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Bacteria in Deep Wells and Surface Waters

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BACTERIA IN DEEP WELLS AND SURFACE WATERS

BY

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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BACTERIA IN DEEP WELLS AND SURFACE WATERS

Of all living things, bacteria are most widely distributed over the surface of the earth. As is the case with higher animals, we find a definite habitat for a particular species of bacteria. There are certain places where some can exist in greater numbers than can others. We sometimes find species in an environment quite different from the normal.

PART I (A)

Bacteria in Deep Wells.

Water coming from underground sources was, until recently, considered sterile. Many older text books claimed that there were no bacteria in water from underground sources. More recently, however, investigators have reported bacteria in deep well waters, until at the present time, most text books grant that a ground water may contain bacteria.

Frankland[1] reports from 6 to 26 cells per cc. in a Kent well sunk in chalk. He gives no information with regard to the methods used in securing the results.

Breunig[22] found from 6 to 30 bacteria per cc. in some artesian wells at Kiel. The medium used is not given.

Hueppe is reported to have found only four in a deep well at Wiesbaden where a special investigation was

carried on. In the Mainz wells Egger found four colonies.

Savage[2] claims that deep well water having been filtered through layers of earth should contain few bacteria and should be subject to very little variation. On gelatin the number of bacteria is usually less than 50 per cc. and on agar less than 1 per cc.

Prescott and Winslow[3] quote analyses of deep wells and springs in the neighborhood of Boston in which the number of bacteria varied from 0 to 12. They reach the conclusion that water absolutely free from bacteria is not ordinarily secured from any source.

Thresh[4] gives many instances of bacteria in ground waters and especially of the intestinal flora coming under his own observation. Some of these will be mentioned later.

The results in the following table indicate the number of bacteria found in some Illinois deep wells:

Sample No.	Depth (ft.)	Agar	Gelatin
27452	282	9	55
27474	400	0	0
27475	160	1	1
27476	160	28	30
27477	120	--	6
27545	211	4	10
27557	895	0	20
27568	113	4	3
27582	2000	1	9
27611	126	0	2
27612	126	0	6
27672	270	4	10

EXPERIMENTAL

It was thot advisable to investigate the character of the colonies found on some of the plates of water from underground sources. These wells are located at different points in the state of Illinois and all are strictly speaking deep wells. Only those samples were considered which were known to be from wells which had been pumped for some time. Very often it was found that a badly contaminated deep well water came from a new well which had been thoroly flushed out.

The work here presented was done on the samples as they came to the State Water Survey for the routine analysis.

Method of Procedure. The water was plated on agar and incubated at 37°C and on gelatin at 20°C. After these plates had been counted, colonies were picked off by means of a sterile platinum wire and transferred to agar slants. These cultures were later plated, in dilution, on gelatin in order to secure a pure culture. The cultural characteristics were then determined by means of the different standard media and the results recorded on the charts of the Society of American Bacteriologists.

Sample A.

A well 1382' deep located at Odell, Illinois, furnished this water. It was comparatively new, but had been pumped for some time before the sample was sent in. The well is cased with iron pipe and the cover is water tight. The water is securec from the St. Peter sandstone.

The number of bacteria on agar was 26 and on gelatin, 1000. The high number of bacteria on gelatin may have been due to the fact that the casing had not been thoroly flushed out when the sample was taken. The two following bacteria were taken from the gelatin plates and subjected to the different kinds of media mentioned below. The colonies were of the same general shape varying only in size. All grew on the surface and were colored slightly brown at their centers. The gelatin plates began to liquefy in less than forty-eight hours.

I. Bacillus subtilus.

Diameter: Less than 1 micron.

Spores: Formed toward middle of the rod.

Motility: Motile when taken from fresh broth medium.

Gram: Positive.

Broth: Turbid with pellicle.

Gelatin plate: Gelatin is liquefied in about 40 hours.
Thru the liquid granules occur.

Potato: Rich wrinkled growth.

Milk: Coagulated.

Indol: Negative.

Nitrates: Not reduced.

Gas: Negative.

2. Bacillus Flourescens liquefaciens.

Diameter: Less than 1 micron.

Spores: No spores could be found.

Motility: Positive.

Gram: Negative.

Broth: Sediment-broth assumes green color.

Agar: Luxuriant growth - agar is turned green.

Gelatin plates: Gelatin rapidly liquefied. Greenish color.

Potato: Scant dark colored growth.

Milk: Coagulated and casein is digested.

Indol: Slightly positive.

Nitrates: Ammonia is produced.

Gas: No gas is formed.

Conclusions. Neither of these forms are of any sanitary significance. Bacillus subtilis is a form abundant on grass. Bacillus floresceus liquefacieus is a common water form.

Sample B

The source of this water was a 90' drilled well. The casing is sunk thru rock and clay and the water is pumped from the rock by an iron pump. Since the cover is of cement and watertight, no surface water can get into the well. The number of bacteria on agar was 7 and on gelatin, 67. The chemical analysis showed a normal water for such a source. The tests for Bacillus colon were negative. The colonies on gelatin were evenly distributed and had begun to liquefy the gelatin in less than 43 hours.

I. Bacillus Vulgatus.

Diameter: Less than 1 micron.

Spores: Negative.

Motility: Positive.

Gram: Positive with pellicle.

Broth: Turbid with pellicle.

Agar: White.

Gelatin plates: Round liquefyers.

Potato: Scanty growth.

Milk: Coagulated. Partial digestion of casein.

Indol: Positive.

Nitrates:

Gas: Negative.

Conclusions. Bacillus vulgatus has no sanitary significance. It is a rather common form.

Sample C

The source of this sample was a 2500' well. The casing was put down thru rock and sandstone and had been pumped for a long time. The count on agar was 30 and on gelatin, 6. Large liquefying colonies of a flourescent green were present as in many of the deep well waters. The colony picked off had the following characteristic.

I. B. arborescens.

Diameter: Slender motile rod.

Spores: Negative.

Motility: Positive.

Gram: Negative.

Broth:

Agar: Yellowish.

Gelatin plates: Rapid liquefyer - Dark opaque colonies with hairy projections.

Potato: Orange colored growth.

Milk: No change.

Indol: Negative.

Nitrates: Reduced.

Gas: No gas.

Conclusions. Bacillus arborescens is found in soil. This might explain its presence in a water.

Sample D

A well drilled in drift 1135' furnished this water.

The well is cased with iron pipe and has a water tight cement cover. No feed lot, privy or stable is near the well. The gelatin plates were covered with liquifiers which made large saucer-like depressions in the medium. From this sample of water the following organism was isolated.

I. Bacillus mycoides.

Diameter: Large bacillus.

Spores: Positive.

Motility: Positive.

Gram: Positive.

Broth: Cloudy with pellicle.

Agar: White growth irregular edge, after spreading.

Gelatine plates: White with many branches.

Potato: White. No discoloration.

Indol: Negative.

Nitrates: Reduced to nitrites and ammonia.

Gas: Negative.

Conclusions. Bacillus mycoides is a common species of bacteria and might easily get into a water.

In all the above cases, it is realized that these different possibilities allowing bacteria to get into a ground water. The well might be a new one which was not thoroly flushed out when the sample was taken. Or, it might have a bad casing allowing surface water to enter.

Since bacteria have been reported in deep wells

from different parts of the world, it seems probable that certain forms do live in deep well waters.

PART I. (B)

Bacillus Colon in Ground Waters.

The presence of Bacillus colon in a water has been accepted by most sanitarians as a sufficient indication that the water has, in some way, received sewage pollution.

Bacillus colon was discovered in 1885 by Emmerich while working on the feces from cholera patients. Since it was found to be present in such large numbers, in that part of the intestine termed the colon, it was given the name Colon Bacillus. When it was first discovered, it was thot to be an inhabitant only of the human intestine. This theory, however, was very soon to be disproved. Flint[5] worked on the feces from the animals in the Chicago Zoological Garden. He found Bacillus colon in the excreta from the snake, llama, white rat, bear and a few others. He concluded that Bacillus colon was not a sufficient basis on which to condemn a water. Belitzer[6] and Dyer and Keith[7a] obtained results to the same effect that Bacillus colon was not only present in the human intestine, but rather widely distributed thru the intestines of most warm blooded animals. Much other work has been reported by various men on Bacillus colon and its ubiquity in warm blooded animals.

Numerous instances are cited of its occurrence in the cold blooded animals. Amyot[22] tried to prove the pres-

ence of Bacillus colon in fish. He could not find it in twenty-three fish, including fourteen varieties. Johnson[24] examined sixty different fishes from the Illinois and Mississippi rivers. He succeeded in isolating Bacillus colon from forty-seven. In forty-one of these the organism was isolated from the intestine. He cites the carrying of Bacillus colon by fish as a method by which a pure water could be apparently polluted. As mentioned before, Flint proved it in the snake. Moore and Wright[25] could not find it in the frog. Eyre[26] reports its presence in the fish and also in some warm blooded animals.

Prescott[76] found an organism similar in all characteristics to Bacillus colon on grains from fields where animal contact was improbable. Even in this case will arise the possibility of birds distributing Bacilli Coli over such areas. One of Prescott's conclusions was that care should be used in interpreting an analysis of a water where Bacillus colon was found to be present.

Metcalf[8] reports Bacillus colon on some South Carolina rice fields.

Smith[9] found colon like bacteria on a field of rye in western Massachusetts.

Since colon organisms have been found in so many different places, even where animal contact was improbable, the question can be raised whether its presence can be taken as an accurate indication of pollution. If it has as wide

spread distribution as it seems to have, it is easy to imagine how it might gain access to a safe potable water. Prescott has indirectly proposed in one of his publications that this organism might originally have been a plant form, and finding the intestine of animals such a favorable abode, had taken up its habitation there.

In 1894 Kruse[10] in a paper in which no experimental date is given, advised against the use of this organism, as an indication of pollution. He said that we were dealing with a group of bacteria and not a single organism. Since it was found in the air, water, and earth, he believed it could not be taken as a sufficient indication of pollution.

Beckman[11] found Bacillus colon in the city supply of Strassburg. This water is taken from deep wells. In his work, he used large quantities.

Maroni[12] after examining some deep and shallow wells about Parma concluded that B. colon had no sanitary significance.

Weissenfeld[13] like Kruse stated Bacillus colon could be found in all waters if large enough quantities were taken. During his work, he studied about thirty samples of a supposedly good water.

On the other hand, we have those who contend that no good water should contain Bacilli coli. Chick[14] has this view. Savage[2] states that sufficient evidence has not yet been produced to discard Bacillus colon as an indication of

pollution. The same statement has been made by Moore.

BACILLUS COLON IN DEEP WELLS

That we should find any indication of pollution in a ground water is queer, but since bacteria have such a wide distribution, it is possible. Many instances are given of contamination of under ground waters.

Horton[15] in examining deep well and spring waters in Ohio often met with organisms resembling Bacillus colon. All shallow wells were excluded and only those wells which were cased, were considered. Bacillus colon was found twice at an interval of a month. The chemical data showed no pollution. Horton concluded that (1) Bacillus colon in ground water should condemn them and (2) the fact that water may come from underground sources should not be a guarantee of its purity.

Nankivell[16] points out that water from wells in chalk are liable to intermittent pollution and should be purified. Microorganisms may get into the water thru fissures and swallow holes from many miles distant, to infect an apparently pure water supply.

Thresh, in his book on water supplies, quotes many instances of finding Bacillus colon in deep well waters. He says, "There are few if any waters in which Bacillus colon cannot be found if a sufficient quantity is taken."

The following work was done on a series of nineteen tubular wells, constituting the supply for a city of about

eleven thousand inhabitants. The positive test for gas formation varied in the water, but were usually present in 1 cc. samples. These wells vary from 80' to 125' in depth. They are all connected to the same pump suction so that it is impossible to secure a sample of water from any one well to see which one is furnishing the gas formers. Water is taken from the gravel deposits of a nearby river.

