SANITARY EXAMINATION OF THE WATER SUPPLIES OF THE VIGO COUNTY SCHOOLS

by

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CHAPTER I

INTRODUCTION

This survey is presented with the hope that it will serve two purposes. The immediate purpose is the scientific analysis and treatment of the wells of Vigo County. The second is of much greater importance in that it discusses the methods for analysis and treatment of any water supply in such a way that any person will be able to make a sanitary analysis of a water supply and administer treatment when necessary.

The reason for basing this report on school wells is apparent. If a source of water is being used by a group of people, it is not necessary to emphasize the necessity of its being pure. Nor will anyone deny that contaminated water is a source of organisms which cause much human illness. If we have pure sources of water at the schools, theoretically, we have one-third of the water problem solved for children of school age. The other two-thirds of the job would be the endless task of treating the wells at all of the homes in the community.

The State Board of Health, realizing the importance of pure water for the schools, makes specific recommendations governing the construction, equipment and maintainance of sanitary features of public school buildings. In this

list of recommendations, Section 18 recommends the following:

> Open or dug wells, or springs, shall not be used. No well shall be within 100 feet of any privy, cesspool, or other known possible source of contamination. The water supply of every school shall be analyzed by the State Board of Health from sample submitted by the school trustee, school board, or the local health officer, whenever in the opinion of the local health officer such analysis shall be necessary, or whenever the State Board of Health shall require such analysis. It is recommended that the water supply at each school shall be analyzed once each year.
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> a. Drinking Fountains--Where pressure water supply is available, sanitary drinking fountains shall have designed fittings which direct the stream of water at an angle away from the jet. The jets shall be protected against fouling by guards which prevent the lips of the user from touching the jets. Drinking fountains shall be equipped with automatic volume regulators which maintain a running stream of uniform height, despite the variations in water pressure.
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> b. The use of open water containers and the common drinking cup in schoolrooms is prohibited by order of the State Board of Health. Water must be kept in a closed container with bubbler attachment or with individual cups for use of the pupils or a combined pump and bubbler fountain shall be installed.

The Sanitary Schoolhouse Law, approved March 3, 1915, states the following:

> All schoolhouses shall be supplied with pure drinking water, and the water supply shall be from driven wells or other sources approved by the health authorities. Only smooth, stout glass or enameled metal drinking cups shall be used; water buckets and tin drinking cups shall be unlawful, and are forbidden; and whenever it is practicable flowing sanitary drinking fountains which do not require drinking cups shall be provided. All schoolhouse wells and pumps shall be supplied with troughs or drains to take away waste water, and under no conditions shall pools or sodden places or small or large mudholes be allowed to exist near a well. When water is not supplied at pumps or from water faucets or sanitary drinking fountains then covered tanks or coolers supplied with spring or self-closing faucets shall be used.

The desirability of conforming to the foregoing recommendations is unquestionable, but they are frequently disregarded because of the additional expense they entail or for other reasons. The most common mistake is in the construction of the equipment about the well. A well-curb can be easily constructed and is relatively inexpensive. Examination shows that many of these are of very poor construction and will permit the water to return to the well. If a curb is made well and a drain trough is placed over it, there is little possibility that the contaminated water can return to the supply.

CHAPTER II

BACTERIOLOGICAL EXAMINATION

The analyses which follow were made according to the methods prescribed by the American Public Health Association. The procedures were taken from the eighth edition of the book STANDARD METHODS FOR THE EXAMINATION OF WATER AND SEWAGE. This book was prepared by the American Public Health Association and is the accepted standard for any sanitary examination of water.

All of the analytical tests were made in the bacteriological and chemical laboratories of Indiana State Teachers College. The water samples were collected in flasks which had been especially prepared in the laboratory. These flasks were sterilized by heating in a 170 degree oven for at least two hours. The samples were collected and rushed to a refrigerator, where they were kept until they were to be analyzed. In no case did the elapsed time between collecting of the sample and innoculation of nutrient agar plates or lactose broth fermentation tubes exceed six hours.

