

**Volunteers' Assessments of Confidence,
Reliability, and Overall Preference to *E. coli* Test Methods
2005 Summary**

Indiana and Iowa

Volunteers in Indiana and Iowa tested a total of 6 *E. coli* test methods during 2005. Four methods were tested in Indiana (Coliscan Easygel® -incubated, 3M Petrifilm™, Coliscan Easygel® -not incubated, and Coliscan MF method), and five methods were tested in Iowa (Coliscan Easygel® -incubated, 3M Petrifilm™, Coliscan Easygel® -not incubated, Colisure® with IDEXX Quanti-Tray/2000™ and Colilert® with IDEXX Quanti-Tray/2000™). At the end of the monitoring season, volunteers were asked to rank their confidence and reliability in the test methods they had used over the past 4-6 months. In the same survey, volunteers were asked which *one* method they preferred for enumerating *E. coli* colonies. This summary provides results of the responses received by the volunteers.

I. Volunteers' mean end of season confidence rankings

We will use mean ranking scores to assess which method the volunteers felt most confidence in using. The method with the smallest mean was the method in which the volunteers had most confidence in using. For Indiana this was the Coliscan Easygel® -Incubated; this was followed by the 3M Petrifilm™. For Iowa this was the Colisure® with IDEXX Quanti-Tray/2000™; close behind was 3M Petrifilm™ and this was followed by Colilert® with IDEXX Quanti-Tray/2000.

Table 1. Mean rankings by INDIANA volunteers regarding confidence in using the *E. coli* testing methods during the 2005 monitoring season.

End of Season Volunteer Confidence in Methods				
	N	Minimum	Maximum	Mean
Coliscan Easygel® - Incubated	5	1	2	1.40
3M Petrifilm™	5	1	3	2.20
Coliscan Easygel® - NOT Incubated	5	2	4	3.20
Coliscan MF method	5	1	4	3.20

Table 2. Mean rankings by IOWA volunteers regarding confidence in using the *E. coli* testing methods during the 2005 monitoring season.

End of Season Volunteer Confidence in Methods				
	N	Minimum	Maximum	Mean
Coliscan Easygel® - Incubated	4	2	5	3.50
3M Petrifilm™	4	1	3	2.25
Coliscan Easygel® - NOT Incubated	4	3	5	4.25
Colisure® with IDEXX Quanti-Tray/2000™	4	1	4	2.00
Colilert® with IDEXX Quanti-Tray/2000	4	1	5	3.00

II. Volunteers' mean end of season reliability rankings

Like with confidence in use of the methods, we analyzed the mean ranking value to assess volunteers' reliability in the methods.

Table 3. Mean rankings by INDIANA volunteers regarding reliability of the *E. coli* testing methods during the 2005 monitoring season.

2005 End of Season Volunteer Assessment of the Reliability of Test Methods

	N	Minimum	Maximum	Mean
Coliscan Easygel® - Incubated	5	1	2	1.40
3M Petrifilm™	5	1	3	2.20
Coliscan Easygel® - NOT Incubated	5	2	4	3.20
Coliscan MF method	5	1	4	3.20

Table 4. Mean rankings by IOWA volunteers regarding reliability of the *E. coli* testing methods during the 2005 monitoring season.

2005 End of Season Volunteer Assessment of the Reliability of Test Methods

	N	Minimum	Maximum	Mean
Coliscan Easygel® - Incubated	4	3	5	3.75
3M Petrifilm™	4	1	3	2.25
Coliscan Easygel® - NOT Incubated	4	4	5	4.50
Colisure® with IDEXX Quanti-Tray/2000™	4	1	2	1.50
Colilert® with IDEXX Quanti-Tray/2000	4	1	5	3.00

III. Results of Single Question Survey – Which one method was preferred by volunteers at the end of the season

At the close of the 2005 monitoring season, project staff in Indiana and Iowa asked their volunteers to identify the *one* method they preferred for enumerating *E. coli* colonies. In Iowa 3 volunteers indicated they preferred Colisure® Method with IDEXX Quanti-Tray™, with one volunteer selecting 3M Petrifilm™. In Indiana, 3 volunteers indicated they preferred the Coliscan Easygel® (Incubated). One volunteer chose the 3M Petrifilm™ and one volunteer chose the Coliscan MF method.

Detailed Information About Difficulties and Lessons Learned By Volunteers During 2005

Lessons Learned/Difficulties Identified Coliscan Easygel® (incubated) (Iowa and Indiana results)

Pros

- Easy to use and gave good results.
- Provided the option of using different water amounts.
- Not particularly difficult to use.
- Easy for determining E. coli bacteria
- Coliforms seemed pretty good.