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The following table shows both the chemical and bacterial analyses of the water from June 25, 1906 - May, 1914. It shows a variation in quality which may be due to the intermittent entrance of another kind of water. The chlorine which is a valuable constituent by which to judge the quality of a water varies between 61 and 35 p.p.m. Similar variations can be seen in the residue, oxygen consumed and nitrogen content. The number of bacteria is not excessive, but the almost constant presence of gas formers is a bad indication.

These wells are located near a large river carrying a very highly polluted water. The drainage from the surrounding country is towards the river, since the land slopes gradually away from it. No observations had ever been made to determine the direction in which the ground water moved. The soil is of sand and gravel. There is no impervious stratum to protect the ground water from the surface water which might get in.

Since the soil is so sandy, we may assume that

there is more or less in filtration of surface water. We can hardly assume that the same standard of purity would be secured here as would be the case with a sand filter. There would not be a proper rate of filtration nor a satisfactory arrangement of sand and gravel to secure the highest efficiency. Old wells, fissures and cess pools might allow polluted water to get into the water bearing stratum with insufficient purification.

There is a possibility of some underground connections with the badly polluted river. The following table has been prepared to show the relation of the constituents of the river water, the city water and water from two wells and of about the same depth, located within a half a block of the city wells.

	River	City Wells	Private Well No.1	Private Well No.2
Laboratory No.	27414	27416	27261	27262
Date	4/20/'14	4/20/'14	3/28/'14	3/28/'14
Turbidity	50	0	2	0
Color	30	0	0	0
Odor	2 c	1 v	1 e	1 e
Residue	332	498	357	617
Chlorine	13.6	42.	10	14
Oxygen Consumed	7.3	..5	1.6	1.5
Nitrogen as Free Ammonia	3.520	.024	.000	.000
Alb. Ammonia	3.200	.000	.000	.000
Nitrates	2.64	.000	.004	.000
Nitrites	.030	7.20	7.20	13.20
Alkalinity	154.0	274.	246.	264.
Bacteria per cc. Agar	16,000	50	0	8
Gelatin	35,000	80	1	1
Gas Formation 10 cc.		1 +	1 -	1 -
1 cc.	1 +	2 -	2 -	2 -
0.1 cc.	2 +	2 -	2 -	2 -
.01 cc.	2 +			

ANALYSES OF THE MUNICIPAL WATER SUPPLY AT PEKIN

Tap at pumping station.

Laboratory No.	Date Recd.	Appearance: Turbidity.	Appearance: Color.	Appearance: Odor.	Residue on Evaporation.	Chlorine in Chlorides.	Oxygen Consumed.	Nitrogen as Ammonia: Free	Nitrogen as Ammonia: Albuminoid.	Nitrogen as: Nitrites.	Nitrogen as: Nitrates.	Alkalinity.	Bacteria per cc.: Gelatine.	Bacteria per cc.: Agar.	Colon Bacillus.: 10 cc.	Colon Bacillus.: 1.0 cc.	Colon Bacillus.: 0.1 cc.	Indol.
	1906																	
14548	Jun 25	0	0	0	418	47.5	2.	.046	.092	.000	1.36	262.	30	--	1-	2-	2-	--
14814	Aug 20	0	0	0	546	47.5	1.95	.028	.052	.003	5.2	259.9	--	--	--	--	--	--
	1911																	
22746	Dec 5	2	0	1v	485	38.	.4	.004	.034	.000	7.6	270.	50	--	1+	1+1-	2-	+
22807	Dec 19	0	0	0	469	35.	.4	.000	.000	.000	24.0	272.	92	--	1+	1?1-	1+1-	+
	1912																	
22868	Jan 16	0	0	2v	584	56.	1.0	.000	.058	.000	10.	278.	--	--	1+	2+	2+	--
22869	Jan 16	0	0	0	534	61.	.4	.000	.048	.000	10.8	278.	--	--	1+	2+	2+	-
22888	Jan 24	--	--	--	--	--	--	--	--	--	--	--	73	--	1+	2+	2-	-
22889	Jan 24	--	--	--	--	--	--	--	--	--	--	--	55	--	1?	2-	2-	-
22890	Jan 24	--	--	--	--	--	--	--	--	--	--	--	82	---	1+	1-1+	2-	-
22984	Feb 13	0	0	0	480	41.	.5	.012	.016	.000	8.0	272.	36	--	1+	2+	2-	-
23227	Apr 16	0	0	1E	595	40.	.3	.000	.000	.000	10.0	288.	58	--	1+	2+	2-	+
23355	May 13	0	0	1	487	43.	.8	.000	.000	.000	1.04	280.	31	--	1+	2+	2-	+
23463	Jun 10	0	2	1M	537	35.	.1	.000	.032	.000	12.00	274.	155	--	1+	2+	1-1+	+
23961	Sep 16	0	0	0	530	48.	1.6	.000	.004	.000	6.80	270.	--	90	1+	1-1+	2-	-
24146	Sep 21	0	0	0	483	53.	1.4	.040	.016	.000	7.20	352.	32	1	1+	2-	2-	-
24498	Dec 17	0	0	0	472	33.	1.4	.000	.032	.000	7.00	270.	22	1	1+	2-	2-	-
	1913																	
24674	Jan 29	2	0	0	520	43.	.5	.000	.024	.007	9.00	272.	52	0	1+	1+1-	2-	-
24798	Feb 24	0	0	0	507	27.	.2	.008	.056	.000	7.60	270.	5	15	1+	2-	2-	-
24891	Mar 18	0	0	0	497	46.	1.2	.016	.032	.000	7.20	410.	2	3	1-	2-	2-	-

ANALYSES OF THE MUNICIPAL WATER SUPPLY AT PEKIN (CONTINUED)

Tap at pumping station.

Nitrogen as

Laboratory No.	Date Recd.	Appearance			Residue on Evaporation.	Chlorine in Chlorides.	Oxygen Consumed.	Ammonia		Nitrites.	Nitrates.	Alkalinity.	Bacteria per cc.		Colon Bacillus.			Indol.
		Turbidity.	Color.	Odor.				Free.	Albumin-oid.				Gelatin.	Agar.	10 cc.	1.0 cc.	0.1 cc.	
25043	Apr 15	1	0	0	532	35.	.7	.000	.014	.003	6.00	262.	4	5	1+	1-1+	2-	-
25282	May 19	0	0	0	580	38.	.6	.000	.048	.003	7.20	276.	19	4	1+	2-	2-	-
25454	Jun 17	0	4	0	528	43.	.7	.032	.016	.000	8.00	272.	7	0	1+	1+1-	1-1+	+
25625	Jul 14	0	3	0	551	31.	1.1	.024	.064	.000	9.2	268.	35	210	1+	1-1+	2-	+
26016	Sep 2	1	3	0	526	36.	1.5	.004	.024	.000	12.00	262.	30	59	1+	1-1+	2-	+
26127	Sep 23	0	0	0	518	34.	1.5	.028	.052	.000	8.80	268.	76	30	1-	2-	2-	+
26324	Oct 21	0	0	0	505	38.	.7	.000	.014	.005	9.20	264.	103	9	1+	1+1-	2-	+
26498	Nov 18	0	0	0	517	38.	.9	.000	.024	.000	10.00	260.	37	58	1-	1+1-	2-	-
26747	Dec 29 1914	0	0	1v	482	39.	.4	.000	.038	.002	.12	260.	9	10	1+	2+	2+	+
26900	Jan 19	0	0	0	502	42.	.8	.000	.000	.000	7.20	266.	25	14	1-	1+1-	2-	+
27081	Feb 17	0	0	0	498	43.	.8	.000	.000	.000	6.80	268.	20	0	1+	2-	2-	-
27210	Mar 17	0	0	0	496	42.	1.0	.000	.024	.000	9.60	270.	140	9	1-	2-	2-	0
27591	May 18	0	0	0	515	40.	1.0	.000	.028	.000	.28	276.	3	0	1-	2-	2-	-

The analytical results show a decided difference between the river water and that taken from the wells. Pollution from this source is improbable, altho a small amount of river water might account for the variation in the city well water.

Gas forming bacteria have been present in 10 cc. samples of the water in over 90 per cent. of the analyses made. The 1 cc. samples are positive in nearly 50 per cent. of the samples analyzed. In the 0.1 cc. samples a much better showing is made with only 9 positive tests from 64. At six different times, gas formers were isolated and found to have characteristics agreeing with Bacillus colon. Very rarely were there any liquefying bacteria present, but flourescent colonies quite often appeared. Some of these proved to Bacillus flourescens liquifacieus and Bacillus flourescens non-liquefacieus, as indicated below.

ISOLATION OF BACTERIA

The methods of isolation were those commonly used and recommended by the Society of American Bacteriologists. The samples of water sent to the State Water Survey are packed in ice. Some of the samples which were studied were taken by representatives of the State Water Survey, by which means, we hoped to eliminate the danger of contamination by having inexperienced men take the samples. When the sample was brought to the laboratory it was plated on litmus lactose agar, plain agar and plain gelatin. The red colonies devel-

oping on litmus lactose agar were picked off and purified by the usual methods.

Confirmatory tests were made according to the chart of the Society of American Bacteriologists. In addition, Endo's medium and Russell's medium was used. Both of these special media gave reactions characteristic of Bacillus colon.

The following characterisitics were assumed to be typical of Bacillus colon:

Shape: Bacillus 2 - 3 microns X .5 micron.

Motility: Motile with flagella.

Spore: Negative.

Gram: Positive.

Gelatin colonies: Small, thin colonies. No liquefaction.

Gelatin stab: Thin growth more vigorous at the surface.

Agar stab: Very scanty growth.

Agar stant: Thick growth.

Litmus milk: Acid and coagulation after a few days.

Indol: Present after 3 days at 37°C.

Potato: Brown growth.

Nitrate: Reduced.

Lactose: Fermented giving gas and acid.

Dextrose: Fermented giving gas and acid.

Laccharose: Fermented giving usually gas and acid.

The chart giving the characters of the gas former isolated from the deep wells, is attached. Characters were not always constant. The motility varied somewhat, but gas formation was constant. The organism from the deep wells

has characters identical with those of Bacillus colon. In this case the bacteriological analysis is more delicate than the sanitary chemical.

Attempt to Trace Source of Pollution.

Realizing that slight contamination was possible from these wells, salt was used as a means of trying to determine the source of pollution.

Different methods have been used to trace pollution of underground waters; chief among these have been salt, lithium salts, flourescein and bacterial suspensions such as Bacillus prodigiosus.

Salt has been used in many instances for such purposes. MacCollie[17] used this method to demonstrate to the citizens of Georgia the results which would be obtained, were a deep well used to dispose of sewage. It was found that a 124' drilled well would carry away an unlimited supply of water and it was proposed to use this as a method of disposing of the sewage. A large amount of salt was put down the well and the chlorine content of the surrounding wells watched. The chlorine increased in the wells and springs in the vicinity, showing that there was underground connection with each well and spring. This demonstrated what would have resulted, had the well been used to carry away the sewage.

Dole[18] in a paper on the use of flourescein in tracing water courses, comes to the following conclusions:

(1) In studying the sanitary character of a well, it is more valuable to study the underground flow than to analyze the water itself.

(2) Foreign substances put into an aquifer and traced from point to point, are of great value in this study.

(3) With the flourescope 1 pt. of flourescein in 10 billion pts. of water can be detected.

(4) Flourescein is a particularly valuable flow indicator for fissured and cavernized rocks.

(5) It progresses at a slightly slower rate than the water in which it is suspended.

(6) It is not decolorized by passage thru sand, gravel manure; it is slightly decomposed by calcareous soils.

(8) It is entirely decolorized by peaty formations and by free acids except by carbonic acid. These conclusions give the limitations of this chemical in tracing ground waters.

M. Trillat[19] has used many colored substances to trace motion of underground waters and claims that flourescein can be detected in dilutions of $\frac{1}{2,000,000,000}$. He claims that before this dye is used in this capacity, a study of the soils should be made to determine the presence of any matters which might decompose the dye.

Marboutin, F[20] gives an account of this dye when used to trace underground waters and comes to about the same conclusions that others have.