Utmost care was exercised in the collecting of samples. If the samples were taken from a manual pump, the pump was operated for at least a ten-minute period before the sample was taken. Faucets and drinking fountains

were allowed to run for a similar period if the sample was to be taken from them. This insured a representive sample. Aside from this precaution, a small kit was prepared and taken into the field on each collecting trip. This kit made possible the qualitative testing for ammonia and chlorides at the well. The sample was not taken until the ammonia and chloride concentration was at a minimum. If these constituents continued to remain in high concentrations after a sufficient period of pumping, they were accepted as present in the supply and recorded in the data. The kit for testing ammonia and chlorides contained the same chemicals and apparatus that were used for testing for them in test and will be described later in this report.

The bacteriological examination was conducted in accordance with good bacteriological technique. All of the equipment was cleaned and sterilized the day previous to the day of collecting samples. Water blanks were prepared by titrating 9.6 cubic centimeters of water into test tubes. These tubes were stoppered with cotton plugs and sterilized in the autoclave for twenty minutes at fifteen pounds pressure. The tube contained 9 cubic-centimeters of sterile water after this period. Dilutions of the sample were prepared in the water blanks by placing 1 cubic-centimeter of the sample which was collected in one of these tubes to get a one-tenth dilution, 1 cubic-centimeter of the one-

tenth dilution into the next tube to get a one-hundredth dilution, and so on until a one-ten-thousandth dilution was obtained. Nutrient agar petri dishes were innoculated with the hundredth, thousandth and ten-thousandth dilutions. The nutrient agar was prepared as follows:

Bacto-Beef Extract .....3 grams

Bacto-Peptone .....8 grams

Bacto-Agar .....13 grams

The above constituents were placed in one liter of water and dissolved by heating. The solution was then placed in test tubes in about 10-cubic-centimeter quantities and stoppered with cotton. These tubes were placed in the autoclave for twenty minutes under fifteen pounds pressure.

After a forty-eight hour period of incubation, the colonies were counted on the agar plates and recorded in the data. In that three plates were made from each dilution, the figure recorded in the data is the average of the three.

The innoculation of nutrient agar plus the innoculation of lactose broth is considered a presumptive test. The nutrient agar plates give an idea as to the number of bacteria in the water, and the lactose broth detects gas-forming bacteria. The lactose broth fermentation tubes were innoculated at the same time as the nutrient agar plates. The fermentation tubes were of the Dunham type.

The lactose broth was prepared as follows:

Bacto-Beef Extract.....8 grams

Bacto-Peptone.....5 grams

Bacto-Lactose.....5 grams

The above compounds were dissolved in one liter of water by heating. The medium was then placed in the fermentation tubes in about 10-cubic-centimeter quantities, stoppered with cotton and autoclaved for twenty minutes at fifteen pounds pressure. Fermentation tubes were innoculated with ten, one and one-tenth cubic centimeter quantities of each sample. Two tubes of each quantity were prepared. The results of the fermentation tube tests were recorded in the data as no gas formed or the percentage of gas formed. When no gas was formed, the analysis was complete. Escherichia Coli, hereafter in this paper designated as E-coli, is a gas producing bacteria. The identification of E-coli is sufficient evidence to consider that a supply of water is not potable. E-coli is not necessarily pathogenic, but its presence in water shows that the water is potentially dangerous and should not be consumed. Aerobacter aeorgenes is also a gas producing bacteria, so the results of the presumptive test are not conclusive, and those samples which produced gas must be carried through to the confirmation tests.

The confirmation test is conducted by innoculation of Endo's agar plates from the fermation tubes which produced

gas. This is done by taking a small portion of the broth from this tube and placing it on the Endo's medium. Endo's agar is prepared as follows:

Bacto-Peptone.....	10 grams
Bacto-Lactose.....	10 grams
Dipotassium Phosphate.....	3.5 grams
Bacto-Agar.....	15 grams
Sodium Sulfite.....	2.5 grams
Bacto-Basic Fuchsin.....	0.4 grams

These ingredients were dissolved in one liter of water and then prepared as the nutrient agar. The normal color of basic fuchsin is red. The reaction of sodium sulfite and basic fuchsin resulted in a very light shade of pink. When colon bacillus ferments lactose, an acid results. An intermediate step in the formation of this acid is the production of acetaldehyde. In the presence of acetaldehyde, basic fuchsin is always red. After the Endo's agar plates have been incubated for a forty-eight hour period, any colonies of E-coli are readily noticeable, in that they will have a distinct red color. When red colonies were observed, the tests were carried through to the completion stage to completely identify these colonies as E-coli or aerogenes.

