THE OCCURRENCE OF DIFFERENT TYPES OF THE COLON-AEROGENES GROUP IN WATER[1]

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Recent work on the colon-aerogenes group of bacteria (Levine, 1916; Rogers, Clark and Davis, 1914; Rogers, Clark and Evans, 1914 and 1915; Winslow and Kligler, 1916) has confirmed and amplified the view held by most bacteriologists that the group includes two distinct types or species represented by *B. coli* and *B. aerogenes*. Although the distinction between the two types has not been very clearly defined, the former has been considered as the predominant organism of the feces of warm blooded animals.

The work cited has demonstrated that there is a real difference between the two colon types and that this difference can be detected by certain simple laboratory tests.

The *B. coli* type is characterized by the production of almost exactly equal volumes of hydrogen and carbon dioxide in the anaerobic fermentation of glucose, the failure to give the Voges-Proskauer reaction, the formation of indol from tryptophane and the comparatively low fermentative ability of a greater part of the cultures.

The *B. aerogenes* type, on the other hand produces an excess of carbon dioxide over hydrogen in the anaerobic fermentation

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The writer wishes to acknowledge his indebtedness to his colleagues, particularly to Dr. William M. Clark for assistance and for many suggestions.

Since this paper was written, the writer has had the privilege of reading two manuscripts by Winslow and Cohen. They cover much the same ground as this paper and the results and conclusions drawn are substantially in accord with those given here.

of glucose, gives a positive Voges-Proskauer reaction, usually fails to form indol from tryptophane and ferments many carbohydrates and alcohols. The tendency to produce rapidly a hydrogen ion concentration which will inhibit alkali formation and consequently any subsequent reduction of the acidity has been made use of by Clark (1915) to distinguish the *B. coli* type from *B. aerogenes* by the methyl red test.

The *B. coli* type is found abundantly in bovine feces while the *B. aerogenes* type is very rare. In human feces the latter type is more common, but apparently subject to considerable variation. These results agree with the conclusions of some of the earlier workers, particularly Clemesha (1912) who states that sucrose +, dulcite −, adonite −, inuline −, V. and P. + organisms are rare in feces (about 1 to 2 per cent). These characters agree with those of the typical high ratio or *B. aerogenes* cultures found in feces.

The work in our laboratories on the colon-aerogenes bacteria of human feces also shows a possible line of demarcation between two varieties of the *B. aerogenes* type. All of the *B. aerogenes* cultures isolated from human feces fermented adonite. Many formed indol, 60 per cent fermented starch and in other ways showed a higher fermentative ability than *B. aerogenes* cultures from other sources.

On the other hand the collection of cultures from grains, consisting very largely of *B. aerogenes* cultures, contained very few adonite fermenters. Those that did ferment adonite agreed very closely with the adonite fermenters of feces while the non fermenters were less active in the formation of indol and in the fermentation of sucrose, raffinose, starch, mannite and glycerine. Disregarding, for the present, any question of the value of the differences enumerated for purposes of classification we believe that it is safe to use them as an indication of the original source of the culture.

It does not necessarily follow that the only source of *B. coli* or the adonite fermenting *B. aerogenes* is the intestine of warm blooded animals but the relative frequency with which they occur there makes it highly probable that any culture with these characters, wherever found, came originally from this habitat.

The value from a sanitary standpoint of an ability to distinguish between different types of colon bacilli rests on the answer which we can give to certain questions. First we are concerned with possibilities of any type of colon bacillus existing normally outside of the digestive tract of animals. If a colon culture of any kind is isolated from water does it necessarily indicate fecal contamination or must we distinguish between those of fecal and those of nonfecal origin?

Secondly what is the fate of the fecal colon bacillus in water? Does it multiply or decrease rapidly? Does the typical fecal colon bacillus become attenuated so that its characters change and it can no longer be recognized?

The first question is partially answered by the results of our comparison of the characters of fecal colon with those from other sources, particularly from grains. The marked variation of the grain cultures from the fecal types indicates that water may receive bacteria of the colon-aerogenes group from other than fecal sources.

Some light has been thrown on the second problem by the observations of Clemesha (1912). He found that the different types of colon did not decrease in water at an equal rate and that in some cases there was an actual increase.