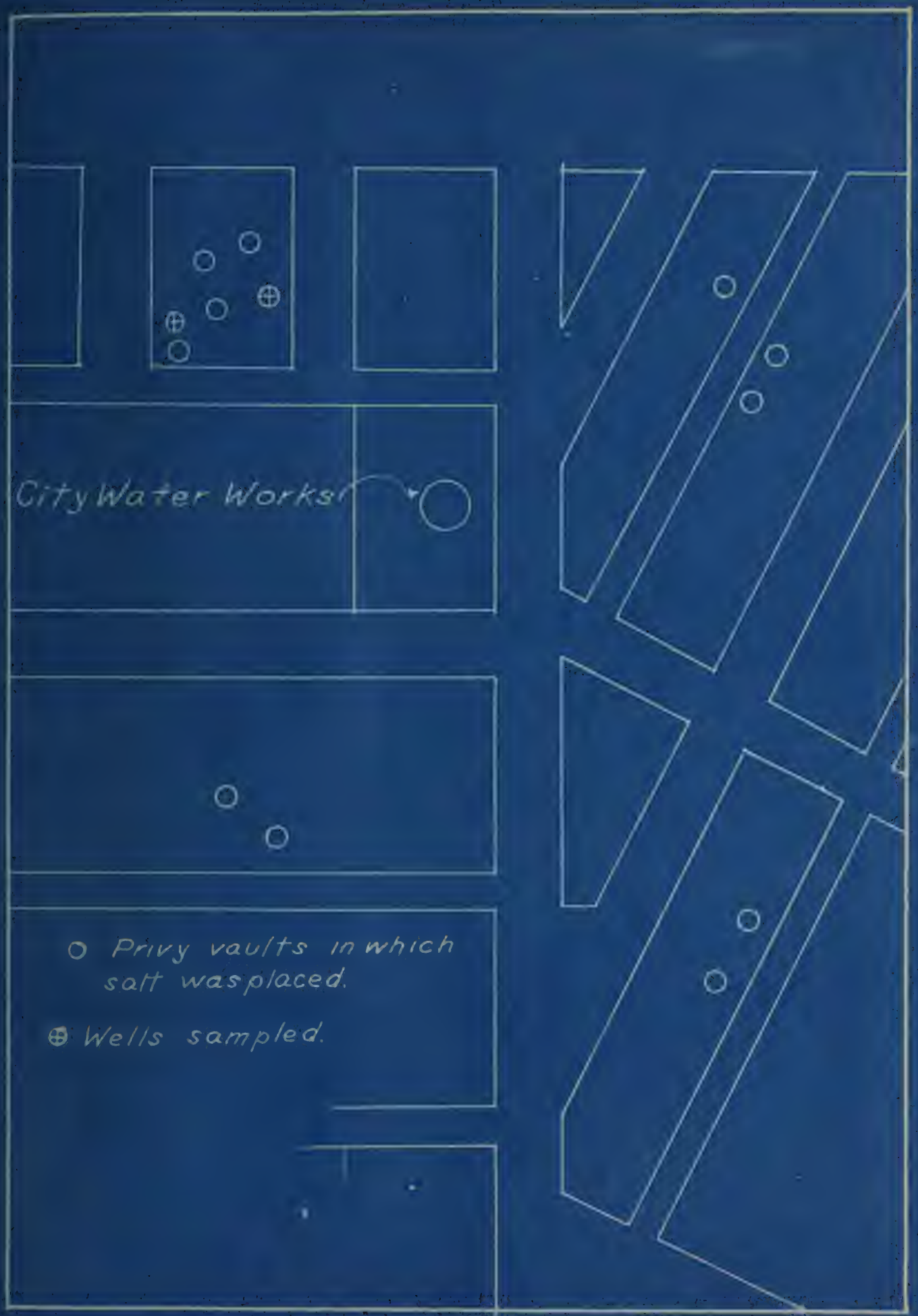
Martel[21] shows that this dye even in very concen-

trated solutions decolorizes rather quickly when kept in the sunlight. When it is kept in complete darkness, which would be the case in the earth, it did not change even after long periods of time.

Gehrmann[22] reports some work of Alba Orlandi and Roudelli, who used a suspension of Bacillus prodigrosus. They found that this organism found its way thru soil two hundred meters, when poured on the ground. In the same paper is quoted the work of Pfulil, who found that it took the same organism a short time to pass thru twenty-four feet of gravel. Gehrmann also reports an instance, coming under his own observation, where wells two to three hundred feet deep located too near an old canal were subject to entrance of contaminated water. No experimental data is given in this ppper.

In the wells on which this work was done, it was thot best not to use flouresoein on account of the possibility of coloring the water too much. Since these wells furnish the only supply for a city of9897 inhabitants, and since it is difficult to remove this chemical effectively under ordinary conditions, salt was used in an attempt to see if there was any seepage from the surface.

One ton of fine salt was evenly divided between eleven privy vaults. This was placed in them on the same afternoon, the chlorine content of the water having been previously determined. The plot showing the location of these privys with respect to the water works is given.


City Water Works
○ Privy vaults in which salt was placed.
⊕ Wells sampled.

When this experiment was started the chlorine content was 42 pts. per mil. From the table it will be seen that this changed scarcely at all.

Date	From	To	CC	P.P.M.	Remarks.
March 16	22.5	26.7	4.2	42	No salt.
" 17	22.5	26.9	4.4	44	Salt added.
" 17	21.	25.3	4.3	43	
" 18	21.6	26.	4.4	44	
" 18	22.	26.5	4.5	45	
" 19	21.	25.3	4.3	43	
" 19	21.7	26.1	4.4	44	10 gals. water added to all vaults.
" 20	21.6	26.1	4.5	45	
" 20	21.8	26.2	4.4	44	
" 21	22.8	27.2	4.4	44	
" 21	21.5	26.	4.5	45	
" 23	18.5	22.8	4.3	43	
" 23	20.5	24.8	4.3	43	
" 24	19.	23.4	4.4	44	
" 24	19.	23.3	4.3	43	
" 25	22.	26.2	4.2	42	
" 25	22.5	26.8	4.3	43	
" 26	20.5	24.7	4.2	42	
" 26	20.	24.2	4.2	42	
" 27	21.	25.2	4.2	42	Heavy rain night of 3/26.
" 27	24.5	28.8	4.3	43	
" 28	21.5	25.8	4.3	43	
" 28	20.5	24.7	4.2	42	
" 30	21.5	25.8	4.3	43	Heavy rain night of 3/29
" 30	21.	25.2	4.2	42	
" 31	20.5	24.7	4.2	42	
" 31	20.5	24.7	4.2	42	
April 1	20.5	24.7	4.2	42	Heavy rain night of 3/31
" 2	21.	25.2	4.2	42	
" 3	21.	25.2	4.2	42	
" 4	22.5	26.8	4.3	43	
" 6	21.5	25.8	4.3	43	All day rain from 6:00 A.M.
" 7	22.	26.2	4.2	42	
" 8	22.	26.2	4.2	42	
" 9	21.5	25.7	4.2	42	
" 10	23.	27.2	4.2	42	
" 11	22.	26.3	4.3	43	
" 13	23.	27.2	4.2	42	
" 14	22.	26.2	4.2	42	
" 15	20.	24.2	4.2	42	
" 16	20.5	24.6	4.1	41	
" 17	22.5	26.6	4.1	41	
" 18	22.5	26.6	4.1	41	Pumped for fire 2 hrs.
" 20	22.5	26.5	4.	40	sample taken 45 min.
" 23	22.	26.1	4.1	41	after fire out.
" 25	20	24.1	4.1	41	

Since there was no increase in the chlorine content, it is quite apparent that there is no direct connection with pollution from surface sources.

PART II.

Surface Waters.

At one of the large filtration plants on the Mississippi river two kinds of bacteria have been troublesome.

Brief Description of Filter Plant. The plant is of the rapid sand type furnishing about 4,000,000 gallons of water per day. The water is taken from the Mississippi river and has a normal chemical content for that water. The raw water is coagulated with alum, filtered and disinfected with calcium hypochlorite.

On the plates made from the sterilized water, large liquifiers were quite numerous. On the raw water these colonies were also present, but since a higher dilution was used, they were not so numerous.

Plates of the treated water have at times contained thousands of small colonies. These have made their appearance periodically. They are described later by chart.

A few of the monthly analyses and the average reduction in bacteria are shown in the following table:

DATE	R A W		STERILIZED		GENERAL EFFLUENT	
1913	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin
Jan.	770	2600	10	85	6	116
Feb.	740	2200	47	60	12	340
March 3	70	5500	20	7000	5	17
March 23	680	82000	162	1240	6	7
April 11	530	6500	90	155	0	0
May 3	340	2600	43	760	6	440
May 12	1700	3800	35	36	3	25
July 1	233	50000	800	7400	9700	3000
July 14	1200	1200	33	65	34	89
Aug.	1500	6900	250	220	97	96
Sept.	1250	3000	114	300	210	60
Oct.	310	7600	76	260	45	80
Dec. 3	1600	5100	325	550	70	63
Dec. 30	86	430	36	35	1	2
1914 Jan.	320	3600	43	52	4	10
Feb.	130	640	55	110	2	2

These analyses show the effect of treatment on the bacteria content. The presence of large liquefiers often made counting difficult. This was often the case before the plates had been incubated fifteen hours. After isolating them from several different samples, they were found to be Bacillus flourescens liquefacieus. This organism is present in most waters, both those on the surface and those from the ground.

It was noticed here as in other cases that where liquefiers were present on gelation that the agar colonies would spread making accurate counting difficult.

Just how these bacteria get into the treated water is difficult to say. Apparently, all are not killed by hypochlorite. They may develop in some way in a part of the plant after the water has been filtered.

The following table gives results which were obtained by treating the strain with hypochlorite. The counts were made from agar incubated at 37°C.

Available Chlorine (pts.per mil)	Untreated	Treated
.0	121	118
.1	170	170
.2	98	105
.4	120	116
.6	230	225
.8	60	75
1.0	200	190
1.2	175	100
1.4	150	130

The results show that this strain is not affected by calcium hypochlorite. Filtration does not entirely remove them for they are found in the sterilized water and in the general effluent. They have no sanitary significance and so can be looked on merely as peculiarities of this water.

On the same water there developes at times thousands of minute colonies. These grow slowly at 20°C on gelatin. When these appeared on the gelatin plates colonies were taken off and put on agar slants. After the culture was purified, the characteristics were determined on the standard media. The attached chart shows them. This organism is not a gas form and so is not a member of the colon group. According to Migula's classification it has the characteristics of Micrococcus candicans.

Source Pekin Well Water Date of Isolation ..... Name Bacillus Coli. Group No. (1) .....

DETAILED FEATURES.

NOTE—Underscore required terms. Observe notes and glossary of terms on opposite side of card.

I. MORPHOLOGY (2)

1. Vegetative Cells, Medium used. Agar.... temp. 37°C..., age. 24 hrs. ... days
Form, *round, short rods, long rods, short chains, long chains, filaments, commas, short spirals, long spirals, clostridium, cuneate, clavate, curved.*
Limits of Size. 1μ X 3μ
Size of Majority.....
Ends, *rounded, truncate, concave.*

Agar Hanging-Block: Orientation (grouping)..... Chains (No. of elements)..... *Short chains, long chains* Orientation of Chains, *parallel, irregular.*

2. Sporangia, medium used.....temp.....
age.....days
Form, *elliptical, short rods, spindled, clavate, drumsticks.*
Limits of Size..... Size of Majority.....

Agar Hanging-Block: Orientation (grouping)..... Chains (No. of elements)..... Orientation of Chains, *parallel, irregular.*

Location of Endospores, *central, polar.*

3. Endospores.
Form, *round, elliptical, elongated.*
Limits of Size.....
Size of Majority.....
Wall, *thick, thin.*
Sporangium wall, *adherent, not adherent.*
Germination, *equatorial, oblique, polar, bipolar, by stretching.*

4. Flagella No.....Attachment *polar, bipolar, peritrichiate.* How Stained.....

5. Capsules, present on.....
6. Zoogloea, Pseudozoogloea.

7. Involution Forms, on.....in...days at...°C.
8. Staining Reactions.
1:10 watery fuchsin, gentian violet, carbol fuchsin, Loeffler's alkaline methylene blue.
Special Stains
Gram..... Glycogen .....
Fat..... Acid fast .....
Neisser.....