When it was necessary to go to the completion tests, red colonies were transplanted, by means of a wire loop, from the Endo's medium to nutrient agar slants. Nutrient

agar slants were prepared by placing the tubes of nutrient agar in a reclining position while the agar was liquid, and permitting it to solidify along the side of the tube so that it would be easily accessable from the top of the tube.

After the nutrient agar slants had been incubated for forty-eight hours, the colonies had grown so that they could be transplanted into the various mediums for the tests that followed.

The first step in the completion was Gram's stain. This was conducted with stock solutions in the laboratory by placing a colony, which had been produced on the agar slant, upon a slide. Then the slide was flodded with crystal violet stain solution for one minute. All of the bacteria were stained violet, for all bacteria behave much alike with simple basic stains. Next, the excess of the violet stain was washed off with water, and the slide was flooded with iodine solution for one minute. The iodine formed a compound with the violet which is of a deep blue-black color. After this treatment, all of the bacteria were still of the same color, now a blue-black. Again the slide was washed with water and then alcohol was applied. Alcohol will dissolve the violet-iodine color out of the Gram negative bacteria more quickly than from the Gram positive bacteria. The alcohol should be used for a period of about thirty seconds. If any Gram positive

bacteria were present, they would still be colored a shade varying from violet to blue-black, while the Gram negative bacteria would be colorless. Being colorless,they could not easily be seen, so a counterstain, red safranin was applied for ten seconds. After this, the Gram negative bacteria appeared red and the Gram positive ones appeared blue-black. Gram negative bacteria are E-coli. In the data, the results of the Gram Stain test were recorded as positive or negative.

A second step in the completion test was the brilliant green bile test. Brilliant green bile was the medium used and was prepared as follows:

Bacto-Peptone..... 10 grams

Bacto-Lactose..... 10 grams

Bacto-Oxgall..... 20 grams

Bacto-Brilliant Green..... 0.0133 grams

The above ingredients were dissolved in one liter of water, placed in test tubes, stoppered and autoclaved. Colonies were then transplanted from the agar slants to the brilliant green bile medium. These tubes were examined after twenty-four and forty-eight hours to determine any growth of the colonies. This brilliant green bile is a selective medium and inhibits the growth of organism other than E-coli and that group. In the data, results of this test were recorded as growth or no growth.

The third test of the completion was a sodium citrate test. The sodium citrate medium was prepared as follows:

Sodium ammonium phosphate..... 1.5 grams
Potassium dihydrogen phosphate..... 1 gram
Magnesium sulfate..... 0.2 grams
Sodium citrate..... 2.5 grams

The above ingredients were dissolved in one liter of water and then distributed in test tubes in five cubic centimeter quantities. The tubes were sterilized in the autoclave as were the bile tubes. This medium was innoculated by means of a wire loop. Colonies were obtained from the nutrient agar slants. After a seventy-two hour incubation period, the tubes were examined and were reported in the data as growth present or not present. In this citrate medium, aerogenes grew,but the coli colonies did not.

The last test was a methyl red test. The medium used for this test was a methyl red Voges and Proskauer medium. This medium was prepared as follows:

Buffered peptone..... 7 grams
Bacto-dextrose..... 5 grams
Pipotassium phosphate..... 5 grams

The above ingredients were dissolved in one liter of water. The solution was heated for twenty minutes while being stirred, filtered, and distributed in sterilized test tubes

in 10 cubic centimeter quantities. The tubes were sterilized in the autoclave for about thirty minutes under fifteen pounds pressure. An indicator solution was prepared by dissolving 0.1 grams of methyl red in 300 cubic centimeters of alcohol and diluting to 500 cubic centimeters with distilled water. The 10 cubic centimeter portions were innoculated from the agar slants. These tubes were incubated for forty-eight hours and then the contents of each tube was divided into two tubes, each containing a five cubic centimeter portion. The original tube was permitted to incubate an additional forty-eight hours, and the 5 cubic centimeter sample which was withdrawn was used for the Voges and Proskauer test. Five drops of the indicator were added to these tubes. Five cubic centimeters of a 10% potassium hydroxide solution were then added to the sample, and the solution was exposed to the air. These tubes were examined at two, twelve and twenty-four hour intervals. If acetyl-methyl-carbinol had been produced, an eosin-pink color appeared in the liquid, and the organism was of the aerogenes type. If the solution remained colorless, the organism was of the colon bacillus type. The development of the color in this test is due to the formation of acetyl-methyl-carbinol from dextrose by aerogenes. The methyl red tubes which were permitted to incubate seventy-two hours were then treated with five drops of the indicator solution. If the