The characters used by Clemesha differ somewhat from those which we have used and only rough comparisons can be made. We have studied this problem by observations on water held in bottles, and on cultures in parchment sacs in running water, by watching the progressive changes in streams and by examination of individual samples from various sources.

While it is realized that the results are not sufficiently comprehensive to permit any positive conclusions it is hoped that they may present something of value.

CHANGES IN SAMPLES HELD IN BOTTLES

In considering bacterial changes in water samples held in bottles, one should remember that the conditions are not necessarily comparable with those obtained in streams or reservoirs.

The concentrations, both of food-stuffs and products of metabolism, are likely to be much greater in the bottle and the results may vary accordingly.

A number of bottles containing 100 cc. of sterile water to which a loopful of human feces had been added were left in a 20° incubator for several months.

These bottles were used for another purpose and from the earlier plates made only a few cultures were isolated. Later a total colon count was made and a considerable number of cultures isolated. In table 1 are given the results from a bottle which was held at 20° for 278 days.

TABLE 1

Changes in colon content in water held at 20°

AGE	COLON GROUP PER CUBIC CENTIMETER	CULTURES ISOLATED		P_H LIMIT OF B. COLI CULTURES						INDOL BY B. COLI CULTURES	
		B. aerogenes	B. coli	4.8	4.9	5.0	5.2	5.4	5.6	+	−
days											
0		0	3	1	2					3	0
17		1	8	2	5	1				8	0
31	4,700,000	1	6	2	3	1				5	0
47	2,100,000	0	9			9				9	0
78	3,530,000	0	12			11	1			11	1
109	2,530,000	4	46								
137	2,090,000	7	58								
160		8	32				23	9		24	1
199	174,000	11	31	2		26	1	1	1	23	8
278	38,750	39	1				1				

The total number of colon forms decreased slowly in this period. At first there was a great preponderance of *B. coli* over *B. aerogenes*. Even after 100 days there were ten times as many *B. coli* as *B. aerogenes*. But this relation was gradually changed until at 278 days there was only one *B. coli* in the 40 cultures isolated.

Another change which may be of some significance is found in the apparent decrease in limiting hydrogen ion concentration reached by the *B. coli* cultures. The P_H value of 5.0 to 4.8 observed in the first cultures isolated is normal for freshly iso-

lated *B. coli* cultures. In the *B. coli* cultures isolated after they had been exposed for a long time to the unfavorable conditions of the water there was some evidence of lowered vitality, in the large numbers of cultures which carried the hydrogen ion concentration[2] to 5, 5.2 or 5.4. What may possibly be another evidence of attenuation is seen in the number of *B. coli* cultures which fail to form indol among those isolated after prolonged exposure to the water.

Many water bacteriologists consider weak gas formation an indication of "attenuation" and therefore evidence of a remote contamination.

This view is supported by some observations on six cultures of the *B. aerogenes* type isolated from this bottle of water. Three of these cultures were isolated when the sample was 47 days old and three at 278 days. All of these cultures gave a normal fermentation in glucose. The three cultures isolated at 47 days produced from 10 cc. of lactose broth approximately 2.7 cc. of gas consisting of carbon dioxide and hydrogen in the ratio of 1.53, 1.54 and 1.48 respectively. This is the normal fermentation for the *B. aerogenes* type. The three cultures isolated at 278 days gave, under similar conditions, 3.77, 3.78 and 3.77 cc. of gas with a ratio of 0.59, 0.56 and 0.51 respectively. These cultures were of course, not identical with those isolated at the earlier date and the evidence of "attenuation" is thus purely circumstantial.

However all of these observations considered together point to a slow change, which may be described as a loss of function, in colon cultures held for a long time in water. This change was perceptible only after the culture had been in water for many weeks.

These results may seem at variance with conclusion reached by Browne who studied changes in bottled water under similar conditions. The conflict is probably only apparent.

Browne's sample was held only 73 days and in that period there was little evidence of change in our sample. Moreover

[2] It should be remembered that the numbers on Sorensen's scale run *inversely as the hydrogen ion concentration.*

Browne used the MacConkey-Jackson system of classification which is merely the possible arrangements of plus and minus signs under sucrose and dulcite and has no relation to the varieties arranged by nature.