II. CULTURAL FEATURES (3)

1. Agar Stroke.
Growth, *invisible, scanty, moderate, abundant.*
Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
Elevation of growth, *flat, effuse, raised, convex.*
Lustre, *glistening, dull, cretaceous.*
Topography, *smooth, contoured, rugose, verrucose.*
Optical Characters, *opaque, translucent, opalescent, iridescent.*
Chromogenesis (8)
Odor, *absent, decided, resembling.....*
Consistency, *slimy, butyrous, viscid, membranous, coriaceous brittle.*
Medium *grayed, browned, reddened, blued, greened.*

2. Potato.
Growth, *scanty, moderate, abundant, transient, persistent.*
Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
Elevation of growth, *flat, effuse, raised convex.*
Lustre, *glistening, dull, cretaceous.*
Topography, *smooth, contoured, rugose, verrucose,*
Chromogenesis (8)..... Pigment in water *insoluble, soluble;* other solvents.....
Odor, *absent, decided, resembling.....*
Consistency, *slimy, butyrous, viscid, membranous, coriaceous, brittle.*
Medium *grayed, browned, reddened, blued, greened.*

3. Loeffler's Blood Serum.
Stroke *invisible, scanty, moderate, abundant.*
Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
Elevation of growth, *flat, effuse, raised, convex.*
Lustre, *glistening, dull, cretaceous.*
Topography, *smooth, contoured, rugose, verrucose.*
Chromogenesis (8).....
Medium *grayed, browned, reddened, blued, greened.*
Liquefaction begins in.....d, complete in.....d.

4. Agar Stab.
Growth *uniform, best at top, best at bottom; surface growth scanty, abundant; restricted, widespread.*
Line of puncture *filiform, beaded, papillate, villous, plumose, arborescent: liquefaction.*

5. Gelatin Stab.
Growth *uniform, best at top, best at bottom.*
Line of puncture, *filiform, beaded, papillate, villous, plumose, arborescent.*
Liquefaction, *crateriform, napiform, infundibuliform, saccate, stratiform;* begins in.....d, complete in.....d.
Medium *fluorescent, browned.....*

6. Nutrient Broth.
Surface growth, *ring, pellicle, flocculent, membranous, none.*
Clouding *slight, moderate, strong; transient, persistent; none; fluid turbid.*
Odor, *absent, decided, resembling.....*
Sediment, *compact, flocculent, granular, flaky, viscid on agitation, abundant, scant.*

7. Milk.
Clearing without coagulation.
Coagulation *prompt, delayed, absent.*
Extrusion of whey begins in.....days.
Coagulum *slowly peptonized, rapidly peptonized.*
Peptonization begins on.....d, complete on.....d
Reaction, 1d..., 2d. 2.3 4d...., 10d....., 20d....
Consistency, *slimy, viscid, unchanged.*
Medium *browned, reddened, blued, greened.*
Lab ferment, *present, absent.*

8. Litmus Milk.
Acid, alkaline, acid then alkaline, no change.
Prompt reduction, no reduction, partial slow reduction.

9. Gelatin Colonies.
Growth, *slow, rapid.*
Form, *punctiform, round, irregular, ameboid, mycelioid, filamentous, rhizoid.*
Elevation, *flat, effuse, raised, convex, pulvinate, crateriform (liquefying).*
Edge, *entire, undulate, lobate, erose, lacerate, fimbriate, filamentous, floccose, curled.*
Liquefaction, *cup, saucer, spreading.*

10. Agar Colonies. 37°C.
Growth, *slow, rapid, temperature.....*
Form, *punctiform, round, irregular, ameboid, mycelioid, filamentous, rhizoid.*
Surface *smooth, rough, concentrically ringed, radiate, striate.*
Elevation, *flat, effuse, raised, convex, pulvinate, umbonate.*
Edge, *entire, undulate, lobate, erose, lacerate, fimbriate, floccose, curled.*
Internal structure, *amorphous, finely-, coarsely-granular, grumose, filamentous, floccose, curled.*

11. Starch Jelly.
Growth, *scanty, copious.*
Diastasic action, *absent, feeble, profound.*
Medium stained.....

12. Silicate Jelly (Fermi's Solution).
Growth *copious, scanty, absent.*
Medium stained.....

13. Cohn's Solution.
Growth, *copious, scanty, absent.*
Medium *fluorescent, non-fluorescent.*

14. Uschinsky's Solution.
Growth *copious, scanty, absent.*
Fluid *viscid, not viscid.*

15. Sodium Chloride in Bouillon.
Per cent inhibiting growth.....

16. Growth in Bouillon over Chloroform, *unrestrained, feeble, absent.*

17. Nitrogen. Obtained from *peptone, asparagin, glycocoll, urea, ammonia salts, nitrogen.*

18. Best media for long-continued growth.....
.....

19. Quick tests for differential purposes.....
.....
.....

III. PHYSICAL AND BIOCHEMICAL FEATURES.

Fermentation-Tubes containing peptone-water or Sugar-free bouillon and	Dextrose	Saccharose	Lactose	Mallose	Glycerin	Mannit					
Gas production, in per cent.											
$\left(\frac{H}{CO_2}\right)$											
Growth in closed arm	1.6%	2.2%	1.60		1.15						
Amount of acid produced 1d.											
" " " " 2d.											
" " " " 4d.											

2. Ammonia production, *feeble, moderate, strong, absent, masked by acids.*
3. Nitrate in nitrate broth,
Reduced, not reduced.
Presence of nitrites.....ammonia.....
" " nitrates.....free nitrogen.....
4. Indol production, *feeble, moderate, strong.*
5. Toleration of Acids: *Great, medium, slight.*
Acids tested.....
6. Toleration of NaOH: *Great, medium, slight.*
7. Optimum reaction for growth in bouillon, stated in terms of Fuller's scale.....
8. Vitality on culture media: *brief, moderate, long.*
9. Temperature relations:
Thermal death-point (10 minutes exposure in nutrient broth when this is adapted to growth of organism).....C.
Optimum temperature for growth.....C.: or best growth at 15° C, 20° C, 25° C, 30° C, 37° C, 40° C, 50° C, 60° C.
Maximum temperature for growth.....C.
Minimum temperature for growth.....C.
10. Killed readily by drying: resistant to drying.
11. Per cent killed by freezing (salt and crushed ice or liquid air).....
12. Sunlight: Exposure on ice in thinly sown agar plates: one-half plate covered (time 15 minutes), *sensitive, not sensitive.*
Per cent killed.....
13. Acids produced .....
14. Alkalies produced .....
15. Alcohols .....
16. Ferments; *pepsin, trypsin, diastase, invertase, pectase, cytase, tyrosinase, oxidase, peroxidase, lipase, catalase, glucase, galactase, lab,* etc.....
.....
17. Crystals formed: .....
18. Effect of germicides:

Substance	Method used.	Minutes	Temperature	Killing quantity	Amt. required to restrain growth

IV. PATHOGENICITY.

1. Pathogenic to Animals
Insects, crustaceans, fishes, reptiles, birds, mice, rats, guinea pigs, rabbits, dogs, cats, sheep, goats, cattle, horses, monkeys, man.....
2. Pathogenic to Plants :
.....
.....
.....
3. Toxins, *soluble, endotoxins.*
4. Non-toxin forming.
5. Immunity bactericidal.
6. Immunity non-bactericidal.
7. Loss of virulence on culture media: *prompt, gradual, not observed* in.....months.

BRIEF CHARACTERIZATION

Mark + or O, and when two terms occur on a line erase the one which does not apply unless both apply.

MORPHOLOGY. (2)	Diameter over 1μ	—
	Chains, filaments	
	Endospores	—
	Capsules	—
	Zoogloea, Pseudozoogloea	
	Motile	+
	Involution forms	
	Gram's Stain	—
CULTURAL FEATURES. (3) — Broth	Cloudy, turpid	+
	Ring	—
	Pellicle	—
	Sediment	—
Agar	Shining	+
	Dull	—
	Wrinkled	—
	Chromogenic	—
Gel. Plate	Round	+
	Proteus-like	—
	Rhizoid	—
	Filamentous	—
	Curled	—
Stab Gel.	Surface-growth	+
	Needle-growth	+
Potato	Moderate, ~~absent~~	+
	Abundant	
	Discolored	+
	Starch destroyed	+
	Grows at 37° C.	+
	Grows, in Cohn's Sol.	
	Grows in Uschinsky's Sol.	
BIOCHEMICAL FEATURES. — Liquefaction	Gelatin (4)	—
	Blood-serum	—
	Casein	+
	Agar, mannan	—
Milk	Acid curd	+
	Rennet curd	—
	Casein peptozized	+
	Indol (5)	+
	Hydrogen sulphide	
	Ammonia (5)	
	Nitrates reduced (5)	+
	Fluorescent	—
	Luminous	—
DISTRIBUTION	Animal pathogen, epizoon	
	Plant pathogen, epiphyte	
	Soil	
	Milk	
	Fresh water	+
	Salt water	
	Sewage	
	Iron bacterium	
	Sulphur bacterium	

DESCRIPTIVE CHART—SOCIETY OF AMERICAN BACTERIOLOGISTS

Prepared by F. D. CHESTER
F P. GORHAM
ERWIN F. SMITH } *Committee on Methods of Identification of Bacterial Species.*

ENDORSED BY THE SOCIETY FOR GENERAL USE AT THE ANNUAL MEETING, DEC. 31, 1907.

GLOSSARY OF TERMS.

AGAR HANGING BLOCK, a small block of nutrient agar cut from a poured plate, and placed on a cover-glass, the surface next the glass having been first touched with a loop from a young fluid culture or with a dilution from the same. It is examined upside down, the same as a hanging drop.

AMEBOID, assuming various shapes like an ameba.

AMORPHOUS, without visible differentiation in structure.

ARBORESCENT, a branched, tree-like growth.

BEADED, in stab or stroke, disjointed or semi-confluent colonies along the line of inoculation.

BRIEF, a few days, a week.

BRITTLE, growth dry, friable under the platinum needle.

BULLATE, growth rising in convex prominences, like a blistered surface.

BUTYROUS, growth of a butter-like consistency.

CHAINS.
Short chains, composed of 2 to 8 elements.
Long chains, composed of more than 8 elements.

CILIATE, having fine, hair-like extensions like cilia.

CLOUDY, said of fluid cultures which do not contain pseudozoogloeae.

COAGULATION, the separation of casein from whey in milk. This may take place quickly or slowly, and as the result either of the formation of an acid or of a lab ferment.

CONTOURED, an irregular, smoothly undulating surface, like that of a relief map.

CONVEX, surface the segment of a circle, but flattened.

COPROPHYL, dung bacteria.

CORIACEOUS, growth tough, leathery, not yielding to the platinum needle.

CRATERIFORM, round, depressed, due to the liquefaction of the medium.

CRETACEOUS, growth opaque and white, chalky.

CURLED, composed of parallel chains in wavy strands, as in anthrax colonies.

DIASTASIC ACTION, Same as DIASTATIC, conversion of starch into water-soluble substances by diastase.

ECHINULATE, in agar stroke a growth along line of inoculation, with toothed or pointed margins; in stab cultures growth beset with pointed outgrowths.

EFFUSE, growth thin, veily, unusually spreading.

ENTIRE, smooth, having a margin destitute of teeth or notches.

EROSE, border irregularly toothed.

FILAMENTOUS, growth composed of long, irregularly placed or interwoven filaments.

FILIFORM, in stroke or stab cultures a uniform growth along line of inoculation.

FIMBRIATE, border fringed with slender processes, larger than filaments.

FLOCCOSE, growth composed of short curved chains, variously oriented.

FLOCCULENT, said of fluids which contain pseudozoogloeae, i. e., small adherent masses of bacteria of various shapes and floating in the culture fluid.

