is the first report with these filters, we tested how well they worked. So, Table 1 is different parameters in how we filtered, and how many we counted in 1 cm.

Table 1 headings:

Expt number, grams of photo film that were used+acetone+ethanol, speed of filtration (seconds), how many bacteria went through the filter in 1 cm, and % of bacteria that were lost through the filter. Done with 10 cm3 of water. We filtered *Bacillus prodiiosum* with a known concentration. For all these bacteria, we used Koch on agar as well – 5 days. The results when we used 30-36 cm3 of ethanol gives reasonable results. <<Tatiana pointed out that 40 looks good too>>

Page 5

These results look reasonable and the filter works in short time and doesn’t require strong vacuum.

We discuss different filters and how low ethanol content may give a decrease in filtering. But there are some difficulties when we prepare these filters, especially when we do a small number of filters. The quality of ethanol and acetone can lead to low filter quality. Temperature during drying is also important, as is humidity. In further studies we use filters prepared with methods 3, 4, or 5. The quality of the filters were detected by looking at them – bad filters were dismissed. We used 10-25 minutes for 100 cm3 water filtering. Thicker filters resulted in better retention of bacteria, but in this case we had to apply higher vacuum pressure. And, it was not very convenient in the field.

Table 2 was water from an aquarium, 5 cm3, filter numbers in Arabic are same as Roman ones in Table 1. You can see the number of bacteria after they filter it through all different filters. Again, filter 1 is not so good.

25 cm3 from different lakes showed pretty similar results with the different filter methods. Filtered replicates show that the same filters give good precision. Replicates were good.

Table 3. describes how we can get better filters. We suggest to use the filter by attaching it to glass to make it flatter to get better distribution of bacteria. – use this way to prepare the filter- the side that was facing the glass was smoother and better to use – so make sure to filter on the even side (because it’s missing the ridges).

For Table 4, we took water samples from 2 different places within the city. 2 different water sources – plus building tap water. Water taken from the source was not purified in any way except a bit of chlorine. We used the direct method and Koch method – during Koch we counted bacteria on nutritional agar in 48 hrs at 20-22°C. Same conditions for Koch method were used in other experiments.

Table 4

Headings: Where water was collected, number of bacteria in 1 cm3 direct count, number of bacteria in 1 cm3 by Koch, ratio between the two.

Water from reservoir

* Reservoir left after sand was removed for building purposes (before chlorine)
* Tap water (after chlorine, with no other purification)

Table 5 Headings. Direct, Koch, ratio

Biofilter

--Incoming water

--III filter

--V filter (coming out)

Bio channel

--incoming

--III step

--IV step

We see that the direct count showed approximately similar amounts of bacteria before and after chlorine in Table 3, but Koch showed a significant decrease in cfu with chlorine.

In table 4, during direct count we cannot distinguish live from dead. If somehow some antiseptic or any antibiotic goes into the water, you cannot say exactly how many were killed, by using the direct method.

Table 5 in biofilter vs. biochannel. These are lab models where we conducted purification of fecal water. The biofilter 5-stories of boxes tall and boxes were filled with porous material. Through this material, the fecal water goes through, but the biochannel was tilted relative to the straight up and down biofilter. So, still gravity flow, but tilted, so not much difference between the two. Both contained the same porous material.

Table 6. headings: date, description where samples were collected – all were along one river, direct counts, Koch, ratio

River Ural (p. YpaA): village names up river vs. down river. Power station.

Table 7. headings same as table 6, I think

Lake Oa. MayeAbl close to surface, close to bottom, (24 meters deep), y cebephoro depera is north side of lake, then south side of lake surface, then bottom, also bottom middle of lake. Then there are other locations around the lake.

We found less variability when we compared samples that were collected close to the bank. There was more variability between samples collected in the middle of different lakes. The direct method showed more bacteria in lake water during summer than during winter. In some lakes there’s a lot of algae, but the bigger lake (Mouzleh) has less microbes which were detected by direct method. During summer we found the biggest difference between Koch and direct counts, so we cannot suggest correct sanitary state, especially during summer. This number was unique for every lake, and for summer and for winter (referring to table 7).

Table 8 is depth, temp, and direct method in the biggest lake Mouzleh.

We wanted to, but couldn’t detect dissolved oxygen in the water, so we don’t present the data. And the Table 8 results from surface could be taken from Table 7, where surface was collected from same spot. We saw changes in temperature in depths 10 and 13. And we detect less bacteria in these spots. But below, the number of bacteria increased close to bottom. However, it was twice as low as that closest to surface. We describe the temperature changes in different lakes. And we saw decreases in bacterial count when temperature changes.

Table 9. is artesian borehole n (wells\_ for drinking. Date,

First row: Number 4 fountain located on the left side of Ural River close to village and water coming from second horizon goes to water well number 4. Mixes with water existing in this well. This is water close to water in river.

Second row is mixed between artesian water and water from river.

Third row: water borehole from depth 65.8 m

Fourth row: water spring

Fifthe row: different village’s water spring

Sixth row: also water spring (2nd spring 40 liters/second water flow

7th row: 3rd 60 liters/second water flow

8th: samples from ground water

Discussion:

Some environments (cultured mild) give similar results with Koch and direct. But natural environments – lakes and rivers - give different results suggesting that Koch method doesn’t give the possibility to detect the total number of bacteria. For these environments we need to use microscopy.

Method Koch represents the method with cfu counts. The results depend on temperature, media, oxygen, time of cultivation, so method Koch is a method for detection of a physiological group of bacteria which is characteristic of this environment. The ones grown on plates can tell us about contamination. Koch is good for identifying a particular physiological group, for example coliform genus. This is the same as the detection of different microbes in soil. The limitation of plating is that not all bacteria grow on this media.

We need to test different media and conditions to grow more bacteria from these environments. And, the direct method has advantages in comparison to Koch of speed, simplicity, and ease to conduct.

Describe suggestions for agar media. In guts, some bacteria grew in 48 hrs but others grew later, but they count in 48 hours, so you miss them. In this case, direct count is better.

Characteristic of water purity should be done on petri dishes in 24 hours, supported by numerous paralell counts. Usually waste water gives similar results under these conditions. However, pure water at 37°C gives lower results.

Direct and Koch methods are similar for wastewater because of higher concentration of bacteria. However, this is not the case with pure lake water. With pure water the methods are very different.

We cannot establish coefficient for counts between direct vs. Koch. If such a coefficient is found, it will be different in different water sources and during different times of year. This coefficient could not be used to make conclusion about sanitation and water purity. Low coefficients may be detected for purer water, and higher for contaminated or waste water.

During the study, purification in biofilter may give additional characteristics of the water to Koch and direct count method coefficients.

The biggest negative for the direct counts is distinguishing between alive and dead. The aim of this study was to find an easy and fast method of detection of sanitary characteristics and we found that in just half of this study. We decide that the direct count could be used in field conditions, but direct count cannot be used for conclusions of purity of water. Need to use old methods.

To give sanitary conclusions, we need to use old method, with modifications to get more bacteria. Look for a new method which will detect all microflora present in water, not just any narrow physiological group (for example just *E coli* or coli titres), but we want to find a method to detect all bacteria.