CULTURES HELD IN WATER IN PERMEABLE SACS

Conditions more nearly approximating those found when sewage is emptied into streams were obtained by holding cultures or fecal matter in parchment sacs suspended in running water. This was repeated several times both in the laboratory and in small streams, but nearly every experiment came to an untimely end through overheating, freshets or other causes before very complete results were obtained.

TABLE 2

Changes in colon bacteria in running water

AGE	COLON GROUP PER CUBIC CENTIMETER	RATIO B. AEROGENES TO B. COLI
days		
0	190,000	1:2.3
1	130,000	1:4
2	19,000	1:3.3
3	9,000	1:1.2
4	20	1:1.1
7	30	1:0.11

In all cases in which the temperature of the water was relatively high there was an increase in the colon bacteria of both types.

Table 2 shows the results obtained by holding a small amount of dilute sewage in a parchment sac in running water. The sac was made by folding parchment paper around a bottle from which the bottom had been removed. This bottle properly protected, was held in running tap water at a temperature of 16 to 20.6° C.

There was no increase in numbers observed but otherwise these results agreed with those obtained on other sacs under similar conditions. The total number decreased far more rapidly than was the case in the bottle held in the incubator at 20° and

the change in the ratio of *B. aerogenes* to *B. coli* was correspondingly abrupt. The initial determinations showed three or four times as many *B. coli* as *B. aerogenes* but at 7 days there were ten times as many *B. aerogenes* as *B. coli.*

There is a possibility that on account of imperfections in the parchment there was a mechanical loss of bacteria. The results obtained were however consistent and in accord with those reported by other investigators. Moreover it is very improbable that a mechanical loss would have resulted in the relative changes observed in the abundance of the two species.

RELATIVE CHANGES IN COLON-AEROGENES BACTERIA IN POLLUTED STREAMS

It is difficult to even approximate the total number of colon-aerogenes bacteria in a polluted stream but the relative number of *B. coli* and *B. aerogenes* may be obtained with a fair degree of correctness by isolating a considerable number of colonies and determining the group to which they belong by the methyl red test.

This was done on two representative streams. The samples, which were collected by following down the stream in an automobile, were held in ice water and taken to the laboratory at once. They were plated on asparagin agar, a medium on which colon types grow well, but which is not favorable to many other bacteria particularly the streptococci.

One of these streams was Wolf Creek, a rather sluggish stream originating in swamps and flowing through Grove City, a town of about 4000 inhabitants. On its entire course through the town it is rendered stagnant by a dam and is polluted by houses and stables on its banks. At the lower limits of the town it receives the untreated city sewage. Below Grove City it flows through a partly wooded farming country and for 15 miles receives no sewage. Samples were collected at approximately 2 mile intervals and a number of colon cultures obtained from each by direct plating. There is of course an element of chance in picking cultures in this way but the results shown in table 3 are probably fairly representative.

Above the city where there was no sewage pollution all of the cultures isolated were *B. aerogenes.* After flowing through the town, by which it was badly polluted, there was a preponderance of cultures of the *B. coli* type. Two miles below the city sewer there was only 1 *B. aerogenes* culture in 11 isolated. This ratio changed rapidly however, and *B. aerogenes* soon outnumbered *B. coli* though the latter type was still present ten miles below the sewer.

Rock Creek, the second stream investigated, runs into the Potomac river between Washington and Georgetown. It is not so well adapted to this study as Wolf Creek because it receives sewage at various points and the self purification cannot be so

TABLE 3

Relative numbers of B. aerogenes and B. coli in Wolf Creek

SAMPLE NO.	MILES	POLLUTION	CULTURES ISOLATED	RATIO B. AEROGENES TO B. COLI
1	0		7	All aerogenes
2	1.1	Private sewers	15	1.1: 1
3	1.1	City sewer	18	0.8: 1
4	3.1		11	0.1: 1
5	5.1		20	9: 1
6	7.1		10	All aerogenes
7	9.1		9	3.5: 1
8	11.3		5	4: 1

satisfactorily observed. In its upper course it flows through an agricultural country and receives no direct sewage. A few miles above the District of Columbia line the untreated sewage of the village of Kensington is emptied into the stream. There are probably some private sewers before it enters Rock Creek Park in which it is protected from contamination with the exception of two small tributaries, Broad Branch and Piney Branch, both of which are evidently polluted.