FLUORESCENT, having one color by transmitted light and another by reflected light.

GRAM'S STAIN, a method of differential bleaching after gentian violet, methyl violet, etc. The + mark is to be given only when the bacteria are deep blue or remain blue after counterstaining with Bismark brown.

GRUMOSE, clotted.

INFUNDIBULIFORM, form of a funnel or inverted cone.

IRIDESCENT, like mother-of-pearl. The effect of very thin films.

LACERATE, having the margin cut into irregular segments as if torn.

LOBATE, border deeply undulate, producing lobes (see undulate.)

LONG, many weeks, or months.

MAXIMUM TEMPERATURE, temperature above which growth does not take place.

MEDIUM, several weeks.

MEMBRANOUS, growth thin, coherent, like a membrane.

MINIMUM TEMPERATURE, temperature below which growth does not take place.

MYCELIOID, colonies having the radiately filamentous appearance of mold colonies.

NAPIFORM, liquefaction with the form of a turnip.

NITROGEN REQUIREMENTS, the necessary nitrogenous food. This is determined by adding to *nitrogen-free* media the nitrogen compound to be tested.

OPALESCENT, resembling the color of an opal.

OPTIMUM TEMPERATURE, temperature at which growth is most rapid.

PELLICLE, in fluid bacterial growth either forming a continuous or an interrupted sheet over the fluid.

PEPTONIZED, said of curds dissolved by trypsin.

PERSISTENT, many weeks, or months.

PLUMOSE, a fleecy or feathery growth.

PSEUDOZOOGLOEAE, clumps of bacteria, not dissolving readily in water, arising from imperfect separation, or more or less fusion of the components, but not having the degree of compactness and gelatinization seen in zoogloeae.

PULVINATE, in the form of a cushion, decidedly convex.

PUNCTIFORM, very minute colonies, at the limit of natural vision.

RAISED, growth thick, with abrupt or terraced edges.

RHIZOID, growth of an irregular branched or root-like character, as in *B. mycoides*.

RING, Same as RIM, growth at the upper margin of a liquid culture, adhering more or less closely to the glass.

REPAND, wrinkled.

RAPID, Developing in 24 to 48 hours.

SACCATE, liquefaction the shape of an elongated sack, tubular, cylindrical.

SCUM, floating islands of bacteria, an interrupted pellicle or bacterial membrane.

SLOW, requiring 5 or 6 days or more for development.

SHORT, applied to time, a few days, a week.

SPORANGIA, cells containing endospores.

SPREADING, growth extending much beyond the line of inoculation, i. e., several millimeters or more.

STRATIFORM, liquefying to the walls of the tube at the top and then proceeding downwards horizontally.

THERMAL DEATH-POINT, the degree of heat required to kill young fluid cultures of an organism exposed for 10 minutes (in thin-walled test tubes of a diameter not exceeding 20 mm.) in the thermal water-bath. The water must be kept agitated so that the temperature shall be uniform during the exposure.

TRANSIENT, a few days.

TURBID, cloudy with flocculent particles; cloudy plus flocculence.

UMBONATE, having a button-like, raised center.

UNDULATE, border wavy, with shallow sinuses.

VERRUCOSE, growth wart-like, with wart-like prominences.

VERMIFORM-CONTOURED, growth like a mass of worms, or intestinal coils.

VILLOUS, growth beset with hair-like extensions.

VISCID, growth follows the needle when touched and withdrawn, sediment on shaking rises as a coherent swirl.

ZOOGLOEAE, firm gelatinous masses of bacteria, one of the most typical examples of which is the *Streptococcus mesenterioides* of sugar vats (*Leuconostoc mesenterioides*), the bacterial chains being surrounded by an enormously thickened firm covering, inside of which there may be one or many groups of the bacteria.

NOTES.

(1) For decimal system of group numbers see Table 1. This will be found useful as a quick method of showing close relationships inside the genus, but is not a sufficient characterization of any organism.

(2) The morphological characters shall be determined and described from growths obtained upon at least one solid medium (nutrient agar) and in at least one liquid medium (nutrient broth). Growths at 37° C shall be in general not older than 24 to 48 hours, and growths at 20° C not older than 48 to 72 hours. To secure uniformity in cultures, in all cases preliminary cultivation shall be practiced as described in the revised Report of the Committee on Standard Methods of the Laboratory Section of the American Public Health Association, 1905.

(3) The observation of cultural and bio-chemical features shall cover a period of at least 15 days and frequently longer, and shall be made according to the revised Standard Methods above referred to. All media shall be made according to the same Standard Methods.

(4) Gelatin stab cultures shall be held for 6 weeks to determine liquefaction.

(5) Ammonia and indol tests shall be made at end of 10th day, nitrite tests at end of 5th day.

(6) Titrate with $\frac{N}{20}$ NaOH, using phenolphthalein as an indicator: make titrations at same times from blank. The difference gives the amount of acid produced.

The titration should be done after boiling to drive off any Co_2 present in the culture.

(7) Generic nomenclature shall begin with the year 1872 (Cohn's first important paper).

Species nomenclature shall begin with the year 1880 (Koch's discovery of the poured plate method for the separation of organisms).

(8) Chromogensis shall be recorded in standard color terms.

TABLE I.

A NUMERICAL SYSTEM OF RECORDING THE SALIENT CHARACTERS OF AN ORGANISM. (GROUP NUMBER.)

100.	Endospores produced
200.	Endospores not produced
10.	Aerobic (Strict)
20.	Facultative anaerobic
30.	Anaerobic (Strict)
1.	Gelatin liquefied
2.	Gelatin not liquefied
0.1	Acid and gas from dextrose
0.2	Acid without gas from dextrose
0.3	No acid from dextrose
0.4	No growth with dextrose
.01	Acid and gas from lactose
.02	Acid without gas from lactose
.03	No acid from lactose
.04	No growth with lactose
.001	Acid and gas from saccharose
.002	Acid without gas from saccharose
.003	No acid from saccharose
.004	No growth with saccharose
.0001	Nitrates reduced with evolution of gas
.0002	Nitrates not reduced
.0003	Nitrates reduced without gas formation
.00001	Fluorescent
.00002	Violet chromogens
.00003	Blue "
.00004	Green "
.00005	Yellow "
.00006	Orange "
.00007	Red "
.00008	Brown "
.00009	Pink "
.00000	Non-chromogenic
.000001	Diastasic action on potato starch, strong
.000002	Diastasic action on potato starch, feeble
.000003	Diastasic action on potato starch, absent
.0000001	Acid and gas from glycerine
.0000002	Acid without gas from glycerine
.0000003	No acid from glycerine
.0000004	No growth with glycerine

The genus according to the system of Migula is given its proper symbol which precedes the number thus: (7)

BACILLUS COLI (Esch.) Mig.	becomes	B.	222.111102
BACILLUS ALCALIGENES Petr.	"	B.	212.333102
PSEUDOMONAS CAMPESTRIS (Pam.) Sm.	"	Ps.	211.333151
BACTERIUM SUICIDA Mig.	"	Bact.	222.232203

Source Water Date of Isolation Name Micrococcus Candicans Group No. (1)

DETAILED FEATURES.

NOTE—Underscore required terms. Observe notes and glossary of terms on opposite side of card.

I. MORPHOLOGY (2)

1. Vegetative Cells, Medium used..Agar.....
   temp....., age.....days
   Form, *round, short rods, long rods, short chains, long chains, filaments, commas, short spirals, long spirals, clostridium, cuneate, clavate, curved.*
   Limits of Size...1μ..
   Size of Majority.....
   Ends, *rounded, truncate, concave.*
   Agar Hanging-Block: Orientation (grouping)..... Chains (No. of elements)..... *Short chains, long chains* Orientation of Chains, *parallel, irregular.*
2. Sporangia, medium used.....temp.....
   age.....days
   Form, *elliptical, short rods, spindled, clavate, drumsticks.*
   Limits of Size..... Size of Majority.....
   Agar Hanging-Block: Orientation (grouping)..... Chains (No. of elements)..... Orientation of Chains, *parallel, irregular.*
   Location of Endospores, *central, polar.*
3. Endospores.
   Form, *round, elliptical, elongated.*
   Limits of Size.....
   Size of Majority.....
   Wall, *thick, thin.*
   Sporangium wall, *adherent, not adherent.*
   Germination, *equatorial, oblique, polar, bipolar, by stretching.*
4. Flagella No.....Attachment *polar, bipolar, peritrichiate.* How Stained.....
5. Capsules, present on.....
6. Zoogloea, Pseudozoogloea.
7. Involution Forms, on.....in...days at...°C.
8. Staining Reactions.
   1:10 watery fuchsin, gentian violet, carbol fuchsin, Loeffler's alkaline methylene blue.
   Special Stains
   Gram..... Glycogen .....
   Fat..... Acid fast .....
   Neisser.....

II. CULTURAL FEATURES (3)

1. Agar Stroke.
   Growth, *invisible, scanty, moderate, abundant.*
   Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
   Elevation of growth, *flat, effuse, raised, convex.*
   Lustre, *glistening, dull, cretaceous.*
   Topography, *smooth, contoured, rugose, verrucose.*
   Optical Characters, *opaque, translucent, opalescent, iridescent.*
   Chromogenesis (8)
   Odor, *absent, decided, resembling.....*
   Consistency, *slimy, butyrous, viscid, membranous, coriaceous brittle.*
   Medium *grayed, browned, reddened, blued, greened.*
2. Potato.
   Growth, *scanty, moderate, abundant, transient, persistent.*
   Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
   Elevation of growth, *flat, effuse, raised, convex.*
   Lustre, *glistening, dull, cretaceous.*
   Topography, *smooth, contoured, rugose, verrucose,*
   Chromogenesis (8) Yellow Pigment in water *insoluble, soluble;* other solvents.....
   Odor, *absent, decided, resembling.....*
   Consistency, *slimy, butyrous, viscid, membranous, coriaceous, brittle.*
   Medium *grayed, browned, reddened, blued, greened.*
3. Loeffler's Blood Serum.
   Stroke *invisible, scanty, moderate, abundant.*
   Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
   Elevation of growth, *flat, effuse, raised, convex.*
   Lustre, *glistening, dull, cretaceous.*
   Topography, *smooth, contoured, rugose, verrucose.*
   Chromogenesis (8).....
   Medium *grayed, browned, reddened, blued, greened.*
   Liquefaction begins in.....d, complete in.....d.
4. Agar Stab.
   Growth *uniform, best at top, best at bottom; surface growth scanty, abundant; restricted, widespread.*
   Line of puncture. *filiform, beaded, papillate, villous, plumose, arborescent: liquefaction.*
5. Gelatin Stab.
   Growth *uniform, best at top, best at bottom.*
   Line of puncture, *filiform, beaded, papillate, villous, plumose, arborescent.*
   Liquefaction, *crateriform, napiform, infundibuliform, saccate, stratiform;* begins in.....d, complete in.....d.
   Medium *fluorescent, browned.....*
6. Nutrient Broth.
   Surface growth, *ring, pellicle, flocculent, membranous, none.*
   Clouding *slight, moderate, strong; transient, persistent; none; fluid turbid.*
   Odor, *absent, decided, resembling.....*
   Sediment, *compact, flocculent, granular, flaky, viscid on agitation, abundant, scant.*
7. Milk.
   Clearing without coagulation.
   Coagulation *prompt, delayed, absent.*
   Extrusion of whey begins in.....days.
   Coagulum *slowly peptonized, rapidly peptonized.*
   Peptonization begins on.....d, complete on.....d
   Reaction, 1d...., 2d...., 4d...., 10d...., 20d....
   Consistency, *slimy, viscid, unchanged.*
   Medium *browned, reddened, blued, greened.*
   Lab ferment, *present, absent.*
8. Litmus Milk.
   Acid, alkaline, acid then alkaline, no change.
   Prompt reduction, no reduction, partial slow reduction.
9. Gelatin Colonies.
   Growth, *slow, rapid.*
   Form, *punctiform, round, irregular, ameboid, mycelioid, filamentous, rhizoid.*
   Elevation, *flat, effuse, raised, convex, pulvinate, crateriform (liquefying).*
   Edge, *entire, undulate, lobate, erose, lacerate, fimbriate, filamentous, floccose, curled.*
   Liquefaction, *cup, saucer, spreading.*
10. Agar Colonies.
    Growth, *slow, rapid, temperature.....*
    Form, *punctiform, round, irregular, ameboid, mycelioid, filamentous, rhizoid.*
    Surface *smooth, rough, concentrically ringed, radiate, striate.*
    Elevation, *flat, effuse, raised, convex, pulvinate, umbonate.*
    Edge, *entire, undulate, lobate, erose, lacerate, fimbriate, floccose, curled.*
    Internal structure, *amorphous, finely-, coarsely-granular, grumose, filamentous, floccose, curled.*
11. Starch Jelly.
    Growth, *scanty, copious.*
    Diastasic action, *absent, feeble, profound.*
    Medium stained.....
12. Silicate Jelly (Fermi's Solution).
    Growth *copious, scanty, absent.*
    Medium stained.....
13. Cohn's Solution.
    Growth, *copious, scanty, absent.*
    Medium *fluorescent, non-fluorescent.*
14. Uschinsky's Solution.
    Growth *copious, scanty, absent.*
    Fluid *viscid, not viscid.*
15. Sodium Chloride in Bouillon.
    Per cent inhibiting growth.....
16. Growth in Bouillon over Chloroform, *unrestrained, feeble, absent.*
17. Nitrogen. Obtained from *peptone, asparagin, glycocoll, urea, ammonia salts, nitrogen.*
18. Best media for long-continued growth.....
    .....
19. Quick tests for differential purposes.....
    .....
    .....

