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A comparison of various sizes of inverted vials used in Durham fermentation tubes.

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<https://doi.org/10.18297/etd/1941>

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A COMPARISON OF VARIOUS SIZES OF INVERTED VIALS
USED IN DURHAM FERMENTATION TUBES

A Dissertation

Submitted to the Faculty
of the Graduate School of the University of Louisville
in Partial Fulfillment of the
Requirements for the Degree
of Master of Science

Department of Bacteriology

by

Blanche Mae Welsh

Year

1940

NAME OF STUDENT: Blanche Mae Welsh

TITLE OF THESIS: A Comparison of Various Sizes of
Inverted Vials Used in Durham
Fermentation Tubes

APPROVED BY READING COMMITTEE COMPOSED OF THE
FOLLOWING MEMBERS:

NAME OF DIRECTOR: _____

DATE: May 23, 1940

2004-10-16

ACKNOWLEDGEMENT

I am indebted to the helpful criticism
and valuable suggestions of Dr. J. A. Kennedy
whose direction has made this paper possible.

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INTRODUCTION

I

Bacteriologists have classified bacteria with the aid of various culture media. They have studied the physiological and chemical reactions of the micro-organisms on these media. For example, some micro-organisms decompose carbohydrate broth to produce acid and gas; others are unable to do this. Some bacteria act upon protein, digesting and liquifying it, and others are incapable of breaking down proteins. Some micro-organisms may produce a fat splitting enzyme, and some may reduce nitrates to nitrites. "The use of a relatively small series of fermentation tests, including especially dextrose, lactose, sucrose, starch and inulin, action on litmus milk, and indole formation resulted in the recognition of certain primary divisions within this group (Refik 1896, Grimbert and Legros 1900, Durham 1901, Jordon 1903)." *

In order to determine whether or not bacteria produce acid and gas in carbohydrate media an apparatus for collection of gas, if produced, must be used -- the so-called fermentation tube.

Theobald Smith, who first clearly showed the value of comparative fermentation of the various sugars in identifying and differentiating bacteria, was the first, it would seem, to use a fermentation tube in bacteriology. He employed a tube which was U-shaped to show gas production in his experiments. This tube, which bears his name, has been generally accepted in scientific studies. In his paper, "The Fermentation Tube"

* Quoted from Topley and Wilson, Principles of Bacteriology and Immunology, 2nd edition, 1936, p. 520.

which appeared in the Wilder Quarter Century Book, Smith states that he found the fermentation tube to be an apparatus of considerable antiquity and of uncertain origin; in Detmer's pflanzenphysiologisches Practicum it figured as Kuhne'scher G'dhrungsgefass. It has been more recently utilized by Einhorn for the quantitative determination of sugar in urine and by Doremus for the estimation of urea. But the first application in bacteriology was made by Smith in 1889 for the purpose of securing anaerobiosis, and in several publications (Centralblatt fur Bakteriologie, 1890, VIII, S 389; J. Boston Society of Medical Science, 1899, III, p. 340) he emphasized its fundamental usefulness for the detection of gas formed by bacteria in media containing carbohydrates. The tube devised by Smith was suitable for experimental purposes, which were not too extensive, but when used in large numbers, was found to be expensive due to ease of breaking, its initial cost, difficulty of cleaning, tediousness to work with because of difficulty of filling, and because of the special racks required to hold the tubes.

A distinct advance from the viewpoint of cheapness, ease of manipulation, and freedom from breakage was made when Herbert Durham proposed and later successfully applied in his studies of the colon-typhoid-dysentery group the tube which he describes in his paper: "A Simple Method for Demonstrating the Production of Gas by Bacteria." In this paper he gave a description of

the tube which consisted of an inverted vial placed in a larger test tube. No size for either tube was specified; the only requirement being that the inverted vial must be movable when placed in the larger tube. The media was placed in the larger tube containing the inverted vial and sterilized. The liquid entered the inverted vial after the first or second sterilization. The inoculant was added to the medium, dispersed between the large and the small tube, and after proper incubation, if the organisms were gas formers, gas would be produced in the upper region of the inverted vial.

The Durham tube is now used extensively in sanitary bacteriology and in diagnostic bacteriology. The colon-paratyphoid-typhoid-dysentery groups are so much alike in cultural characteristics that special diagnostic procedures involving inoculation of various kinds of special culture media are necessary in order to determine their exact identity. The five "sugar" media commonly used are dextrose, lactose, mannitol, sucrose, and xylose broth. Carbohydrate media are used to determine whether or not the bacteria under consideration can decompose the particular sugar with production of acid and gas or acid alone. To detect the production of acid, a dye such as brom-cresol-purple is added to the original culture media. In addition to the carbohydrate media, tryptone for indole production and brom-cresol-purple milk are utilized. In the following chart are given the differential characteristics of the enteric micro-organisms.

Biochemical Characteristics of the
colon-paratyphoid-typhoid-dysentery Group

Organism Bacterium	Motil- ity	Russell's Slant Butt		Dex- trose	Lac- tose	Sucr- ose	Mann- itol	Xyl- ose	BCP Milk	Ind- ole
alkaligenes	+	-	-	-	-	-	-	-	-	-
aerogenes	-	A	AG	AG	AG	AG	AG		A	-
coli (ior)	+	A	AG	AG	AG	AG	AG		A	+
coli (is)	+	A	AG	AG	AG	-	AG		A	+
paratyphosum B	+	-	AG	AG	-	-	AG	AG	A-Alk	-
paratyphosum A	+	-	AG	AG	-	-	AG	-	A	-
Morgani #1	+	-	AG	AG	-	-	-	-	A-Alk	+
Newcastle dys.*	-	-	AG	AG	-	-	-	?	A-Alk	-
typhosum	+	-	A	A	-	-	A	-orA	A	-
dispar	-	-	A	A	A slow	A	A	A	A	+
Sonne	-	-	A	A	A slow	-orA	A	-	A	-
Flexner**	-	-	A	A	-	-	A	-	A	+
alkalescens**	-	-	A	A	-	+ or-	A	A	Alk	+
Shiga	-	-	A	A	-	-	-	-	A	-
para-Shiga	-	-	A	A	-	-	-	-	A	-
Schmitz	-	-	A	A	-	-or + slow	-	-	A-Alk	+

- = Negative result BCP = Brom-cresol purple A = Acid formation ior = Communior
+ = Positive result Alk = Alkaline AG = Acid and gas formation is = Communis

*The Newcastle dysentery bacillus ferments dulcitol slowly (3-9)days).

**Alkalescens ferments dulcitol; Flexner does not ferment dulcitol.

Chart taken from Laboratory Manual of Medical Bacteriology and Immunology (1939-1940)
by J. A. Kennedy. (author's permission).

In sanitary bacteriology the colon-aerogenes group is of extreme importance. Since water is subject to sewage contamination, the aim of the bacteriological analysis of a water is to find evidence of excretal pollution. Many pathogenic bacteria die soon after they reach water. The intestinal pathogens, e. g., typhoid bacilli, paratyphoid bacilli, and dysentery bacilli, may be transmitted by drinking water since they do survive for some time in water. The bacteriologist determines the presence or absence of the colon-aerogenes group. The presence of this group renders the water in question a potential source of infection from enteric pathogens and therefore non-potable. The life of the typhoid bacillus and the cholera spirillum in water is comparatively short. Examination of water known to have transmitted or even to have been suspected of transmitting these pathogens is often so delayed that the pathogenic micro-organisms, in the meantime, have perished. For these reasons the search for specific pathogenic bacteria is rarely crowned with success, and their real or apparent absence affords no good ground for judging the general safety of the water. The actual number of bacteria in water is also no absolute criterion of its wholesomeness, although taken together with other factors concerning the source and history of the water, it may be helpful in forming an opinion. The test for the colon-aerogenes group is based upon the fact that these micro-organisms, and especially Bacterium coli, are common inhabitants of the intestinal tract and are found

in great abundance in sewage. Their close biological relationship to the typhoid, paratyphoid, and dysentery bacilli, and the fact that, like the latter micro-organisms, they find their way into sewage chiefly from the discharges of the body renders their presence, especially when in large numbers, particularly suggestive. The test for micro-organisms of the colon-aerogenes group is almost universally conceded to be the most significant determination in the bacteriological examination of water since it affords the most nearly specific test of fecal contamination. The formation of 10% or more of gas in the Durham tube containing lactose broth within 24 hours at 37° C is presumptive evidence of the presence of members of the group. Lactose broth is used since it eliminates many forms unrelated to the colon group. For example, many micro-organisms will ferment glucose that will not ferment lactose and which, consequently, do not belong to the colon-aerogenes group.