Summary of Pros

- Easy to use
- Easy to identify colonies
- Simple
- Can dilute sample

Cons

- 5mL is too much water to use – it's messy and doesn't set up as well.
- Sometimes there are strange and unidentifiable growths and I am not sure whether to count them or not.
- Condensation can be an issue.
- Doing a good job of reading the colonies is definitely a learned skill.
- Making a distinction between teal and blue is difficult.
- Need to have the appropriate lighting.
- Condensation on the lid sometimes made it difficult to read.
- Varied quite a bit, got lots of questionable colonies.
- Plates sometimes grew many pink colonies, which can create problems counting coliforms bacteria colonies.

Summary of Cons

- Hard to count (blue vs. teal hard to distinguish)
- Condensation an issue
- Messy to use
- Questionable results

Suggestions/Observations

- Using 1ml samples facilitates setting up the agar quickly and reduces the likelihood of a math mistake.
- Allowing agar to cool to room temp before pouring the plates considerably reduced the amount of condensation.
- Marking all the plates, containers, bottles, etc. reduces errors and it only takes a minute.
- Adjusting the sample volume can help fine tune the accuracy of the tests
- This is the method utilized by IOWATER volunteers and has proven relatively accurate.

Lessons learned/Difficulties Identified Coliscan Easygel® (not incubated) (Iowa and Indiana results)

Pros

- Reliability of E. coli were relatively good.
- Easy to use.
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<p><u>Summary of Pros</u></p> <ul style="list-style-type: none">▪ Showed good results▪ Easy to use
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Cons

- Profusion of other Coliform bacteria was a bit too much.
- Difficult to distinguish between blue and teal when colonies are young (24 hours).
- The condensation was a negative.
- Does not work in cool houses.
- Varied too much with temperature.
- It takes so long to see results.
- Results varied a little.
- Temperature will be an issue.
- Hard to ensure a constant temperature with this method.

<p><u>Summary of Cons</u></p> <ul style="list-style-type: none">▪ Hard to count (Blue vs. teal hard to distinguish)▪ Time consuming▪ Condensation a problem▪ Regulating Temperature a problem

Suggestions/Observations

- This is my least favorite.
- Not worth much.
- I like incubated better.
- Good alternative for those without an incubator
- Using 1ml samples facilitates setting up the agar quickly and reduces the likelihood of a math mistake.
- After thawing the agar, allow it to cool to room temp before pouring the plates, it reduces considerably the amount of condensation. In addition, the plates firm up quicker.
- Marking all the plates, containers, bottles, etc. reduces errors and it only takes a minute.
- Always turn on the incubator several hours ahead of starting incubation to allow the temp to stabilize.
- Doing a good job of reading the colonies is definitely a learned skill, including making a distinction of the teal blue colonies.
- Again lighting is essential for determining colors.
- We always had a few colors to choose from.
- Some times we got quick results and sometimes it took longer.
- I seemed to have a lot of problems with this. While it wasn't incubated I didn't have the house at that even of a temperature either so there was a lot of variation in temperature between sample runs.

Lessons learned/Difficulties Identified 3M Petrifilm™ (Iowa and Indiana results)

Pros

- Easier to know what to count with this test.
- Very easy to use.
- Good results.
- Far and away the easiest to use.
- Minimal manipulation of equipment and materials.
- Easy to read results.
- It only takes a minute.
- This method is also very easy to perform.
- Probably the easiest and quickest.
- Seemed to be pretty easy to use.
- No condensation to worry about.
- Disposal is easy.
- Extremely easy to read for the nonprofessional.
- Very easy to set up.
- Worked relatively easily, spread well, and seemed to distribute the bacteria over the whole area.
- Mostly no issues or problems.

Summary of Pros

- Simple to do/easy to read
- Least amount of equipment
- Least waste
- Fastest/easy set-up
- Good results

Cons

- Sometimes the liquid spreads outside the well, then you don't feel you have the correct amount and don't know if you count color outside the area the liquid was supposed to be in.
- You had to use only one ml.
- Tend to curl when in the fridge.
- Room for error if you allow air bubbles to get into the film
- When you are reading the results we had some problems in determining if the colony was E.coli or not.
- Drawback may be having to refrigerate the opened package.
- Sometimes the gas bubbles and colors are not clear.
- Counting can be difficult when there are many colonies.
- Colonies may also grow together/merge.

Summary of Cons

- Colonies too small/close together
- Gas bubbles cause problems counting colonies
- Can only use 1 mL water per sample
- Storage of materials

Suggestions/Observations

- The bubbles definitely help but there are color differences that need more explaining as well as bubble separation.
- Keeping something on top of them helps.
- Accurate measurement of the sample is important, as is keeping the sample on a flat level surface to allow the sample to spread across the surface in its own time are important.
- Don't handle the cell part of the film while putting it in the incubator.