The results of the study of Rock Creek are given in table 4. At a point about 10 miles above the district line a sample was taken from which 10 cultures were isolated. All of these were *B. aerogenes.* Two and one-half miles below where the stream passes the small village of Garrett Park nearly one-half of the

cultures isolated were of the *B. coli* type. From a sample taken immediately above the mouth of the Kensington sewer, 3 *B. coli* and 13 *B. aerogenes* cultures, only 2 of which fermented adonite were isolated. A few yards below over half of the cultures isolated were of the *B. coli* type. Of the 13 *B. aerogenes* cultures isolated, 7 fermented adonite which we may assume indicated fecal origin. The fermentation tubes made in the usual way and incubated at 37° did not show a greater number of lactose fermenters immediately below the Kensington sewer than just

TABLE 4

Relative numbers of B. aerogenes and B. coli in Rock Creek

SAMPLE NO.	MILES	POLLUTION	HIGHEST DILUTION SHOWING GAS		CULTURES ISOLATED	RATIO OF B. AEROGENES TO B. COLI	FERMENTATION OF ADONITE BY B. AEROGENES CULTURES	
			24 h.	48 h.			+	−
			cc.	cc.				
1	0		1.0	0.1	10	All aerogenes		
2	2.5	Village without sewers	0.1	0.1	13	1.1 : 1.0		
3	7		0.01	0.01	16	4.3 : 1.0	2	11
4	7	Kensington sewer	0.01	0.01	28	0.86: 1.0	7	6
5	9		0.1	0.01	22	1.0 : 1.0	4	7
6	10.7		0.01	0.01	22	1.0 : 1.0	5	5
7	12.7	Broad Branch	0.1	0.1	17	1.4 : 1.0		
8	14.5	Piney Branch Zoological Park	0.1	0.1	14	1.0 : 1.0		
9	15.7		0.001	0.001	24	1.0 : 1.1		

above it; that is there was gas in 0.01 cc. dilution but none in the 0.001 cc. dilution in each case. Accurate counts could not be made from the plates but they indicated a great increase at this point.

Samples taken at lower points on Rock Creek showed a slight increase in the proportion of *B. aerogenes* but below the mouth of Broad Branch and Piney Branch bacteriological conditions as shown by these tests were nearly as bad as just below the Kensington sewer. This is probably due to a badly polluted condition in Piney Branch.

THE EXAMINATION OF INDIVIDUAL SAMPLES OF SURFACE WATER

In the course of this work about 30 samples of water from a great variety of sources have been examined. These have included samples from grossly polluted streams such as Rock Creek and the Anacostia river and from springs in the Maine woods in which the chance of pollution was remote. With a few exceptions bacteria of the colon-aerogenes type were isolated from these samples without difficulty. The exceptions were a Maine lake without pollution except from a few camps on the shores, a small stream flowing into this lake and which at no point was near a habitation or a highway, a spring flowing out

TABLE 5

Comparison of cultures from grain, water and feces

SOURCE OF CULTURES	TOTAL CULTURES	B. COLI TYPE	B. AEROGENES TYPE	B. CLOACAE TYPE	B. AEROGENES TYPE				B. CLOACAE TYPE				AVERAGE GELATIN LIQUEFICATION
					Indol	Mannite	Dulcite	Adonite	Indol	Mannite	Dulcite	Adonite	
					per cent +	*per cent* +	*per cent* +	*per cent* +	*per cent* +	*per cent* +	*per cent* +	*per cent* +	*mm.*
Grain....	159	8	111	40	7.20	20.72	16.21	12.61	0	97.5	100	0	5
Water....	134	54	67	13	30.98	91.04	23.88	71.21	15.38	84.62	15.38	58.33	16
Feces.....	177	131	46	0	21.74	100.0	21.74	100.0					

of the gravel on the shore of the lake and a well protected spring in Rock Creek Park, Washington. In all other cases at least 2 or 3 colon cultures were obtained by direct plating.

A total of 134 cultures were isolated from these samples. In table 5 the characters of these cultures as a group are compared with those from grains and from human feces. There will be noticed a general tendency for the water cultures to agree with those of fecal origin rather than with those isolated from grains.