.REVIEW OF LITERATURE

William Browne published a paper on "A Comparative Study of the Relative Efficiencies of Certain Fermentation Tubes", in which he compared the Durham and the Smith fermentation tubes using the liquor from oysters. He found the inverted vial more efficient in low dilutions and the Smith tube more efficient in higher dilutions. His most important conclusion was that, due to irregularities in the amount of gas produced in the tubes and the failure of some of the tubes to show any gas production, it was imperative to use the tubes (either Smith or Durham) in duplicate or triplicate.

Later Lee and Fegely in "An Experimental Study on the Relative Efficiencies of Certain Fermentation Tubes" showed the Durham tube to be superior to the Smith tube. At the end of twenty-four hour incubation periods (if efficiency is based on the accuracy obtainable) there was no difference, while at the end of longer periods, efficiency in concentrated solutions favored the Durham tube and in dilute solutions favored the Smith tube. They also came to the conclusion that the most obvious result was irregularity as shown by figures given in their charts.

Ivan Hall, who modified the Durham tube, stated that the greatest objection to the original Durham tube was in the qualitative and quantitative determination of gas; likewise, it was futile in the Smith tube to measure the gas due to varying absorption of CO_2 by the culture media. He sought to overcome the only fault of the Durham tube which would prevent it from

being equal to Smith's. This fault was the irregular results obtained in higher dilutions (as stated in Browne's paper) because of the fact that there was insufficient communication between the inside and outside of the vial due to the square end of the latter which fitted snugly against the bottom of the larger test tube. In the case of non-motile organisms a longer time would be required to reach inside of the inverted vial than to reach the upper part of the Smith tube. Hall prepared a tube which had an opening at a forty-five degree angle, and by a series of experiments concluded that the diagonally cut vial is preferable due to the fact (1) that non-motile organisms could reach inside the vial, (2) that the amount of gas produced is greater, and (3) that the gas is in a larger number of tubes than in either the Smith or the square cut vial. These above factors were considered important in presumptive tests.

In 1917, Graves prepared another tube. His objection to the Hall and Durham tubes was that you had to shake the media sufficiently to get germs into the inner tube which was not heavily inoculated. He found that shaking the tube produced gas in some tubes, while others remained gasless. In comparison with the unshaken tubes, the shaken tubes had a greater percentage of gas. Also he stated that a false negative might be given because the germs could be held near the surface of the medium by surface tension. His inner tube was a vial (30 mm x 5 mm) inverted over a wooden rod (50 mm x 2 mm) in

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a test tube (20 mm x 250 mm) containing 20 cc of media. This allowed free circulation of the medium, and shaking was more thoroughly accomplished because the rod aided in churning and in drawing and expelling the medium. By raising the mouth of the vial, a certain amount of gas, which is formed between the mouth of the inverted vial and the bottom of the larger tube, was thereby entrapped.

Neither the Hall nor the Graves modification of the Durham tube has replaced the original tube, which is now used extensively both in sanitary bacteriology and in diagnostic bacteriology.

In some laboratory supply catalogues the combination of the inverted tube in the larger tube is referred to as the "Dunham". The Arthur H. Thomas Company advertises the tube as a "Durham tube", but, in answer to my inquiry, they write that they "have never been quite sure that the late Dr. E. K. Dunham, of New York, did not devise or use a similar arrangement of tubes because the name "Dunham" was used in connection with it in the Laboratory Apparatus trade for a number of years". After searching the Index Medicus and Quarterly Cumulative Index, I have found no reference to Dr. Dunham in connection with this special tube. In response to my letters, the other companies who advertised the tube as "Dunham" did not give a reason for the use of this name.

Various catalogues also refer to standard and regular size Durham fermentation tubes approved by the American Public Health Association. In reply to my letter about specifications

for the Durham tube, the American Public Health Association answered that no reference to various sizes was given by them, and they concluded that the size had been found to be of little importance because no specified size is included in the directions for performing the test in the "Standard Methods for the Examination of Water".

MATERIALS AND METHODS

The aim of this paper was to determine the most efficient size of inverted vial used in the Durham fermentation tube. A series of fermentation experiments was run on seven sizes of inverted vials. The sizes employed were 9 mm x 40 mm (flat top), 7 mm x 40 mm (flat top), 5 mm x 60 mm, 5 mm x 40 mm, 5 mm x 30 mm, 9 mm x 76 mm, and 7 mm x 45 mm (all five round top). The outer tube measured 184 mm x 18 mm; in all cases the medium used was lactose broth, and the inoculum was Bacterium coli, our strain #71.

The procedure in this experiment was based on the requirements specified for water analysis by the American Public Health Association in its publication Standard Methods for the Examination of Water and Sewage, eighth edition, 1936.

In Standard Methods for the Examination of Water and Sewage it states that ~~any~~ type of fermentation tube may be used provided it allows conformance to the requirement for concentration of nutritive ingredients, which are that lactose broth, prepared according to the directions given (Part VII, Section III E), contains amounts (0.5%) of peptone and lactose which yields an optimum growth of the organisms of the coli-aerogenes group, and twice as much medium as water to be tested must be used.

The ingredients used in the lactose broth are:

Distilled water	1 kg
Beef extract	3 grams
Peptone	5 "
Carbohydrate (lactose)	5 "

To the required quantity of distilled water is added the beef extract and peptone. These are dissolved by heating

slowly on a water bath to 65° C and stirring until dissolved. After dissolving, the pH of the solution is adjusted to 7.0. The solution is then brought to a boil. Water is added to make up the weight lost by boiling. The sugar is then added and dissolved, and the solution is filtered through paper until clear. It is then dispensed in 10cc amounts in the Durham fermentation tubes and sterilized in the autoclave at about 15 pounds of pressure for 15 minutes.

After cooling, the tubes were inoculated with 1 cc of water containing a 4 mm loop of Bacterium coli per 1000 cc of water. The tubes were then placed in the incubator (37° C) for 24 hours. At the end of this time the gas formed in the inverted vial was measured in millimeters. Then the tubes were placed in the incubator and read again at the end of 48 hours.

The readings of the amount of gas formed (in millimeters) was divided by the length of the inverted vial to give the percentage of gas formed. This was then charted.

A series of eight different groups was examined. The first group contained 14 of each of the 7 various sizes of inverted tubes, the second and third had 16, the fourth and fifth had 12, and the sixth, seventh, and eighth had 20 of each size of inverted vial. This made a total of 130 of each of the various sizes or a total of 910 tubes in all.

Following are the charts (Numbers 1 to 8) showing the measurements of gas in millimeters and the percentage of gas

for each group. Also, there is a chart giving the average percentage for each size of tube in each chart.

OBSERVATIONS AND DISCUSSION

Charts in Millimeters

Chart #1

Size of
inverted vial
measured in mm

Measurement of gas (in mm) produced by Bacterium coli in the inverted vial
at the end of 24 hours incubation at 37° C.

9 x 40	12	13	13	12	13	14	13	12.5	11.5	12.5	11	12	11.5	12
7 x 40	14	12	15	11	12	11	10	12	11	11	11	10.5	11	11
5 x 60	11	12	10	11	11	12	13	11	12	12	11	12	11	11
5 x 40	10	9	10	10	10	11	12	10	10	5	9	11	15	8
5 x 30	5	5	5	5	10	8	15	7	7	5	5	5	7	5
7 x 45	13	12	14	15	13	13	14	10	12	11	9	10	13	10
9 x 76	16	15	15	15	15.5	14.5	16	16	15	15	16	16	15.5	16.5

Measurement of gas produced by Bacterium coli in the inverted vial
at the end of 48 hours incubation at 37° C.