solution changed to a pink or red, the colonies were recorded as E-coli. If no color developed, the colonies were considered to be of the aerogenes type. The test depends upon the fact that colon bacilli produce higher acidities than aerogenes types. This indicator changes color at a hydrogen ion concentration between that produced by colon bacilli and that formed by aerogenes.

Table I contains the data of the bacteriological examination.

TABLE I

DATA OF BACTERIOLOGICAL EXAMINATION PRESUMPTIVE TEST

Well No.	Bacterial Count in Dilution of			Lactose Fermentation Tubes with		
	1/100	1/1000	1/10000	.1cc	1cc	10cc
1	1	0	0	*NG	NG	NG
2	1	0	0	NG	NG	NG
3	1	0	0	NG	NG	NG
4	3	0	0	NG	NG	NG
5	Over 200	Over 200	Over 200	NG	NG	NG
6	9	3	1	NG	NG	NG
7	6	1	0	NG	NG	NG
8	6	1	0	NG	NG	NG
9	1	0	0	NG	NG	NG
10	12	7	1	NG	NG	NG
11	5	2	1	NG	NG	NG
12	8	1	0	NG	NG	NG
13	13	3	1	NG	NG	NG
14	5	1	0	NG	NG	NG
15	Approx. 100	12	2	NG	NG	NG
16	35	5	2	48%	37%	45%
17	9	3	1	25%	16%	25%
18	15	2	0	NG	NG	NG
19	12	3	0	NG	25%	70%
20	4	1	0	NG	NG	NG
21	Over 200	Over 200	Over 200	16%	50%	35%

*NG -- No gas

TABLE II

DATA OF BACTERIOLOGICAL EXAMINATION
CONFIRMATION AND COMPLETION TEST

Well No.	Endo's Medium	Gram's Stain	Brilliant Green Bile	Sodium Citrate	Methyl Red Test
16	Red	Negative	Growth	No Growth	Red
17	Red	Negative	Growth	No Growth	Red
19	Red	Negative	Growth	No Growth	Red
21	Red	Negative	Growth	No Growth	Pink

CHAPTER III

CHEMICAL EXAMINATION

The chemical analysis was for the purpose of determining ammonia, nitrite, nitrates and chlorides. The first three of these tests were based on tests appearing in the fifth edition of The Merck-Index.

The ammonia test is the Trillat-Turchet Test, which appeared in Annales de chimie analytique et XX de chimie applique et Revue de chimie analytique reunies (France) in Volume 10, 179 (1906). This test was conducted by adding 3 drops of 10% potassium iodide solution to 20 cubic centimeters of the water in question. This was mixed, and then 2 drops of concentrated sodium hypochlorite was added. A brown color developed if ammonia was present, the depth of color being proportional to the amount of ammonia. If considerable quantities of ammonia are present, a precipitate forms. The brown color is due to the formation of nitrogen triiodide. The test will detect one part of ammonia in five hundred thousand.

The nitrite test was perfected by a Frenchman, Rochaix, and was first published in Repetoire de pharmacie, 1909, 139. Before conducting the test, a reagent was prepared by dissolving 0.2 grams neutral red in one liter of water. Twenty cc. of this reagent and 1-3 cc. of 20%

sulfuric acid was added to 10 cc. of the water in question. If nitrite is present, a violet to blue color develops. This test will detect one part of nitrite per million.

To detect the presence of nitrates in the water, the Loof Test was used. To conduct this test, 5 grams of sodium salicylate is added to the water under examination. When the sodium salicylate is completely dissolved, 10 cc. of concentrated sulfuric acid is added. If nitrate is present, a yellowish to red color forms on mixing.

The determination of chlorides was based on the standard method used in every chemistry class. Silver nitrate was added to 10 cc. of the water under examination. The formation of a white precipitate which was insoluble in nitric acid denoted the presence of chlorides.