In making this comparison it should be remembered that the grain cultures included some which were very probably of fecal origin while the water cultures included some evidently not of fecal origin. The 12 per cent of the *B. aerogenes* cultures from

grains which fermented adonite had all the characters of the *B. aerogenes* cultures from feces. If these tabulations should be made on this basis, it would be found that the cultures of the fecal *B. aerogenes* type from water would agree very closely with those from feces while they would be quite distinct from the grain cultures. There is a decided difference in the characteristics of the liquefying cultures from grains and from feces but in the light of our present knowledge of this sub-group, it would be unsafe to make any definite deductions from these data. Greenfield, (1916) found that of 405 cultures from ground and surface waters 70 per cent were of the *B. coli* type as indicated by the methyl red and Voges-Proskauer tests.

More light can be thrown on the value of a qualitative examination on the colon bacteria by a study of the results from individual samples. Space will not permit a consideration of all the samples but a few representative ones are given.

No. 21. Rock Creek. This is a polluted stream previously described. Two samples were taken from which 13 cultures were isolated. Eleven of these were high ratio, gelatin −, indol −, adonite +, dulcite −, sucrose and salicin +. One differed from these in being indol + and dulcite +. The characters of these cultures agreed very closely with those of the high ratio cultures isolated from human feces.

In view of the results obtained in the survey of Rock Creek it is rather surprising that no *B. coli* cultures were obtained from these samples. The samples from which *B. coli* were isolated were taken about a year later than those giving all *B. aerogenes*. The disappearance of the *B. coli* type may be looked upon as evidence of self purification and in this the time element is an important factor. The rate of flow which varies greatly in a small stream has a direct influence on the time for which sewage is exposed to purifying influences before it reaches a given point. There is also a possibility that the pollution in the lower part of Rock Creek may have become materially increased after the first samples were taken.

No. 10. The Potomac River. The pollution of this river has been very thoroughly studied (Cumming, 1915). The principal source of pollution is the city of Cumberland about 180 miles above Washington. Sewage is emptied into the river or its tributaries at other points nearer Washington but, considering the volume of water flowing in the river they are relatively unimportant.

Ten cultures were obtained from two samples collected near Washington when the river was in normal flow. These included three cultures of the *B. coli* type which were typical in every way except that they had a hydrogen ion limit of 5.2 to 5.4. In this regard they corresponded to the cultures held in water many weeks rather than with freshly isolated fecal cultures.

The 7 *B. aerogenes* cultures included 5 which fermented adonite and starch and otherwise agreed with the fecal type.

No. 30. Anacostia River. One sample was taken at the bridge below Bladensburg when the flow was above normal. This stream is polluted by the sewage of Hyattsville and other smaller villages. All of the nine cultures were of the *B. coli* type and had a hydrogen ion limit of 4.8 to 5.3.

These results indicate a high pollution of recent origin, comparing as they do with results obtained a short distance below the sewer in Rock Creek and Wolf Creek.

No. 28. Pimmit Run. This is a relatively small stream flowing into the Potomac at Chain Bridge. It probably receives no direct sewage but flows through an agricultural country from which it is contaminated by surface wash. No *B. coli* cultures were obtained from the one sample examined but of the 8 *B. aerogenes* cultures isolated 6 fermented adonite and starch and were probably of fecal origin. One fermented starch but not adonite and one fermented neither adonite nor starch.

No. 26. Spring near Chain Bridge. This spring is in a rather sparsely settled suburban district and was carefully protected from surface contamination by tiles and stone work. The source of the water was not evident and the possibility of contamination was a matter of conjecture.

The houses in the vicinity were, for the most part connected with sewers. Five *B. aerogenes* cultures were isolated, all of which fermented adonite and starch and were therefore of the fecal type.

No. 11. Spring near Little Falls. This spring at a camp on the Virginia shore of the Potomac was carefully protected by stone work. The shore is wooded for nearly a half mile from the river. The camps in the vicinity are occupied at irregular intervals and there are no houses within a mile.

The spring flows from gravel at the foot of an abrupt rocky hill and so far as an examination of the surroundings shows there is no reason to expect contamination. Of the 5 cultures isolated 3 were *B. coli* and two were *B. aerogenes* fermenting adonite and starch.

No. 6. Davis Brook. This is a very small stream in the Maine woods. About 1 mile above the point where the sample was taken it flows from a small but very deep pond fed by submerged springs. On the upper end of this pond is a summer camp. Considering the very small flow from the pond there is only a remote probability of any contamination from this camp affecting the stream.