9 x 40	14.5	15	17	15	17	16	16	15	15	17	14	16	17	15
7 x 40	15	15	20	13	13	14	13	13.5	15	16	13	13	16	14
5 x 60	16	16	15	18	16.5	17.5	17	17	15	15	15	17.5	16.5	13.5
5 x 40	12	11	13	18	16.5	17.5	17	13	12	12	13	14.5	16	12
5 x 30	12	11	13	18	13	16	15	10	10	18	8.5	11	8.5	8
7 x 45	14	16	12	14	15	16	13	13	15	14	15	14	16	15
9 x 76	21	23	22	22	20	23	21	25	22	22.5	22	23	24	22

Chart #2

Size of
inverted vial
measured in mm

Measurement of gas (in mm) produced by Bacterium coli in the inverted vial
at the end of 24 hours incubation at 37° C.

9 x 40	11.5	10	11.5	10	9	9	10	11	10	10	10	10	9.5	9	9.5	7.5
7 x 40	9	10	10	10	10	9.5	10	10	8	10	9.5	10	9.5	10	9.5	10
5 x 60	10	10	10.5	10	11	9	9	10	11	10	9	10	9	9	9	12
5 x 30	7	8	5	6	15	6	7	10	10	10	10	12	7	9	8	7
5 x 40	15	13	11	15	5	10	6	10	10	10	10	12	7	9	8	7
7 x 45	10	10	10.5	10	12	10	10.5	11	10	11.5	11	10	8.5	10	9.5	10
9 x 76	13	11.5	14	12	10	13	15	11	15	13	14	15	12.5	11	11.5	11

Measurement of gas (in mm) produced by Bacterium coli in the inverted vial
at the end of 48 hours incubation at 37° C.

9 x 40	17	18	17	17	16	19	16.5	18	16	16	15	16	15	15.5	15	14
7 x 40	14.5	14	15	17	15	16.5	16.5	14	16	13	16	15	14.5	15	14	17
5 x 60	18	17	16.5	16	18	18	16.5	17	19	17	19	16	15	16	15.5	20
5 x 40	20	16.5	16	20	8.5	15.5	20	18	14	18	17	18	13	14	18	15
5 x 30	12	15.5	11	11	18	12.5	13	14	14	13.5	12	15	11	16	12	17
7 x 45	17.5	15.5	16	14.5	19	18	17	18	16.5	17	20	18	14.5	14	16	17
9 x 76	25	20	23	20	19	21	25	21	26	21	23	25	21	18	20	20

Chart #3

Size of
inverted vial
measured in mm

Measurement of gas (in mm) produced by Bacterium coli in the inverted vial
at the end of 24 hours incubation at 37° C.

9 x 40	12	11	11.5	10	9	12	10	10.5	11	12	10	11	10	11	10.5	10
7 x 40	9	10	10	10	9	8	10	10	10	9	9	10	10.5	9.5	10	10
5 x 60	10	10	10.5	11	11	11.5	10.5	10	15	11	10	12	10	9	10	10
5 x 40	11	8	9	9	10	10	11	10	5	8	11	9	9	10	10	6
5 x 30	7	8	6.5	10	5	5	10	6	5	5	6.5	10	7	4	7	8
9 x 76	15	11	12	10	13	12	12	11	13.5	11	12	12	12	13	11	15
7 x 45	12	10	11	10	10	9	10	9.5	10	11	10	9.5	10	11	8	10

Measurement of gas (in mm) produced by Bacterium coli in the inverted vial
at the end of 48 hours incubation at 37° C.

9 x 40	18.5	20	18	17	15.5	22	17	20	18	17	16	16	15.5	17	15	15
7 x 40	14	16	16	17	15	15	16	15	15	15	15	17	13	16	16	13
5 x 60	19	18	17.5	18	20	18	19	19	18	19	22	18	16	17	18.5	20
5 x 40	21	17	20	20	6	13	18	18	14	19	15	17	10	15	16	15
5 x 30	13	14	12	12.5	20	13	14	17	15	14	10	16	12	17	11	16
9 x 76	31	25	29	26	24	25	27	26	30	27	25	29	25	21	25	25
7 x 45	17	16.5	17	15	20	20	18	18	18	18	20	20	15	18	18	18

Size of
inverted vial
measured in mm

Chart #4

Measurement of gas (in mm) produced by Bacterium coli in the inverted vial
at the end of 24 hours incubation at 37° C.

9 x 40	11	11	10	12	11	10.5	11.5	11	12.5	11.5	11	11
7 x 40	10	10	11	12.5	11	11	11.5	11	11	11	11	11
5 x 60	11.5	12	13.5	11	11.5	11	12	12	12	11	12	10
5 x 40	10	10	10	10	7	12	11.5	11	8	6.5	10	6
5 x 30	5	7	10	8	6	6	8	8	8	9	8	7
9 x 76	12	13.5	14	15	16	12	11.5	13	15	13.5	16	16
7 x 45	12.5	13	13	13.5	14	13	12.5	14	12	12.5	11	12

Measurement of gas (in mm) produced by Bacterium coli in the inverted vial
at the end of 48 hours incubation at 37° C.

9 x 40	17	18	17	18	18	17	17	17	17	17	17	16
7 x 40	14	15	16	16	15	15	14	16	16.5	16	17	15
5 x 60	18	19	19	17	19	16	20	20	23	20	19	19
5 x 40	19	15	15	16	12	15	15	16	13	16	17	12
5 x 30	14	12	13	13	11	9	12	13	14	14	15	14
9 x 76	21	22	21	20	23	20	22.5	22	25	25	24	20
7 x 45	19.5	18	20	19	20	18	17	20	17	18	17	16.5

Chart # 5

Size of
inverted vial
measured in mm

Measurement of gas (in mm) produced by Bacterium coli in the inverted vial
at the end of 24 hours incubation at 37° C.

9 x 40	11.5	10	11	12	12.5	11	11	10.5	13	12	11	11.5
7 x 40	13	10	11	10	10	10	11	10	11	10	10	10
5 x 60	10.5	10	12	12.5	10	12	11	10	12	12	11	12
5 x 40	6.5	6	10	7	10	9	8	7	8	8	7	10
5 x 30	5	6	8	6	6	7	7	5	5	8	5	7
9 x 76	15	15	13	16	16	16	15	13	14	14	15	13
7 x 45	11	11	11	11.5	11	12	12	13	11	11	10.5	11

Measurement of gas (in mm) produced by Bacterium coli in the inverted vial
at the end of 48 hours incubation at 37° C.

9 x 40	22	18	15	16	15	16.5	16	18	16	16.5	16	15
7 x 40	14	14	13	16	15	16	16	15	14	15	16	15
5 x 60	18	18	15	18	16	16	18	18	17	17	16.5	16
5 x 40	13	14	16	13	15	14	14	13	15	12	13	12
5 x 30	11	13	14	10	10	15	14	10	11	13	13	10
9 x 76	22	23	23	25	22	23	25	25	22	24	25	20
7 x 45	18	17.5	17	17	19	20	17	18	20	19	17	18

Chart #6

Size of
inverted vial
measured in mmMeasurement of gas (in mm) produced by Bacterium coli in the inverted vial
at the end of 24 hours incubation at 37° C.

9 x 40	8	9	9	9	9	9.5	9.5	9.5	10	9.5
7 x 40	8	8	7	9.5	7	8	8	7	9	9
5 x 60	8	9	9.5	10	9.5	10	9.5	9.5	11	9.5
5 x 40	6	11	5.5	6	9	6	5.5	6	19	8
5 x 30	3	6	3.5	6	3	6	9.5	3.5	5.5	11
9 x 76	16	16	11	14	12.5	11	11	11	12.5	11
7 x 45	9.5	10	9.5	11	9.5	10	9.5	9.5	11	9

Measurement of gas at the end of 24 hours (continued)

9 x 40	9.5	9	9	9.5	9.5	10	9.5	9	9	9
7 x 40	11	9	7	8	9	9.5	9	9	12	6
5 x 60	10	9.5	11	11	10	9.5	11	9.5	10	9.5
5 x 40	9.5	8	6	6	6	5.5	6	8	7	6
5 x 30	4.5	4.5	4.5	12.5	4.5	4.5	3.5	6	3.5	3.5
9 x 76	16	11	14	11	12.5	12.5	16	16	14	17
7 x 45	10	9.5	9.5	10	9.5	9.5	11	9.5	9.5	11

Chart #6 (continued)

Size of
inverted vial
measured in mm

Measurement of gas (in mm) produced by Bacterium coli in the inverted vial
at the end of 48 hours incubation at 37° C.