III. PHYSICAL AND BIOCHEMICAL FEATURES.

Fermentation-Tubes containing peptone-water or Sugar-free bouillon and	Dextrose	Saccharose	Lactose	Maltose	Glycerin	Mannit		
Gas production, in per cent.								
$\left(\frac{H}{CO_2}\right)$	No	Gas						
Growth in closed arm								
Amount of acid produced 1d.								
" " " " 2d.								
" " " " 4d.								

2. Ammonia production, *feeble, moderate, strong, absent, masked by acids.*
3. Nitrate in nitrate broth,
   Reduced, not reduced.
   Presence of nitrites.....ammonia.....
   " " nitrates.....free nitrogen.....
4. Indol production, *feeble, moderate, strong.*
5. Toleration of Acids: *Great, medium, slight.*
   Acids tested.....
6. Toleration of NaOH: *Great, medium, slight.*
7. Optimum reaction for growth in bouillon, stated in terms of Fuller's scale.....
8. Vitality on culture media: *brief, moderate, long.*
9. Temperature relations:
   Thermal death-point (10 minutes exposure in nutrient broth when this is adapted to growth of organism).....C.
   Optimum temperature for growth.....C.: or best growth at 15° C, 20° C, 25° C, 30° C, 37° C, 40° C, 50° C, 60° C.
   Maximum temperature for growth.....C.
   Minimum temperature for growth.....C.
10. Killed readily by drying: resistant to drying.
11. Per cent killed by freezing (salt and crushed ice or liquid air).....
12. Sunlight: Exposure on ice in thinly sown agar plates: one-half plate covered (time 15 minutes), *sensitive, not sensitive.*
    Per cent killed.....
13. Acids produced .....
14. Alkalies produced .....
15. Alcohols .....
16. Ferments; *pepsin, trypsin, diastase, invertase, pectase, cytase, tyrosinase, oxidase, peroxidase, lipase, catalase, glucase, galactase, lab,* etc.....
    .....
17. Crystals formed: .....
18. Effect of germicides:

Substance	Method used.	Minutes	Temperature	Killing quantity	Amt. required to restrain growth

IV. PATHOGENICITY.

1. Pathogenic to Animals
   Insects, crustaceans, fishes, reptiles, birds, mice, rats, guinea pigs, rabbits, dogs, cats, sheep, goats, cattle, horses, monkeys, man.....
2. Pathogenic to Plants :
   .....
   .....
   .....
3. Toxins, *soluble, endotoxins,*
4. Non-toxin forming.
5. Immunity bactericidal.
6. Immunity non-bactericidal.
7. Loss of virulence on culture media: *prompt, gradual, not observed* in.....months.

BRIEF CHARACTERIZATION

Mark + or O, and when two terms occur on a line erase the one which does not apply unless both apply.

MORPHOLOGY. (2)	Diameter over 1μ	—
	Chains, filaments	
	Endospores	—
	Capsules	
	Zoogloea, Pseudozoogloea	
	Motile	
	Involution forms	
	Gram's Stain	+
CULTURAL FEATURES. (3) — Broth	Cloudy, turpid	+
	Ring	—
	Pellicle	+
	Sediment	+
Agar	Shining	—
	Dull	+
	Wrinkled	—
	Chromogenic	—
Gel. Plate	Round	+
	Proteus-like	—
	Rhizoid	—
	Filamentous	—
	Curled	—
Stab Gel.	Surface-growth	+
	Needle-growth	+
Potato	Moderate, absent	+
	Abundant	—
	Discolored	+
	Starch destroyed	—
	Grows at 37° C.	—
	Grows, in Cohn's Sol.	
	Grows in Uschinsky's Sol.	
BIOCHEMICAL FEATURES. — Liquefaction	Gelatin (4)	—
	Blood-serum	—
	Casein	—
	Agar, mannan	—
Milk	Acid curd	—
	Rennet curd	—
	Casein peptozized	—
	Indol (5)	—
	Hydrogen sulphide	
	Ammonia (5)	
	Nitrates reduced (5)	
	Fluorescent	
	Luminous	
	Animal pathogen, epizoon	
	Plant pathogen, epiphyte	
DISTRIBUTION	Soil	
	Milk	
	Fresh water	+
	Salt water	
	Sewage	
	Iron bacterium	
	Sulphur bacterium	

DESCRIPTIVE CHART—SOCIETY OF AMERICAN BACTERIOLOGISTS

Prepared by F. D. CHESTER, F. P. GORHAM, ERWIN F. SMITH } *Committee on Methods of Identification of Bacterial Species.*

ENDORSED BY THE SOCIETY FOR GENERAL USE AT THE ANNUAL MEETING, DEC. 31, 1907.

GLOSSARY OF TERMS.

AGAR HANGING BLOCK, a small block of nutrient agar cut from a poured plate, and placed on a cover-glass, the surface next the glass having been first touched with a loop from a young fluid culture or with a dilution from the same. It is examined upside down, the same as a hanging drop.

AMEBOID, assuming various shapes like an ameba.

AMORPHOUS, without visible differentiation in structure.

ARBORESCENT, a branched, tree-like growth.

BEADED, in stab or stroke, disjointed or semi-confluent colonies along the line of inoculation.

BRIEF, a few days, a week.

BRITTLE, growth dry, friable under the platinum needle.

BULLATE, growth rising in convex prominences, like a blistered surface.

BUTYROUS, growth of a butter-like consistency.

CHAINS.
Short chains, composed of 2 to 8 elements.
Long chains, composed of more than 8 elements.

CILIATE, having fine, hair-like extensions like cilia.

CLOUDY, said of fluid cultures which do not contain pseudozoogloeae.

COAGULATION, the separation of casein from whey in milk. This may take place quickly or slowly, and as the result either of the formation of an acid or of a lab ferment.

CONTOURED, an irregular, smoothly undulating surface, like that of a relief map.

CONVEX, surface the segment of a circle, but flattened.

COPROPHYL, dung bacteria.

CORIACEOUS, growth tough, leathery, not yielding to the platinum needle.

CRATERIFORM, round, depressed, due to the liquefaction of the medium.

CRETACEOUS, growth opaque and white, chalky.

CURLED, composed of parallel chains in wavy strands, as in anthrax colonies.

DIASTASIC ACTION, Same as DIASTATIC, conversion of starch into water-soluble substances by diastase.

ECHINULATE, in agar stroke a growth along line of inoculation, with toothed or pointed margins; in stab cultures growth beset with pointed outgrowths.

EFFUSE, growth thin, veily, unusually spreading.

ENTIRE, smooth, having a margin destitute of teeth or notches.

EROSE, border irregularly toothed.

FILAMENTOUS, growth composed of long, irregularly placed or interwoven filaments.

FILIFORM, in stroke or stab cultures a uniform growth along line of inoculation.

FIMBRIATE, border fringed with slender processes, larger than filaments.

FLOCCOSE, growth composed of short curved chains, variously oriented.

FLOCCULENT, said of fluids which contain pseudozoogloeae, i. e., small adherent masses of bacteria of various shapes and floating in the culture fluid.

FLUORESCENT, having one color by transmitted light and another by reflected light.

GRAM'S STAIN, a method of differential bleaching after gentian violet, methyl violet, etc. The + mark is to be given only when the bacteria are deep blue or remain blue after counterstaining with Bismark brown.

GRUMOSE, clotted.

INFUNDIBULIFORM, form of a funnel or inverted cone.

IRIDESCENT, like mother-of-pearl. The effect of very thin films.

LACERATE, having the margin cut into irregular segments as if torn.

LOBATE, border deeply undulate, producing lobes (see undulate.)

LONG, many weeks, or months.

MAXIMUM TEMPERATURE, temperature above which growth does not take place.

MEDIUM, several weeks.

MEMBRANOUS, growth thin, coherent, like a membrane.

MINIMUM TEMPERATURE, temperature below which growth does not take place.

MYCELIOID, colonies having the radiately filamentous appearance of mold colonies.

NAPIFORM, liquefaction with the form of a turnip.

NITROGEN REQUIREMENTS, the necessary nitrogenous food. This is determined by adding to *nitrogen-free* media the nitrogen compound to be tested.

OPALESCENT, resembling the color of an opal.

OPTIMUM TEMPERATURE, temperature at which growth is most rapid.

PELLICLE, in fluid bacterial growth either forming a continuous or an interrupted sheet over the fluid.

PEPTONIZED, said of curds dissolved by trypsin.

PERSISTENT, many weeks, or months.

PLUMOSE, a fleecy or feathery growth.

PSEUDOZOOGLOEAE, clumps of bacteria, not dissolving readily in water, arising from imperfect separation, or more or less fusion of the components, but not having the degree of compactness and gelatinization seen in zoogloeae.

PULVINATE, in the form of a cushion, decidedly convex.

PUNCTIFORM, very minute colonies, at the limit of natural vision.

RAISED, growth thick, with abrupt or terraced edges.

RHIZOID, growth of an irregular branched or root-like character, as in *B. mycoides*.

RING, Same as RIM, growth at the upper margin of a liquid culture, adhering more or less closely to the glass.

REPAND, wrinkled.

RAPID, Developing in 24 to 48 hours.

SACCATE, liquefaction the shape of an elongated sack, tubular, cylindrical.

SCUM, floating islands of bacteria, an interrupted pellicle or bacterial membrane.

SLOW, requiring 5 or 6 days or more for development.

SHORT, applied to time, a few days, a week.

SPORANGIA, cells containing endospores.

SPREADING, growth extending much beyond the line of inoculation, i. e., several millimeters or more.

STRATIFORM, liquefying to the walls of the tube at the top and then proceeding downwards horizontally.

THERMAL DEATH-POINT, the degree of heat required to kill young fluid cultures of an organism exposed for 10 minutes (in thin-walled test tubes of a diameter not exceeding 20 mm.) in the thermal water-bath. The water must be kept agitated so that the temperature shall be uniform during the exposure.

TRANSIENT, a few days.

TURBID, cloudy with flocculent particles; cloudy plus flocculence.

UMBONATE, having a button-like, raised center.

UNDULATE, border wavy, with shallow sinuses.

VERRUCOSE, growth wart-like, with wart-like prominences.

VERMIFORM-CONTOURED, growth like a mass of worms, or intestinal coils.

VILLOUS, growth beset with hair-like extensions.

VISCID, growth follows the needle when touched and withdrawn, sediment on shaking rises as a coherent swirl.