The chemical analysis was for the purpose of determining external contamination. A well which is being contaminated will contain ammonia and chlorides. If it has been contaminated in the past, nitrites will be present, but if the contamination is very old, all of the nitrogen will be oxidized to nitrates.

TABLE III

DATA OF CHEMICAL EXAMINATION

Well No.	Ammonia	Nitrite	Nitrate	Chloride
1	Trace	Trace	None	None
2	Trace	None	None	Trace
3	None	None	None	None
4	Trace	Trace	None	Trace
5	Trace	Trace	None	None
6	Trace	Trace	None	Slight Trace
7	Trace	None	None	Slight Trace
8	Slight Trace	None	None	Slight Trace
9	Slight Trace	None	None	Slight Trace
10	Present	Present	Trace	Trace
11	Trace	None	None	Trace
12	Present	Present	Trace	Trace
13	Present	Present	Trace	Trace
14	Present	Present	None	Trace
15	Present	Trace	None	Trace
16	Present	Present	None	None
17	Present	Present	Present	None
18	None	None	None	Present
19	Present	Trace	Trace	Present
20	Trace	Trace	None	Trace
21	Present	Present	None	Present

CHAPTER IV

SUMMARY

The wells tested in this report are representative of what may be found in any county in Indiana. Of the wells tested, four contained harmful bacteria and others had bacteria counts which were extremely high and should be lowered. These conditions can be remedied by the proper addition of chemicals.

The source of contamination of the wells tested was not always traceable, but in most cases it was. Most of them were polluted by faulty curbs or the lack of drain troughs to dispose of waste water. One was definitely due to a privy located within fifty feet of the well. The only hope for remedying this situation is the abandonment of the well now in use and digging or drilling another an appropriate distance from this source of contamination. This situation will account for well No. 10 being ranked with the four containing harmful bacteria.

The wells are ranked in Table IV; the best source, in the opinion of the author, is first and so on to the worst. These are compared with the average daily attendance figures recorded as percentages. It had been my intention to correlate these results, but in that there are so many variables in such a table, correlation would be of no

significance. The ranking shows some correlation but the average daily attendance of the first ten, which is 89.6% as compared to that of the last eleven, which is 85%, is more significant.

Examination of the data showed that in every case of polluted water, the tests for ammonia and nitrite were positive. They were positive in other instances also, but if a simplified method for any inexperienced person to examine a well were employed, a contaminated source could be detected by these tests. Treatment by chemicals is harmless to human beings and inexpensive to the person treating the well. In the event that a harmless source is treated, no damage will be done. For these reasons, the simplified analysis, which follows, is based on the detection of ammonia and nitrites.

TABLE IV

RANK OF WELLS AS COMPARED TO AVERAGE DAILY ATTENDANCE

Well Rank	Average Daily Attendance of School
1	97
2	90
3	90
4	95
5	88
6	87
7	87
8	84
9	87
10	91
11	81
12	90
13	83
14	86
15	83
16	83
17 (unpotable)	81-93-86-90-83

I. A SIMPLIFIED EXAMINATION OF WELL WATER

According to the school laws in this state, every source of water used by a public school should be tested at least once each year. A sanitary analysis causes considerable inconvenience and is frequently neglected. This neglect is frequently at the expense of the health of members of the student body.

A simple examination which will detect the presence of organic pollution can be conducted by you at the school.

To 20 cubic centimeters of the water add 3 drops of 10% potassium iodide solution and 2 drops of sodium hypochlorite. If a dark brown color develops, ammonia is present. To another 10-cubic-centimeter sample of the water add 20 cubic centimeters of solution which is prepared by dissolving .2 grams of neutral red in a liter of water. To this mixture add 2 cubic centimeters of 20% sulfuric acid. A violet or blue color develops if nitrite is present. If both ammonia and nitrites are present, it is advisable to treat the well by adding calcium hypochlorite. This should be added in the amount of one tablespoon per one thousand gallons of water.

Examine the well curb thoroughly to see that no water is able to seep through the curb and return to the well. If this condition exists, it is probably a source of

pollution and should be remedied.

The chemicals used in the above tests may be obtained from the drug store if they are unobtainable from the high school laboratory.

Consult the following chart to determine the gallons of water in given well.

Diameter of well in feet	Gallons per foot of Water
1	6
2	24
3	53
4	94
5	147
6	212
7	288
8	376

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