9 x 40	16	15	15	15	15	17	16	16	15	14
7 x 40	15	14	15	15	15	15	16	15	14	16
5 x 60	17	18	17	17	16	17	17	16	19	18
5 x 40	16	20	15	13	15	20	18	13	22	17
5 x 30	8	12	8	11	11	11	18	15	11	16
9 x 76	24	25	29	24	23	24	21	23	29	26
7 x 45	15	17	15	16	17	17	16.5	18	18	16

Measurement of gas at the end of 48 hours (continued)

9 x 40	14	15	14	15	16	16	16	18	16	16
7 x 40	15	15	14	15	15	14	15	15.5	15	16
5 x 60	18	16	18	18	18	17	16	17	17	18
5 x 40	15	13	10	11	13	10	15	16	15	14
5 x 30	10	8	8	20	14	10	11	13	10	8
9 x 76	28	25	26	24	20	25	24	23	22	25
7 x 45	18	17	16	18	18	17	18	17	17	17

Chart #7

Size of
inverted vial
measured in mm

Measurement of gas (in mm) produced by Bacterium coli in the inverted vial
at the end of 24 hours incubation at 37° C.

9 x 76	14	16	15	15	18	16	15	15	14	16
5 x 60	11	10	11	11	12	13	11	11	12	12
5 x 40	7	6	7	9	6	8	6	7	8	9
5 x 30	5	7	2	4	5	5	6	5	5	6
9 x 40	11	10	11	11	11	10	10	11	11	10
7 x 40	10	10	9	10	10	10	9	10	10	10
7 x 45	11	12	11	12	10	12	11	12	11	11

Measurement of gas at the end of 24 hours (continued)

9 x 76	12	10	15	13	15	13	12	14	15	16
5 x 60	11	11	11	12	11	15	12	12	11	10
5 x 40	8	9	10	10	8	15	8	10	11	10
5 x 30	4.5	6	6	4	15	15	5	10	5	5
9 x 40	11	11	11	12	11	11	11	12	10	10
7 x 40	10	10	10	10	11	10	11	10	11	10
7 x 45	12	12	11	12	12	15	11	12	11	11

Chart #7 (continued)

Size of
inverted vial
measured in mm

Measurement of gas (in mm) produced by Bacterium coli in the inverted vial
at the end of 48 hours incubation at 37° C.

9 x 76	26	24	24	26	21	25	27	20	30	26
5 x 60	19	18	20	19	19	20	19	22	19	20
5 x 40	13	13	14	18	14	18	20	13	18	18
5 x 30	13	11	13	9	20	11	12	11	11	18
9 x 40	17	18	17	17	15	17	18	18	18	16
7 x 40	16	16	16.5	16	15	16	15	16	17	16
7 x 45	21	17	18	17	17	18	17	17	18	18

Measurement of gas at the end of 48 hours (continued)

9 x 76	27	26	19	24	25	21	25	25	21	20
5 x 60	21	20	22	17	17	17	19	18	18	20
5 x 40	19	15	18	20	15	17	14	16	18	14
5 x 30	17	11	18	21	11	14	11	22	13	16
9 x 40	18	18	17.5	18	18	17	17	18	17	17
7 x 40	15	16	16	20	15	15	17	15	17	17
7 x 45	20	18	19	18	18	20	17	19	17	18

Chart #8

Size of
inverted vial
measured in mm

Measurement of gas (in mm) produced by Bacterium coli in the inverted vial
at the end of 24 hours incubation at 37° C.

9 x 40	11	10	11	10	11	12	11	11	10	10
7 x 40	11	9	10	11	10	10	11	10	9	10
5 x 60	11	11	10	11	10	10	11	10	12	11
5 x 40	7	7	8	7	6	11	6	14	6	6
5 x 30	9	8	9	5	1	5	10	6	5	5
9 x 76	14	14	15	13	17	18	15	14	15	14
7 x 45	11	12	11	12	11	11	12	11	11	12

Measurement of gas at the end of 24 hours (continued)

9 x 40	10.5	10	11	10	12	11	11	12	11	12
7 x 40	11	9	9	10	9	6	10	10	11	10
5 x 60	12	10	11	12	10	11	11	12	11	11
5 x 40	10	6	6	6	7	10	7	8	7	6
5 x 30	3	8	7	4	4	5	6	4	6	6
9 x 76	15	14	13	14	13	15	14	14	13	15
7 x 45	11	12	12	12	11	11	11	12	12	11

Chart #8 (continued)

Size of
inverted vial
measured in mm

Measurement of gas (in mm) produced by Bacterium coli in the inverted vial
at the end of 48 hours incubation at 37° C.

9 x 40	19	16	18	16	16	16	20	17	16	17
7 x 40	15	15	13	16	15	16	15	15	13	14
5 x 60	19	20	20	19	15	17	19	17	19	16
5 x 40	13	11	13	17	15	17	20	15	15	13
5 x 30	14	10	13	9	10	7	15	15	9	13
9 x 76	22	23	22	26	25	26	25	26	22	21
7 x 45	17	19	17	19	18	17	18	18	17	18

Measurement of gas at the end of 48 hours (continued)

9 x 40	17	16	16	17	17	16	17	18	17	17
7 x 40	15	15	14	15	15	12	14	15	15	16
5 x 60	18	18	18	18	16	17	17	20	18	19
5 x 40	15	15	13	13	18	15	20	18	12	12
5 x 30	10	12	10	9	10	15	14	10	11	12
9 x 76	22	22	21	23	21	20	26	21	24	23
7 x 45	18	16	16	17	17	16	18	18	17	18

Charts in Percentages

Chart #1

Size of
inverted vial
measured in mm

Percentage of gas produced by Bacterium coli in the inverted vial
at the end of 24 hours incubation at 37° C.

9 x 40	30	32.5	30	35	32.5	31.25	28.75	31.25	27.5	30	28.7	30	32.5	32.5
7 x 40	35	30	37.5	27.5	30	27.5	25	30	27.5	27.5	28.5	27.5	27.5	27.5
5 x 60	22	24	22	20	22	24	26	22	24	24	22	24	22	22
5 x 40	20	18	20	20	22	24	20	20	10	18	22	30	20	16
5 x 30	10	10	10	10	20	16	30	14	14	10	10	10	14	10
7 x 45	28.8	26.6	31.1	33.3	28.8	28.8	31.1	22.2	26	24.4	20	22.2	28.8	22.2
9 x 76	21.05	19.73	19.73	19.73	20.4	19	21.05	21.05	19.73	19.73	21.05	21.05	20.4	21.6

Percentage of gas produced by Bacterium coli in the inverted vial
at the end of 48 hours incubation at 37° C.

9 x 40	36.25	37.5	42.50	37.5	42.5	40	40	37.5	37.5	42.5	35	40	42.5	37.5
7 x 40	37.5	37.5	40	32.5	32.5	36	32.5	33.75	37.5	40	32.5	32.5	40	35
5 x 60	32	32	30	36	33	35	34	34	30	30	30	34	33	27
5 x 40	24	22	26	36	26	30	30	26	24	24	26	29	32	24
5 x 30	16	16	20	16	20	30	30	20	20	16	17	16	22	17
9 x 76	28	30.6	29.3	29.3	26.3	30.6	28	25	29.3	29.3	30.6	29.3	31.5	29.3
7 x 45	31.1	35.5	26.6	31.1	33.3	35.5	28.8	28.8	33.3	31.1	33.3	31.1	35.5	33.3

Chart #2

Size of
inverted vial
measured in mm

Percentage of gas produced by Bacterium coli in the inverted vial
at the end of 24 hours incubation at 37° C.