ZOOGLOEAE, firm gelatinous masses of bacteria, one of the most typical examples of which is the *Streptococcus mesenterioides* of sugar vats (*Leuconostoc mesenterioides*), the bacterial chains being surrounded by an enormously thickened firm covering, inside of which there may be one or many groups of the bacteria.

NOTES.

(1) For decimal system of group numbers see Table 1. This will be found useful as a quick method of showing close relationships inside the genus, but is not a sufficient characterization of any organism.

(2) The morphological characters shall be determined and described from growths obtained upon at least one solid medium (nutrient agar) and in at least one liquid medium (nutrient broth). Growths at 37° C shall be in general not older than 24 to 48 hours, and growths at 20° C not older than 48 to 72 hours. To secure uniformity in cultures, in all cases preliminary cultivation shall be practiced as described in the revised Report of the Committee on Standard Methods of the Laboratory Section of the American Public Health Association, 1905.

(3) The observation of cultural and bio-chemical features shall cover a period of at least 15 days and frequently longer, and shall be made according to the revised Standard Methods above referred to. All media shall be made according to the same Standard Methods.

(4) Gelatin stab cultures shall be held for 6 weeks to determine liquefaction.

(5) Ammonia and indol tests shall be made at end of 10th day, nitrite tests at end of 5th day.

(6) Titrate with $\frac{N}{20}$ NaOH, using phenolphthalein as an indicator: make titrations at same times from blank. The difference gives the amount of acid produced.

The titration should be done after boiling to drive off any Co_2 present in the culture.

(7) Generic nomenclature shall begin with the year 1872 (Cohn's first important paper).

Species nomenclature shall begin with the year 1880 (Koch's discovery of the poured plate method for the separation of organisms).

(8) Chromogensis shall be recorded in standard color terms.

TABLE I.

A NUMERICAL SYSTEM OF RECORDING THE SALIENT CHARACTERS OF AN ORGANISM. (GROUP NUMBER.)

100.	Endospores produced
200.	Endospores not produced
10.	Aerobic (Strict)
20.	Facultative anaerobic
30.	Anaerobic (Strict)
1.	Gelatin liquefied
2.	Gelatin not liquefied
0.1	Acid and gas from dextrose
0.2	Acid without gas from dextrose
0.3	No acid from dextrose
0.4	No growth with dextrose
.01	Acid and gas from lactose
.02	Acid without gas from lactose
.03	No acid from lactose
.04	No growth with lactose
.001	Acid and gas from saccharose
.002	Acid without gas from saccharose
.003	No acid from saccharose
.004	No growth with saccharose
.0001	Nitrates reduced with evolution of gas
.0002	Nitrates not reduced
.0003	Nitrates reduced without gas formation
.00001	Fluorescent
.00002	Violet chromogens
.00003	Blue "
.00004	Green "
.00005	Yellow "
.00006	Orange "
.00007	Red "
.00008	Brown "
.00009	Pink "
.00000	Non-chromogenic
.000001	Diastasic action on potato starch, strong
.000002	Diastasic action on potato starch, feeble
.000003	Diastasic action on potato starch, absent
.0000001	Acid and gas from glycerine
.0000002	Acid without gas from glycerine
.0000003	No acid from glycerine
.0000004	No growth with glycerine

The genus according to the system of Migula is given its proper symbol which precedes the number thus: (7)

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PSEUDOMONAS CAMPESTRIS (Pam.) Sm.	"	Ps.	211.333151
BACTERIUM SUICIDA Mig.	"	Bact.	222.232203

Source Mississippi River Date of Isolation Name B. Flouresceens Liquefacens, Group No. (1)

DETAILED FEATURES.

NOTE—Underscore required terms. Observe notes and glossary of terms on opposite side of card.

I. MORPHOLOGY (2)

1. Vegetative Cells. Medium used.. Agar.... temp.....37....., age...1.....days
Form, *round, short rods, long rods, short chains, long chains, filaments, commas, short spirals, long spirals, clostridium, cuneate, clavate, curved.*
Limits of Size.....
Size of Majority.....
Ends, *rounded, truncate, concave.*
Agar Hanging-Block: Orientation (grouping)..... Chains (No. of elements)..... *Short chains, long chains* Orientation of Chains, *parallel,* irregular.

2. Sporangia, medium used.....temp.....
age.....days
Form, *elliptical, short rods, spindled, clavate, drumsticks.*
Limits of Size..... Size of Majority.....
Agar Hanging-Block: Orientation (grouping)..... Chains (No. of elements)..... Orientation of Chains, *parallel, irregular.*
Location of Endospores, *central, polar.*

3. Endospores.
Form, *round, elliptical, elongated.*
Limits of Size.....
Size of Majority.....
Wall, *thick, thin.*
Sporangium wall, *adherent, not adherent.*
Germination, *equatorial, oblique, polar, bipolar, by stretching.*

4. Flagella No.....Attachment *polar, bipolar, peritrichiate.* How Stained.....

5. Capsules, present on.....
6. Zoogloea, Pseudozoogloea.

7. Involution Forms, on.....in...days at...°C.

8. Staining Reactions.
1:10 watery fuchsin, gentian violet, carbol fuchsin, Loeffler's alkaline methylene blue.
Special Stains
Gram..... Glycogen .....
Fat..... Acid fast .....
Neisser.....

II. CULTURAL FEATURES (3)

1. Agar Stroke.
Growth, *invisible, scanty, moderate, abundant.*
Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
Elevation of growth, *flat, effuse, raised, convex.*
Lustre, *glistening, dull, cretaceous.*
Topography, *smooth, contoured, rugose, verrucose.*
Optical Characters, *opaque, translucent, opalescent, iridescent.*
Chromogenesis (8) Greenish.
Odor, *absent, decided, resembling.....*
Consistency, *slimy, butyrous, viscid, membranous, coriaceous brittle.*
Medium *grayed, browned, reddened, blued, greened.*

2. Potato.
Growth, *scanty, moderate, abundant, transient, persistent.*
Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
Elevation of growth, *flat, effuse, raised, convex.*
Lustre, *glistening, dull, cretaceous.*
Topography, *smooth, contoured, rugose, verrucose.*
Chromogenesis (8) Brown Pigment in water *insoluble, soluble;* other solvents.....
Odor, *absent, decided, resembling.....*
Consistency, *slimy, butyrous, viscid, membranous, coriaceous, brittle.*
Medium *grayed, browned, reddened, blued, greened.*

3. Loeffler's Blood Serum.
Stroke *invisible, scanty, moderate, abundant.*
Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
Elevation of growth, *flat, effuse, raised, convex.*
Lustre, *glistening, dull, cretaceous.*
Topography, *smooth, contoured, rugose, verrucose.*
Chromogenesis (8).....
Medium *grayed, browned, reddened, blued, greened.*
Liquefaction begins in.....d, complete in.....d.

4. Agar Stab.
Growth *uniform, best at top, best at bottom; surface growth scanty, abundant; restricted, widespread.*
Line of puncture *filiform, beaded, papillate, villous, plumose, arborescent: liquefaction.*

5. Gelatin Stab.
Growth *uniform, best at top, best at bottom.*
Line of puncture, *filiform, beaded, papillate, villous, plumose, arborescent.*
Liquefaction, *crateriform, napiform, infundibuliform, saccate, stratiform;* begins in.....d, complete in.....d.
Medium *fluorescent, browned.....*

6. Nutrient Broth.
Surface growth, *ring, pellicle, flocculent, membranous, none.*
Clouding *slight, moderate, strong; transient, persistent; none; fluid turbid.*
Odor, *absent, decided, resembling.....*
Sediment, *compact, flocculent, granular, flaky, viscid on agitation, abundant, scant.*

7. Milk.
Clearing without coagulation.
Coagulation *prompt, delayed, absent.*
Extrusion of whey begins in.....days.
Coagulum *slowly peptonized, rapidly peptonized.*
Peptonization begins on.....d, complete on.....d
Reaction, 1d...., 2d...., 4d....., 10d....., 20d....
Consistency, *slimy, viscid, unchanged.*
Medium *browned, reddened, blued, greened.*
Lab ferment, *present, absent.*

8. Litmus Milk.
Acid, alkaline, acid then alkaline, no change.
Prompt reduction, no reduction, partial slow reduction.

9. Gelatin Colonies.
Growth, *slow, rapid.*
Form, *punctiform, round, irregular, ameboid, mycelioid, filamentous, rhizoid.*
Elevation, *flat, effuse, raised, convex, pulvinate, crateriform (liquefying).*
Edge, *entire, undulate, lobate, erose, lacerate, fimbriate, filamentous, floccose, curled.*
Liquefaction, *cup, saucer, spreading.*

10. Agar Colonies.
Growth, *slow, rapid, temperature.....*
Form, *punctiform, round, irregular, ameboid, mycelioid, filamentous, rhizoid.*
Surface *smooth, rough, concentrically ringed, radiate, striate.* Spread
Elevation, *flat, effuse, raised, convex, pulvinate, umbonate.*
Edge, *entire, undulate, lobate, erose, lacerate, fimbriate, floccose, curled.*
Internal structure, *amorphous, finely-, coarsely-granular, grumose, filamentous, floccose, curled.*

11. Starch Jelly.
Growth, *scanty, copious.*
Diastasic action, *absent, feeble, profound.*
Medium stained.....

12. Silicate Jelly (Fermi's Solution).
Growth *copious, scanty, absent.*
Medium stained.....

13. Cohn's Solution.
Growth, *copious, scanty, absent.*
Medium *fluorescent, non-fluorescent.*

14. Uschinsky's Solution.
Growth *copious, scanty, absent.*
Fluid *viscid, not viscid.*

15. Sodium Chloride in Bouillon.
Per cent inhibiting growth.....

16. Growth in Bouillon over Chloroform, *unrestrained, feeble, absent.*

17. Nitrogen. Obtained from *peptone, asparagin, glycocoll, urea, ammonia salts, nitrogen.*

18. Best media for long-continued growth.....
.....

19. Quick tests for differential purposes.....
.....
.....

III. PHYSICAL AND BIOCHEMICAL FEATURES.

Fermentation-Tubes containing peptone-water or Sugar-free bouillon and	Dextrose	Saccharose	Lactose	Maltose	Glycerin	Mannit				
Gas production, in per cent.										
$\left(\frac{H}{CO_2}\right)$										
Growth in closed arm										
Amount of acid produced 1 d.										
" " " " 2d.										
" " " " 4d.										

No Gas

2. Ammonia production, *feeble, moderate, strong, absent, masked by acids.*
3. Nitrate in nitrate broth,
Reduced, not reduced.
Presence of nitrites.....ammonia.....
" " nitrates.....free nitrogen.....
4. Indol production, *feeble, moderate, strong.*
5. Toleration of Acids: *Great, medium, slight.*
Acids tested.....
6. Toleration of NaOH: *Great, medium, slight.*
7. Optimum reaction for growth in bouillon, stated in terms of Fuller's scale.....
8. Vitality on culture media: *brief, moderate, long.*
9. Temperature relations:
Thermal death-point (10 minutes exposure in nutrient broth when this is adapted to growth of organism).....C.
Optimum temperature for growth.....C.: or best growth at 15° C, 20° C, 25° C, 30° C, 37° C, 40° C, 50° C, 60° C.
Maximum temperature for growth.....C.
Minimum temperature for growth.....C.
10. Killed readily by drying: resistant to drying.
11. Per cent killed by freezing (salt and crushed ice or liquid air).....
12. Sunlight: Exposure on ice in thinly sown agar plates: one-half plate covered (time 15 minutes), *sensitive, not sensitive.*
Per cent killed.....
13. Acids produced .....
14. Alkalies produced .....
15. Alcohols .....
16. Ferments: *pepsin, trypsin, diastase, invertase, pectase, cytase, tyrosinase, oxidase, peroxidase, lipase, catalase, glucase, galactase, lab,* etc.....
.....
17. Crystals formed: .....
18. Effect of germicides:

Substance	Method used	Minutes	Temperature	Killing quantity	Amt. required to restrain growth

IV. PATHOGENICITY.

1. Pathogenic to Animals
Insects, crustaceans, fishes, reptiles, birds, mice, rats, guinea pigs, rabbits, dogs, cats, sheep, goats, cattle, horses, monkeys, man.....
2. Pathogenic to Plants :
.....
.....
.....
3. Toxins, *soluble, endotoxins,*
4. Non-toxin forming.
5. Immunity bactericidal.
6. Immunity non-bactericidal.
7. Loss of virulence on culture media: *prompt, gradual, not observed* in.....months.