One of the 4 cultures isolated was of the *B. coli* type. Two of the *B. aerogenes* cultures did not ferment adonite or starch and one fermented starch but the fermentation of adonite was not determined.

Lactose broth tubes inoculated with 1 cc. of water gave small amounts of gas in forty-eight hours.

No. 5. Small Brook. This is a very small stream flowing through dense woods except that at about a mile above where the sample was taken it crosses a highway.

Lactose broth inoculated with 10 cc. of water gave a small amount of gas in forty-eight hours. Of the 4 gas forming cultures isolated 2 were of the *B. coli* type, one was a high ratio liquefier and one was a *B. aerogenes* which failed to ferment adonite.

CONCLUSIONS

Through its greater resistance to the unfavorable conditions found in water the *B. aerogenes* type is able to survive longer than *B. coli.* This was apparent in the water held in bottles, in the sewage held in parchment sacs, in running water and in polluted streams. From this we may draw the inference that water near the source of pollution should contain a greater proportion of *B. coli* to *B. aerogenes* than after the processes of self purification have had an opportunity to act. This was found to be the case in two sewage polluted streams. In each case the gas forming bacteria isolated above the source of pollution consisted largely of *B. aerogenes* cultures, while immediately below the sewers a majority of the cultures isolated were of the *B. coli* type.

At lower points on the streams the proportion of *B. aerogenes* increased again. In the only case in which suitable determinations were made it was found that a similar relation existed between the fecal and nonfecal types of *B. aerogenes.*

The assumption that the two types, *B. coli* and the fecal *B. aerogenes*, are distinctively fecal organisms without other habitat may make it difficult to explain their occurrence in certain samples of water in which the chances of contamination seem very remote. We have isolated both the *B. coli* type and the fecal *B. aerogenes* type from water in which the chances of pollution from dwellings or wash from farm lands is almost completely excluded. In some cases the fact that all of the cultures isolated belonged to one or the other of the two fecal types would point to a source of contamination not found by physical examination of the surroundings.

In no case was the possibility of contamination by animals completely excluded. This is especially true of the springs and brooks in the Maine woods. Deer and moose frequent water courses in the warm months and there are a number of kinds of small animals which make their homes along the banks.

Even a protected spring may be exposed to the visits of squirrels and similar animals. It is possible that the occasional colon bacillus of the fecal type found in waters presumably free from pollution may be accounted for in this way.

The possibility of fish as a source of intestinal bacteria in water is suggested by the work of Browne, (Browne 1917), who found *B. coli* in the intestinal tract of 39.8 per cent of scup examined. The feeding habits of the fish may determine the presence or absence of colon-aerogenes bacteria in its digestive tract but in no case is it likely that fish would account for more than occasional cultures.

There is also a possibility that the digestive tract of animals is not the only source of the so called fecal type of colon. At the present time there is little or no evidence that this is the case. It is true that some of our water cultures were not true to the fecal type and therefore might suggest a different variety or source. These differences were very slight, consisting for the most part in failure to form indol or in a hydrogen ion limit slightly lower than that of the typical culture.

Whatever the final conclusion may be in regard to the occurrence of these occasional cultures the fact remains that there

are two types of the colon-aerogenes group which occur in fecal matter in large numbers. While it is possible that they may also live in the soil or other material from which they may be carried to water, their presence in water is strong presumptive evidence that the water was polluted with fecal matter. One of these types has certain distinctive characters which render identification easy; the other type is not so well marked but may be identified with reasonable certainty and without great difficulty.

In one way the recent contributions to the knowledge of the colon-aerogenes group has not changed the methods of water bacteriology. The presence of any particular kind of bacteria in water is merely an indication of the existence of certain conditions and the bacteriologist must weigh all the available evidence on the basis of his experience and make his decision accordingly. However, the method which we now have of separating the colon-aerogenes group into varieties which have a very definite relation to habitat should be of material assistance in forming an opinion of the potability of a water. The value of this ability to separate the varieties of the colon-aerogenes group is much more evident if a sufficient number of cultures can be isolated from each sample to establish the relative numbers of the different types. This, we believe will prove to be of much greater value than the mere determination of the presence of colon or of any one variety of colon.

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