9 x 40	28.75	25	28.75	25	25.5	22.5	25	27.5	25	25	25	25	23.75	25.5	23.75	25
7 x 40	22.5	25	25	25	25	23.75	25	25	25	23.75	25	25	23.75	25	23.75	22.5
5 x 60	22	20	21	20	20	18	18	20	22	18	18	20	18	18	18	24
5 x 40	10	20	12	20	20	20	22	20	10	10	12	20	10	20	12	20
5 x 30	14	16	10	12	10	12	14	20	20	12	14	20	10	20	12	20
9 x 76	17.1	15.13	18.43	15.9	13.15	17.10	19.73	17.10	18.42	19.73	16.44	14.47	15.13	14.47	14.47	19.73
7 x 45	22.2	22.2	23.3	22.2	26.6	22.2	23.3	24.4	22.2	25.5	24.4	22.2	18.8	22.2	21.1	22.2

Percentage of gas produced by Bacterium coli in the inverted vial
at the end of 48 hours incubation at 37° C.

9 x 40	42.5	45	42.5	42.5	40	47.5	41.25	45	40	40	37.5	40	37.5	38.75	37.5	35
7 x 40	36.25	35	37.5	42.5	37.5	42.5	41.25	35	40	32.5	40	37.5	36.25	41.25	37.5	35
5 x 60	36	34	33	32	36	36	33	34	38	34	38	32	30	32	31	40
5 x 40	34	36	26	28	40	32	32	40	17	31	40	36	28	36	36	30
5 x 30	24	27	22	22	36	25	26	28	28	27	24	30	22	32	24	32
9 x 76	32.89	26.31	30.26	26.31	25	27.63	30.26	27.63	34.21	27.63	30.26	27.63	23.68	26.31	27.63	26.31
7 x 45	38.8	34.4	35.5	32.2	42.2	40	37.7	40	36.6	37.7	44.4	40	32.2	31.1	35.5	37.7

Chart #3

Size of
inverted vial
measured in mmPercentage of gas produced by Bacterium coli in the inverted vial
at the end of 24 hours incubation at 37° C.

9 x 40	30	27.5	27.8	25	22.5	30	25	25.1	27.5	30	25	27.5	25	27.5	25.1	25
7 x 40	22.5	25	25	25	22.5	20	25	25	25	22.5	22.5	25	25.1	23.7	25	25
5 x 60	20	20	21	22	22	23	21	20	30	22	20	24	20	16	20	20
5 x 40	22	16	18	18	20	20	22	20	10	16	22	18	18	20	20	12
5 x 30	14	16	13	20	10	10	20	12	10	10	13	20	14	8	14	14
9 x 76	19.73	14.47	15.98	13.51	17.10	15.98	15.98	14.47	17.7	14.47	15.98	16.4	15.98	17.10	14.47	19.73
7 x 45	26.6	22.2	24.4	22.2	22.2	20	22.2	21.1	22.2	24.4	22.2	21.1	22.2	24.4	17.7	22.2

Percentage of gas produced by Bacterium coli in the inverted vial
at the end of 48 hours incubation at 37° C.

9 x 40	46.25	50	45	42.5	38.75	55	42.5	50	45	42.5	40	40	38.75	42.5	37.5	37.5
7 x 40	35	40	40	42.5	37.5	37.5	40	37.5	37.5	37.5	37.5	42.5	32.5	40	40	32.5
5 x 60	38	36	35	36	40	36	34	38	36	38	44	36	32	34	33	40
5 x 40	42	34	40	40	12	26	36	36	34	38	30	34	20	30	32	30
5 x 30	26	28	22	25	40	26	28	34	30	28	20	22	24	34	32	32
9 x 76	41.89	32.89	38.1	34.4	31.57	32.89	34.21	39.47	35.52	32.89	38.15	32.89	37.62	32.89	32.89	35.52
7 x 45	37.7	36.6	37.7	33.3	44.4	44.4	40	40	40	44.4	44.4	33.3	40	40	40	40

Chart #4

Size of
inverted vial
measured in mm

Percentage of gas produced by Bacterium coli in the inverted vial
at the end of 24 hours incubation at 37° C.

9 x 40	28.5	27.5	27.5	30	28.5	27.5	28.75	27.5	31.25	28.75	31.25	28.5
7 x 40	25	25	27.5	31.25	27.5	27.5	28.75	27.5	27.5	27.5	27.5	27.5
5 x 60	23	24	27	22	23	22	24	24	24	22	24	20
5 x 40	20	20	20	20	14	24	23	22	16	15	20	12
5 x 30	16	10	14	20	16	12	12	16	16	18	16	15
9 x 76	15.87	17.75	18.42	19.73	21.05	15.78	15.13	17.1	19.73	17.75	21.05	21.05
7 x 45	27.7	27.7	27.7	30	33.3	28.8	27.7	33.3	26.6	27.7	24.4	26.6

Percentage of gas produced by Bacterium coli in the inverted vial
at the end of 48 hours incubation at 37° C.

9 x 40	42.5	45	42.5	45	45	42.5	42.5	42.5	42.5	42.5	42.5	40
7 x 40	35	37.25	40	40	37.25	37.25	35	40	41.25	40	42.5	37.25
5 x 60	36	38	38	35	38	32	40	40	46	40	38	38
5 x 40	38	30	30	32	24	30	30	32	26	32	34	32
5 x 30	28	24	26	26	22	18	24	26	28	28	30	28
9 x 76	27.63	28.9	27.63	26.31	26.31	31.58	26.31	29.6	28.9	32.89	32.89	31.57
7 x 45	44.4	43.3	40	42.2	44.4	40	37.7	44.4	37.7	40	37.7	36.6

Chart #5

Size of
inverted vial
measured in mm

Percentage of gas produced by Bacterium coli in the inverted vial
at the end of 24 hours incubation at 37° C.

9 x 40	28.75	25	27.5	30	31.25	27.5	27.5	26.25	32.5	30	27.5	28.75
7 x 40	32.5	25	27.5	25	25	25	27.5	25	27.5	25	25	25
5 x 60	21	20	24	25	20	24	22	20	24	24	22	24
5 x 40	13	12	20	14	20	18	18	14	16	16	14	20
5 x 30	10	12	13	12	12	14	14	10	10	18	10	14
9 x 76	19.75	19.75	17.1	21.05	21.05	21.05	19.73	17.1	18.42	18.42	19.73	17.1
7 x 45	24.4	24.4	24.4	25.5	24.4	26.6	26.6	28.8	24.4	24.4	23.3	24.4

Percentage of gas produced by Bacterium coli in the inverted vial
at the end of 48 hours incubation at 37° C.

9 x 40	55	45	37.5	40	37.5	40	41.25	40	45	40	41.25	37.5
7 x 40	35	35	32.5	40	37.5	40	40	37.5	35	37.5	40	37.5
5 x 60	36	36	30	36	32	32	36	36	34	33	34	32
5 x 40	26	28	32	26	30	28	28	26	30	26	24	24
5 x 30	22	26	28	20	20	30	26	20	22	26	26	20
9 x 76	28.9	31.58	31.58	32.89	28.9	31.58	32.89	32.89	28.9	31.59	32.89	26.31
7 x 45	40	38.8	37.7	37.7	42.2	44.4	37.7	40	42.2	44.4	37.7	40

Chart #6

Size of
inverted vial
measured in mm

Percentage of gas produced by Bacterium coli in the inverted vial
at the end of 24 hours incubation at 37° C.