BRIEF CHARACTERIZATION

Mark + or O, and when two terms occur on a line erase the one which does not apply unless both apply.

MORPHOLOGY. (2)	Diameter over 1μ	−
	Chains, filaments	+
	Endospores	−
	Capsules	−
	Zoogloea, Pseudozoogloea	−
	Motile	+
	Involution forms	−
	Gram's Stain	−
CULTURAL FEATURES. (3)	Broth: ~~Cloudy~~, turpid	+
	Broth: Ring	−
	Broth: Pellicle	+
	Broth: Sediment	+
	Agar: Shining	
	Agar: Dull	+
	Agar: Wrinkled	−
	Agar: Chromogenic	+
	Gel. Plate: Round	+
	Gel. Plate: Proteus-like	−
	Gel. Plate: Rhizoid	−
	Gel. Plate: Filamentous	−
	Gel. Plate: Curled	−
	Stab Gel.: Surface-growth	+
	Stab Gel.: Needle-growth	+
	Potato: Moderate, absent	−
	Potato: Abundant	+
	Potato: Discolored	+
	Potato: Starch destroyed	
	Grows at 37° C.	+
	Grows, in Cohn's Sol.	
	Grows in Uschinsky's Sol.	
BIOCHEMICAL FEATURES.	Liquefaction: Gelatin (4)	+
	Liquefaction: Blood-serum	−
	Liquefaction: Casein	−
	Liquefaction: Agar, mannan	−
	Milk: Acid curd	−2
	Milk: Rennet curd	
	Milk: Casein peptozized	
	Indol (5)	+2
	Hydrogen sulphide	
	Ammonia (5)	
	Nitrates reduced (5)	
DISTRIBUTION	Fluorescent	
	Luminous	
	Animal pathogen, epizoon	
	Plant pathogen, epiphyte	
	Soil	
	Milk	+
	Fresh water	+
	Salt water	
	Sewage	+
	Iron bacterium	
	Sulphur bacterium	

DESCRIPTIVE CHART—SOCIETY OF AMERICAN BACTERIOLOGISTS

Prepared by F. D. CHESTER, F. P. GORHAM, ERWIN F. SMITH } *Committee on Methods of Identification of Bacterial Species.*

ENDORSED BY THE SOCIETY FOR GENERAL USE AT THE ANNUAL MEETING, DEC. 31, 1907.

GLOSSARY OF TERMS.

AGAR HANGING BLOCK, a small block of nutrient agar cut from a poured plate, and placed on a cover-glass, the surface next the glass having been first touched with a loop from a young fluid culture or with a dilution from the same. It is examined upside down, the same as a hanging drop.

AMEBOID, assuming various shapes like an ameba.

AMORPHOUS, without visible differentiation in structure.

ARBORESCENT, a branched, tree-like growth.

BEADED, in stab or stroke, disjointed or semi-confluent colonies along the line of inoculation.

BRIEF, a few days, a week.

BRITTLE, growth dry, friable under the platinum needle.

BULLATE, growth rising in convex prominences, like a blistered surface.

BUTYROUS, growth of a butter-like consistency.

CHAINS.
Short chains, composed of 2 to 8 elements.
Long chains, composed of more than 8 elements.

CILIATE, having fine, hair-like extensions like cilia.

CLOUDY, said of fluid cultures which do not contain pseudozoogloeae.

COAGULATION, the separation of casein from whey in milk. This may take place quickly or slowly, and as the result either of the formation of an acid or of a lab ferment.

CONTOURED, an irregular, smoothly undulating surface, like that of a relief map.

CONVEX, surface the segment of a circle, but flattened.

COPROPHYL, dung bacteria.

CORIACEOUS, growth tough, leathery, not yielding to the platinum needle.

CRATERIFORM, round, depressed, due to the liquefaction of the medium.

CRETACEOUS, growth opaque and white, chalky.

CURLED, composed of parallel chains in wavy strands, as in anthrax colonies.

DIASTASIC ACTION, Same as DIASTATIC, conversion of starch into water-soluble substances by diastase.

ECHINULATE, in agar stroke a growth along line of inoculation, with toothed or pointed margins; in stab cultures growth beset with pointed outgrowths.

EFFUSE, growth thin, veily, unusually spreading.

ENTIRE, smooth, having a margin destitute of teeth or notches.

EROSE, border irregularly toothed.

FILAMENTOUS, growth composed of long, irregularly placed or interwoven filaments.

FILIFORM, in stroke or stab cultures a uniform growth along line of inoculation.

FIMBRIATE, border fringed with slender processes, larger than filaments.

FLOCCOSE, growth composed of short curved chains, variously oriented.

FLOCCULENT, said of fluids which contain pseudozoogloeae, i. e., small adherent masses of bacteria of various shapes and floating in the culture fluid.

FLUORESCENT, having one color by transmitted light and another by reflected light.

GRAM'S STAIN, a method of differential bleaching after gentian violet, methyl violet, etc. The + mark is to be given only when the bacteria are deep blue or remain blue after counterstaining with Bismark brown.

GRUMOSE, clotted.

INFUNDIBULIFORM, form of a funnel or inverted cone.

IRIDESCENT, like mother-of-pearl. The effect of very thin films.

LACERATE, having the margin cut into irregular segments as if torn.

LOBATE, border deeply undulate, producing lobes (see undulate.)

LONG, many weeks, or months.

MAXIMUM TEMPERATURE, temperature above which growth does not take place.

MEDIUM, several weeks.

MEMBRANOUS, growth thin, coherent, like a membrane.

MINIMUM TEMPERATURE, temperature below which growth does not take place.

MYCELIOID, colonies having the radiately filamentous appearance of mold colonies.

NAPIFORM, liquefaction with the form of a turnip.

NITROGEN REQUIREMENTS, the necessary nitrogenous food. This is determined by adding to *nitrogen-free* media the nitrogen compound to be tested.

OPALESCENT, resembling the color of an opal.

OPTIMUM TEMPERATURE, temperature at which growth is most rapid.

PELLICLE, in fluid bacterial growth either forming a continuous or an interrupted sheet over the fluid.

PEPTONIZED, said of curds dissolved by trypsin.

PERSISTENT, many weeks, or months.

PLUMOSE, a fleecy or feathery growth.

PSEUDOZOOGLOEAE, clumps of bacteria, not dissolving readily in water, arising from imperfect separation, or more or less fusion of the components, but not having the degree of compactness and gelatinization seen in zoogloeae.

PULVINATE, in the form of a cushion, decidedly convex.

PUNCTIFORM, very minute colonies, at the limit of natural vision.

RAISED, growth thick, with abrupt or terraced edges.

RHIZOID, growth of an irregular branched or root-like character, as in *B. mycoides*.

RING, Same as RIM, growth at the upper margin of a liquid culture, adhering more or less closely to the glass.

REPAND, wrinkled.

RAPID, Developing in 24 to 48 hours.

SACCATE, liquefaction the shape of an elongated sack, tubular, cylindrical.

SCUM, floating islands of bacteria, an interrupted pellicle or bacterial membrane.

SLOW, requiring 5 or 6 days or more for development.

SHORT, applied to time, a few days, a week.

SPORANGIA, cells containing endospores.

SPREADING, growth extending much beyond the line of inoculation, i. e., several millimeters or more.

STRATIFORM, liquefying to the walls of the tube at the top and then proceeding downwards horizontally.

THERMAL DEATH-POINT, the degree of heat required to kill young fluid cultures of an organism exposed for 10 minutes (in thin-walled test tubes of a diameter not exceeding 20 mm.) in the thermal water-bath. The water must be kept agitated so that the temperature shall be uniform during the exposure.

TRANSIENT, a few days.

TURBID, cloudy with flocculent particles; cloudy plus flocculence.

UMBONATE, having a button-like, raised center.

UNDULATE, border wavy, with shallow sinuses.

VERRUCOSE, growth wart-like, with wart-like prominences.

VERMIFORM-CONTOURED, growth like a mass of worms, or intestinal coils.

VILLOUS, growth beset with hair-like extensions.

VISCID, growth follows the needle when touched and withdrawn, sediment on shaking rises as a coherent swirl.

ZOOGLOEAE, firm gelatinous masses of bacteria, one of the most typical examples of which is the *Streptococcus mesenteroides* of sugar vats (*Leuconostoc mesenterioides*), the bacterial chains being surrounded by an enormously thickened firm covering, inside of which there may be one or many groups of the bacteria.

NOTES.

(1) For decimal system of group numbers see Table 1. This will be found useful as a quick method of showing close relationships inside the genus, but is not a sufficient characterization of any organism.

(2) The morphological characters shall be determined and described from growths obtained upon at least one solid medium (nutrient agar) and in at least one liquid medium (nutrient broth). Growths at 37° C shall be in general not older than 24 to 48 hours, and growths at 20° C not older than 48 to 72 hours. To secure uniformity in cultures, in all cases preliminary cultivation shall be practiced as described in the revised Report of the Committee on Standard Methods of the Laboratory Section of the American Public Health Association, 1905.

(3) The observation of cultural and bio-chemical features shall cover a period of at least 15 days and frequently longer, and shall be made according to the revised Standard Methods above referred to. All media shall be made according to the same Standard Methods.

(4) Gelatin stab cultures shall be held for 6 weeks to determine liquefaction.

(5) Ammonia and indol tests shall be made at end of 10th day, nitrite tests at end of 5th day.

(6) Titrate with $\frac{N}{25}$ NaOH, using phenolphthalein as an indicator: make titrations at same times from blank. The difference gives the amount of acid produced.

The titration should be done after boiling to drive off any Co_2 present in the culture.

(7) Generic nomenclature shall begin with the year 1872 (Cohn's first important paper).

Species nomenclature shall begin with the year 1880 (Koch's discovery of the poured plate method for the separation of organisms).

(8) Chromogensis shall be recorded in standard color terms.

TABLE I.

A NUMERICAL SYSTEM OF RECORDING THE SALIENT CHARACTERS OF AN ORGANISM. (GROUP NUMBER.)

100.	Endospores produced
200.	Endospores not produced
10.	Aerobic (Strict)
20.	Facultative anaerobic
30.	Anaerobic (Strict)
1.	Gelatin liquefied
2.	Gelatin not liquefied
0.1	Acid and gas from dextrose
0.2	Acid without gas from dextrose
0.3	No acid from dextrose
0.4	No growth with dextrose
.01	Acid and gas from lactose
.02	Acid without gas from lactose
.03	No acid from lactose
.04	No growth with lactose
.001	Acid and gas from saccharose
.002	Acid without gas from saccharose
.003	No acid from saccharose
.004	No growth with saccharose
.0001	Nitrates reduced with evolution of gas
.0002	Nitrates not reduced
.0003	Nitrates reduced without gas formation
.00001	Fluorescent
.00002	Violet chromogens
.00003	Blue "
.00004	Green "
.00005	Yellow "
.00006	Orange "
.00007	Red "
.00008	Brown "
.00009	Pink "
.00000	Non-chromogenic
.000001	Diastasic action on potato starch, strong
.000002	Diastasic action on potato starch, feeble
.000003	Diastasic action on potato starch, absent
.0000001	Acid and gas from glycerine
.0000002	Acid without gas from glycerine
.0000003	No acid from glycerine
.0000004	No growth with glycerine

The genus according to the system of Migula is given its proper symbol which precedes the number thus: (7)

BACILLUS COLI (Esch.) Mig.	becomes	B.	222.111102
BACILLUS ALCALIGENES Petr.	"	B.	212.333102
PSEUDOMONAS CAMPESTRIS (Pam.) Sm.	"	Ps.	211.333151
BACTERIUM SUICIDA Mig.	"	Bact.	222.232203

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