9 x 40	20	22.5	22.5	22.5	22.5	23.75	23.75	23.75	25	23.75
7 x 40	20	20	17.5	23.75	17.5	20	20	17.5	22.5	22.5
5 x 60	16	18	19	20	19	20	19	19	22	19
5 x 40	12	22	11	12	18	12	11	12	38	16
5 x 30	6	12	7	12	6	12	19	7	11	22
9 x 76	21.1	21.1	14.47	18.4	16.4	14.47	14.47	14.47	16.4	14.47
7 x 45	21.1	22.2	21.1	24.4	21.1	22.2	21.1	21.1	24.4	21.1

Percentage of gas produced at the end of 24 hours (continued)

9 x 40	22.5	22.5	23.75	23.75	25	23.75	23.7	22.5	22.5	22.5
7 x 40	27.5	22.5	17.5	20	22.5	23.75	22.5	22.5	30	15
5 x 60	20	19	22	22	20	19	22	19	20	19
5 x 40	19	16	12	12	12	11	12	16	14	12
5 x 30	9	9	9	25	9	9	7	12	7	7
9 x 76	21.1	14.47	18.4	14.47	16.4	16.4	21.1	21.1	18.4	22.3
7 x 45	22.2	21.1	21.1	22.2	21.1	21.1	24.4	21.1	21.1	24.4

Chart #6 (continued)

Size of
inverted vial
measured in mm

Percentage of gas produced by Bacterium coli in the inverted vial
at the end of 48 hours incubation at 37° C.

9 x 40	40	37.5	37.5	37.5	37.5	42.5	40	40	37.5	35
7 x 40	37.5	35	37.5	37.5	40	37.5	40	37.5	35	40
5 x 60	34	36	34	34	32	34	34	32	38	36
5 x 40	32	36	31	26	30	40	36	26	36	34
5 x 30	16	24	16	22	22	22	36	30	22	31
9 x 76	31.57	38.1	31.57	30.2	31.57	32.8	27.6	30.2	38.1	34.2
7 x 45	33.3	37.7	33.3	35.5	37.7	37.7	36.6	40	40	35.5

Percentage of gas produced at the end of 48 hours (continued)

9 x 40	35	37.5	35	37.5	40	40	40	45	40	40
7 x 40	37.5	37.5	35	37.5	37.5	35	37.5	38.7	37.5	40
5 x 60	36	32	36	36	36	34	32	34	34	36
5 x 40	30	26	20	22	26	20	30	32	30	28
5 x 30	20	16	16	40	18	20	22	26	20	16
9 x 76	36.8	32.8	34.2	31.57	26.3	32.8	31.57	30.2	28.9	32.8
7 x 45	40	37.7	35.5	40	40	37.7	40	37.7	37.7	37.7

Chart #7

Size of
inverted vial
measured in mm

Percentage of gas produced by Bacterium coli in the inverted vial
at the end of 24 hours incubation at 37° C.

9 x 40	27.5	25	27.5	27.5	27.5	25	25	27.5	27.5	25
7 x 40	25	25	22.5	25	25	25	22.5	25	25	25
5 x 60	22	20	22	22	24	26	22	22	24	24
5 x 40	14	12	14	18	12	16	12	14	16	18
5 x 30	10	14	4	8	10	10	12	10	10	12
9 x 76	18.4	21	19.7	19.7	23.6	19.7	19.7	18.4	21	15.7
7 x 45	24.4	26.6	24.4	26.6	22.2	26.6	24.4	26.6	24.4	24.4

Percentage of gas produced at the end of 24 hours (continued)

9 x 40	27.5	27.5	27.3	30	27.5	27.5	27.5	30	27.5	25
7 x 40	25	25	25	25	27.5	25	27.5	25	27.5	25
5 x 60	22	22	20	24	22	30	24	24	22	20
5 x 40	16	18	20	20	16	30	16	20	22	20
5 x 30	9	12	12	8	30	30	10	20	10	10
9 x 76	13.1	19.7	17.1	19.7	17.1	15.7	21	18.4	19.7	21
7 x 45	26.6	26.6	24.4	26.6	26.6	33.3	24.4	26.6	24.4	24.4

Chart #7 (continued)

Size of
inverted vial
measured in mm

Percentage of gas produced by Bacterium coli in the inverted vial
at the end of 48 hours incubation at 37° C.

9 x 40	42.5	45	42.5	42.5	37.5	42.5	45	45	45	40
7 x 40	40	40	41.2	40	37.5	40	37.5	40	42.5	40
5 x 60	38	36	40	38	38	40	38	44	38	40
5 x 30	26	18	26	18	40	22	24	22	22	36
5 x 40	26	26	28	36	28	36	40	26	36	36
9 x 76	34.2	31.5	31.5	34.2	27.6	32.8	35.5	39.4	39.4	34.2
7 x 45	44.4	37.7	40	37.7	37.7	40	37.7	37.7	40	40

Percentage of gas produced at the end of 48 hours (continued)

9 x 40	45	45	43.7	45	45	42.5	42.5	45	42.5	42.5
7 x 40	37.5	40	40	50	37.5	37.5	42.5	37.5	42.5	42.5
5 x 60	42	40	44	34	34	34	38	36	38	40
5 x 30	34	22	36	40	22	28	22	44	26	22
5 x 40	38	30	36	40	30	38	28	32	36	28
9 x 76	35.5	34.2	25	31.5	32.8	27.6	32.8	31.5	27.6	26.3
7 x 45	44.4	40	42.2	40	40	44.4	37.7	42.2	37.7	40

Chart #8

Size of
inverted vial
measured in mm

Percentage of gas produced by Bacterium coli in the inverted vial
at the end of 24 hours incubation at 37° C.

9 x 40	27.5	25	27.5	25	27.5	30	27.5	27.5	25	25
7 x 40	27.5	22.5	25	27.5	25	25	27.5	25	22.5	27.5
5 x 60	22	22	20	22	20	20	22	20	24	22
5 x 40	14	14	16	14	12	22	12	28	12	12
5 x 30	18	16	18	10	2	10	20	12	10	10
9 x 76	18.4	18.4	19.7	15.7	21.5	23.7	19.4	18.4	19.4	18.4
7 x 45	24.4	26.6	24.4	26.6	24.4	24.4	26.6	24.4	24.4	26.6

Percentage of gas produced at the end of 24 hours (continued)

9 x 40	26.25	25	27.5	25	30	27.5	27.5	30	27.5	30
7 x 40	27.5	22.5	22.5	25	27.5	15	25	25	27.5	25
5 x 60	24	20	22	24	20	22	22	24	22	22
5 x 40	20	12	12	12	14	20	14	16	14	12
5 x 30	6	16	14	8	8	10	12	8	12	12
9 x 76	19.4	18.4	15.7	18.4	15.7	19.4	18.4	18.4	15.7	19.4
7 x 45	24.4	26.6	26.6	26.6	24.4	24.4	24.4	26.6	26.6	24.4

Chart #8 (continued)

Size of
inverted vial
measured in mm

Percentage of gas produced by Baoterium coli in the inverted vial
at the end of 48 hours incubation at 37° C.

9 x 40	47.5	40	45	40	40	40	50	42.5	40	42.5
7 x 40	37.5	37.5	32.5	40	37.5	40	37.5	37.5	32.5	35
5 x 60	38	40	40	38	30	34	38	34	38	32
5 x 40	26	22	26	32	30	32	40	30	30	26
5 x 30	28	20	26	18	20	14	30	30	18	26
9 x 76	28.9	31.5	28.9	34.2	32.8	34.2	32.8	34.2	28.9	27.8
7 x 45	37.7	42.2	37.7	42.2	40	37.7	40	40	37.7	40

Percentage of gas produced at the end of 48 hours (continued)

9 x 40	42.5	40	40	42.5	42.5	40	42.5	45	42.5	42.5
7 x 40	37.5	37.5	35	37.5	37.5	30	35	37.5	37.5	40
5 x 60	36	36	36	36	32	34	34	40	36	38
5 x 40	30	30	26	26	36	30	40	36	24	24
5 x 30	20	24	20	18	20	30	28	20	22	24
9 x 76	28.9	28.9	27.8	31.5	27.8	26.3	27.8	34.2	33.02	31.5
7 x 45	40	35.5	35.5	37.7	37.7	35.5	40	40	37.7	40

Discussion of Charts

Chart #1

At the end of 24 hours incubation at 37 °C:

1. Inner vials 9 mm x 40 mm and 7 mm x 45 mm indicated the highest percentages of gas.
2. Inner vials 5 mm x 30 mm, 5 mm x 40 mm, 9 mm x 76 mm and 5 mm x 60 mm indicated the lowest percentages of gas, the 5 mm x 30 mm size showing the lowest percentage.
3. The highest percentage of gas, 37.5%, was found in the 7 mm x 40 mm size.
4. The lowest percentage, 10%, was found in the 5 mm x 30 mm size.
5. The 9 mm x 40 mm size had the highest percentage of gas, 9 vials out of a possible 14.

At the end of 48 hours incubation at 37 °C:

1. The 9 mm x 40 mm and the 7 mm x 40 mm vials gave the highest percentage of gas formation.
2. The lowest percentage of gas was formed in the 5 mm x 60 mm, 7 mm x 45 mm, 9 mm x 76 mm, 5 mm x 40 mm and 5 mm x 30 mm vials.
3. The highest percentage of gas formed was 42.5%-in the 9 mm x 40 mm size.
4. The lowest was 16% - in the 5 mm x 30 mm size.
5. The 9 mm x 40 mm size had the highest percentage of gas, 11 out of the 14 tubes used.

Conclusion: For total efficiency the 9 mm x 40 mm size was the most efficient. The 5 mm x 30 mm size ranked the lowest and was therefore the least efficient.

Chart #2

At the end of 24 hours incubation at 37° C:

1. The lowest percentage of gas was formed in the vials 5 mm x 30 mm, 5 mm x 40 mm, 9 mm x 76 mm, and 5 mm x 60 mm. Of these the lowest percentage of gas was in the 5 mm x 30 mm size.
2. The highest percentage of gas was found in vials 9 mm x 40 mm, 7 mm x 40 mm, and 7 mm x 45 mm.
3. The highest percentage of 28.75% was collected in the 9 mm x 40 mm size.
4. The lowest percentage, 10%, was found in the 5 mm x 30 mm size.
5. The 9 mm x 40 mm size had the highest percentage of gas in 14 out of 16 trials.

At the end of 48 hours incubation at 37° C:

1. The highest percentages of gas were shown in the inner vials 9 mm x 40 mm, 7 mm x 40 mm, and 7 mm x 45 mm.
2. The lowest percentages were shown in the 5 mm x 30 mm, 5 mm x 40 mm, 9 mm x 76 mm, and 5 mm x 60 mm.
3. The highest percentage, 47.5%, was found in the 9 mm x 40 mm size.
4. The lowest percentage, 22%, was found in the 5 mm x 30 mm size.
5. The 9 mm x 40 mm size had the highest percentage of gas in 10 out of 16 trials.

Conclusion: The 9 mm x 40 mm size of inner vials was the most efficient and the 5 mm x 30 mm size was the least efficient in collecting gas.

Chart #3

At the end of 24 hours incubation at 37° C:

1. The vials with the lowest percentage of gas were the 5 mm x 30 mm, 9 mm x 76 mm, 5mm x 60 mm, and 5 mm x 40 mm vials.
2. The vials with the highest percentage of gas were the 9 mm x 40 mm, 7 mm x 40 mm, and 7 mm x 45 mm vials.
3. The highest percentage of gas, 27.8%, was collected in the 9 mm x 40 mm size.
4. The lowest percentage of gas, 10%, was collected in the 5 mm x 30 mm size.
5. The 9 mm x 40 mm size had the highest percentage of gas in 15 out of 16 trials.

At the end of 48 hours incubation at 37° C:

1. The tubes having the lowest percentage were the 5 mm x 30 mm, 5 mm x 40 mm, 9 mm x 76 mm, and 5 mm x 60 mm vials.
2. The highest percentage of gas were found in the 9 mm x 40 mm, 7 mm x 40 mm, and 7 mm x 45 mm vials.
3. The highest percentage of gas, 55%, was collected in the 9 mm x 40 mm size.
4. The lowest percentage of gas, 12%, was collected in the 5 mm x 40 mm size.
5. The 9 mm x 40 mm size had the highest percentage of gas in 11 out of 16 trials.

Conclusion: The 9 mm x 40 mm size was the vial most efficient and the 5 mm x 30 mm size the least efficient in collection of gas.

Chart #4

At the end of 24 hours incubation at 37° C:

1. The vials with the lowest percentages of gas were the 5 mm x 30 mm, 5 mm x 40 mm, and 5 mm x 60 mm sizes.
2. The vials having the highest percentages of gas were the 9 mm x 40 mm, 7 mm x 40 mm, and 7 mm x 45 mm sizes.
3. The highest percentage of gas, 33.35%, was collected in the 7 mm x 45 mm size.
4. The lowest percentage of gas, 10%, was collected in the 5 mm x 30 mm size.
5. The 9 mm x 40 mm size had the highest percentage of gas formation in 8 out of 12 trials.

At the end of 48 hours incubation at 37° C:

1. The vials with the lowest percentages of gas were the 5 mm x 30 mm, 9 mm x 76 mm, 5 mm x 40 mm, and 5 mm x 60 mm sizes.
2. The vials with the highest percentages of gas were the 9 mm x 40 mm, 7 mm x 45 mm, and 7 mm x 40 mm.
3. The highest percentage of gas, 45%, was collected in 9 mm x 40 mm size.
4. The lowest percentage of gas, 18%, was collected in the 5 mm x 30 mm size.
5. The 9 mm x 40 mm size had the highest percentage of gas formation in 9 out of 12 trials.

Conclusion: The 9 mm x 40 mm size was the most efficient and the 5 mm x 30 mm the least efficient in collecting gas.

Chart #5

At the end of 24 hours incubation at 37° C:

1. The highest percentages of gas were attained by the 9 mm x 40 mm, 7 mm x 40 mm, and 7 mm x 45 mm vials.
2. The lowest percentages of gas were attained by the 5 mm x 30 mm, 5 mm x 40 mm, 5 mm x 60 mm, and 9 mm x 76 mm vials.
3. The highest percentage of gas, 32.5%, was collected in the 9 mm x 40 mm size.
4. The lowest percentage of gas, 10%, was collected in the 5 mm x 30 mm size.
5. The 9 mm x 40 mm size had the highest percentage of gas in 10 out of 12 trials.

At the end of 48 hours incubation at 37° C:

1. The highest percentages of gas were collected in the 9 mm x 40 mm, 7 mm x 45 mm, and 7 mm x 40 mm vials.
2. The lowest percentages of gas were collected in the 5 mm x 30 mm, 5 mm x 40 mm, 9 mm x 76 mm, and 5 mm x 60 mm vials.
3. The highest percentage of gas, 55%, was collected in the 9 mm x 40 mm size.
4. The lowest percentage of gas, 20%, was collected in the 5 mm x 30 mm size.
5. The 9 mm x 40 mm size had the highest percentage of gas in 9 out of 12 trials.

Conclusion: The 9 mm x 40 mm size was the most efficient and the 5 mm x 30 mm the least efficient in collecting gas.

Chart #6

At the end of 24 hours incubation at 37 °C:

1. The highest percentage of gas was formed in the vials 9 mm x 40 mm, 7 mm x 45 mm, and 7 mm x 40 mm.
2. The lowest percentage of gas was formed in the 5 mm x 30 mm, 5 mm x 40 mm, 9 mm x 76 mm, and 5 mm x 60 mm vials.
3. The highest percentage of gas, 25%, was collected in the 9 mm x 40 mm size.
4. The lowest percentage of gas, 6%, was collected in the 5 mm x 30 mm size.
5. The 9 mm x 40 mm size had the highest percentage of gas in 16 out of 20 trials.

At the end of 48 hours incubation at 37 °C:

1. The highest percentages of gas were found in the 9 mm x 40 mm, 7 mm x 40 mm, and 7 mm x 45 mm vials.
2. The lowest percentages of gas were found in the 5 mm x 30 mm, 5 mm x 40 mm, 9 mm x 76 mm, and 5 mm x 60 mm vials.
3. The highest percentage of gas, 45%, was collected in the 9 mm x 40 mm size.
4. The lowest percentage of gas, 16%, was collected in the 5 mm x 30 mm size.
5. The 9 mm x 40 mm size had the highest percentage of gas in 10 out of 20 trials.

Conclusion: The 9 mm x 40 mm size was the most efficient and the 5 mm x 30 mm the least efficient in collecting gas.

Chart #7

At the end of 24 hours incubation at 37 ° C:

1. The vials having the highest percentage of gas formation were the 9 mm x 40 mm, 7 mm x 40 mm, and 7 mm x 45 mm sizes.
2. The lowest percentages of gas were found in the 5 mm x 30 mm, 5 mm x 40 mm, 9 mm x 76 mm, and 5 mm x 60 mm vials.
3. The highest percentage of gas, 33.3%, was collected in the 7 mm x 45 mm size.
4. The lowest percentage of gas, 4%, was collected in the 5 mm x 30 mm size.
5. The 9 mm x 40 mm size attained the highest percentage of gas in 14 out of 20 trials.

At the end of 48 hours incubation at 37 ° C:

1. The vials having the lowest percentages of gas were the 5 mm x 30 mm, 5 mm x 40 mm, 5 mm x 60 mm, and 9 mm x 76 mm sizes.
2. The highest percentages of gas were found in the 9 mm x 40 mm, 7 mm x 45 mm, and 7 mm x 40 mm sizes.
3. The highest percentage of gas, 45.6%, was collected in the 9 mm x 40 mm size.
4. The lowest percentage of gas, 18%, was collected in the 5 mm x 30 mm size.
5. The 9 mm x 40 mm size had the highest percentage of gas in 17 out of 20 trials.

Conclusion: The 9 mm x 40 mm size was the most efficient and the 5 mm x 30 mm the least efficient in collecting gas.

Chart #8

At the end of 24 hours incubation at 37° C:

1. The lowest percentages of gas were found in the 5 mm x 30 mm, 5 mm x 40 mm, 9 mm x 76 mm, and 5 mm x 60 mm vials.
2. The highest percentages of gas were found in the 9 mm x 40 mm, 7 mm x 45 mm, and 7 mm x 40 mm vials.
3. The highest percentage of gas, 30%, was collected in the 9 mm x 40 mm size.
4. The lowest percentage of gas, 6%, was collected in the 5 mm x 30 mm size.
5. The 9 mm x 40 mm size had the highest percentage in 13 out of 20 trials.

At the end of 48 hours incubation at 37° C:

1. The lowest percentages of gas were found in the 5 mm x 30 mm, 5 mm x 40 mm, 9 mm x 76 mm, and 5 mm x 60 mm vials.
2. The highest percentages of gas were found in the 9 mm x 40 mm, 7 mm x 45 mm, and 7 mm x 40 mm vials.
3. The highest percentage of gas, 47.5%, was collected in the 9 mm x 40 mm size.
4. The lowest percentage of gas, 14%, was collected in the 5 mm x 30 mm size.
5. The 9 mm x 40 mm size had the highest percentage of gas in 20 out of 20 trials.

Conclusion: The 9 mm x 40 mm size was the most efficient and the 5 mm x 30 mm the least efficient in collecting gas.

Chart of Averages

Chart of Averages

Size of
inverted vial
measured in mm

Average percentage of gas produced by Bacterium coli in the inverted vial
at the end of 24 hours incubation at 37° C.

9 x 40	30.9	24.6	26.6	27.9	28.5	23.1	27.1	27.2
7 x 40	29.1	25	23.9	27.5	26.2	21.25	25.1	24.9
5 x 60	22.8	19.6	20.1	23.3	22.5	19.65	22.9	21.8
5 x 40	20	17.9	18.3	18	16.1	15	17.2	15.1
5 x 30	13.4	14.7	13.1	15.1	12.4	10.85	12.5	11.7
7 x 45	26.4	22.8	22.4	28.6	25.9	21.9	25.2	25.3
9 x 76	20.7	16.6	16.18	19.2	19.8	17.5	18.9	18.6

Average percentage of gas produced at the end of 48 hours incubation.

9 x 40	39.2	40.3	43.3	41.6	42.9	38.8	43.3	42.4
7 x 40	35.6	37.9	38.05	37.2	38.5	37.5	40.3	36.6
5 x 60	32.1	34.2	36.6	33.9	38.2	34.5	36.5	36
5 x 40	27.0	32.7	32.1	27.3	28.3	28.5	32.5	29.8
5 x 30	19.7	26.8	28.0	24	25.6	23.25	22.4	22.8
7 x 45	32.1	37.25	39.8	40.56	40.5	37.5	40.1	38.7
9 x 76	29.0	27.79	35.1	30.9	29.26	32.19	32.2	30.59

Discussion of Averages

Discussion of Averages

After averaging the percentages for each size tube in each group of the series, it is evident that the 9 mm x 40 mm size vial had the highest average percentage of gas formation at the end of 24 hours incubation at 37° C. in each chart with the exception of Chart #4 in which the 7 mm x 45 mm size had an average percentage of 28.6 as compared to the average percentage of 27.9 for the 9 mm x 40 mm size. At the end of 48 hours incubation the 9 mm x 40 mm size had the highest percentage of gas in all charts.

The inner vials 9 mm x 40 mm, 7 mm x 40 mm, and 7 mm x 45 mm were the most efficient in indicating gas production according to the chart of averages. The 7 mm x 45 mm size, having 4 out of 8 vials with the second highest percentage at the end of 24 hours incubation and 5 out of 8 tubes second at the end of 48 hours incubation, ranked second to the 9 mm x 40 mm size. The 7 mm x 40 mm size had 4 out of 8 second highest average percentages at the end of 24 hours incubation but ranked second only 3 out of 8 possible times at the end of 48 hours incubation.

The 5 mm x 30 mm and 5 mm x 40 mm vials had the lowest average percentages of gas formation in all groups both for 48 and 24 hour incubations. The 5 mm x 60 mm and 9 mm x 76 mm vials, although having lower average percentages than the 9 mm x 40 mm, 7 mm x 40 mm, and 7 mm x 45 mm vials, were found to be more efficient than the 5 mm x 30 mm and 5 mm x 40 mm vials.

SUMMARY AND CONCLUSIONS

The following table summarizes the number of times the 9 mm x 40 mm (flat top) inverted vial attained the highest percentage of gas during 24 and 48 hours of incubation and the total number of tubes of this size used.

24 Hours Incubation

Group	Total No. of Tubes Used	No. of times 9 mm x 40 mm size attained highest percentage
Chart #1	14	9
2	16	14
3	16	15
4	12	8
5	12	10
6	20	16
7	20	14
8	20	13
	<u>130</u>	<u>99</u>

48 Hours Incubation

1	14	11
2	16	10
3	16	11
4	12	9
5	12	9
6	20	10
7	20	17
8	20	20
	<u>130</u>	<u>97</u>

Out of 130 trials the 9 mm x 40 mm size (flat top) inverted vial showed the highest percentage of gas production 99 times at the end of 24 hours incubation. At the end of 48 hours incubation the 9 mm x 40 mm size (flat top) inverted vial had the highest percentage of gas 97 times.

NOTE: The decrease in the number of 9 mm x 40 mm vials which obtained the highest percentage of gas production at the end of 48 hours was due to the fact that tubes of other sizes which were near the 9 mm x 40 mm in gas production at the end of 24 hours replaced the 9 x 40 at the end of

48 hours incubation by showing a more rapid increase in gas production, to a small extent.

Conclusions

1. On the basis of averages the 9 mm x 40 mm size inverted vial was found to yield the highest percentage of gas formation after 24 hours and 48 hours incubations at 37° C.
2. With regard to total efficiency, the 9 mm x 40 mm size would rank as the most efficient. Out of 130 tubes used 99 attained the highest percentages after 24 hours incubation, and 97 had the highest percentages after 48 hours incubation.
3. The 5 mm x 40 mm and 5 mm x 30 mm vials were the least efficient having the lowest average percentages of gas and the lowest percentages in each group.
4. The 7 mm x 40 mm and 7 mm x 45 mm inverted vials although not as efficient as the 9 x 40 mm size were found to be more efficient than the 5 mm x 60 mm and 9 mm x 76 mm inverted vials.

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