- Is my favorite for field testing.
- I like it.
- Didn't seem to have a "scientific feel" to it.

Lessons learned/Difficulties identified with Colisure® Method with IDEXX Quanti-Tray™ (Iowa results)

Pros

- Best kit all around.
- Easy to use at any dilution and at 100% sample strength.
- The only kit that can see *E. coli* and coliforms concentrations at less than 100 cfu/ 100 ml.
- This is very good for a groups or club.
- Mostly no issues.
- It is relatively easy to read.
- Works well, seems to change color relatively quickly and decisively.
- Fluorescence is more complete and uniform.

Summary of Pros

- Best overall kit
- Easy to use
- Florescent part works well

Cons

- The necessity for the sealer and incubator make it cost prohibitive for general field use.
- Sometimes it is difficult to decide which the re/dark red colored cells to include
- Sometimes red and magenta are very similar.
- Disposal also would be a problem for most.

Summary of Cons

- More equipment necessary
- Generates waste that's not easy to dispose of
- Magenta color differentiation difficult

Suggestions/Observations

- Most accurate (in my opinion)

Lessons learned/Difficulties identified with Colilert® Method with IDEXX Quanti-Tray™ (Iowa results)

Pros

- Very good for a club or group
- It was, of the two IDEXX methods, the easiest to read (yellow is yellow, even if pale).
- Incubation time was less
- Mostly no issues.

Summary of Pros

- Easy to use
- Reading/distinguishing colors not hard
- Takes less time

Cons

- Problems exist for disposal.
- Seems fine, but a lot of the cells change after the 24 hours
- Partial fluorescence can make this test inaccurate and difficult to use.
- Sometimes the yellow color is so faint it is difficult to decide whether to count or not.
- After storing it for a while, almost all the cells turn color.

Summary of Cons

- Colors hard to distinguish, yellow too pale
- Generates waste that's not easy to dispose of
- Counting differences after different times.

Suggestions/Observations

- Most accurate (in my opinion)
- This is my favorite
- I don't know how reliable it was, I'll leave that to the experts.

Lessons learned/Difficulties identified with Coliscan MF Method (Indiana results)

Pros

- I didn't seem to have the color determination problems as with Easy Gel and it seemed like the colonies were much more distinct and easy to count.

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| <p><u>Summary of Pros</u></p> <ul style="list-style-type: none">▪ Easy to read colonies and distinguish colors |
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Cons

- It's messy
- A very "busy" test with many places for errors to creep onto the system.
- This method was very difficult to read, especially for beginners.
- It required a different type of agar, creating one place for error.
- There was so much equipment and manipulation required.
- The Petri dishes were very tight to take apart, requiring both hands.
- The top of the Petri dishes had a frosted area for marking, but it did obscure colonies.
- Because these dishes were incubated right side up, condensation was always a problem, both in reading the results and in terms of handling and decontamination.
- It was possible to believe the top of the two-part unit (filter apparatus) was sealed and have water run out on the lab desk...less than ideal.
- Using the vacuum pump hose on the filter units created enough torque that you almost needed three hands to keep something from turning over.
- The method was too time consuming for the results.
- Sterilization of the equipment is somewhat suspect.
- This method takes the most time and allows for the most chance of contamination, error etc.
- The results are also difficult to read because of the case and light penetration.
- There are several color variations that also make it difficult to read.

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| <p><u>Summary of Cons</u></p> <ul style="list-style-type: none">▪ Time consuming▪ Bulky equipment/tricky to use▪ Color variations make it difficult to read▪ Messy to use |
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Suggestions/Observations

- This was my least favorite method
- I believe you would need additional training to make this as effective as the others.
- My least favorite method.
- Using the other methods I don't see a need to use a method like this. S
- Seems silly to add distilled water to a sample so you can dilute it.
- I don't like the apparatus to pump the water.
- I got pretty creative using my knees as a third grasping appendage.
- Using the other methods I don't see a need to use a method like this.
- I believe you would need additional training to make this as effective as the others.

Michigan, Minnesota, Ohio and Wisconsin

Volunteers in Michigan, Minnesota, Ohio and Wisconsin tested only 2 *E. coli* test methods during 2005: the Coliscan Easygel® -Incubated and 3M Petrifilm™. Following initial training sessions, volunteers were asked to rank how easy it was to learn how to use the methods. At the end of the monitoring season, volunteers were asked to rank their confidence and reliability in the two test methods they had used over the past 4-6 months. In the same survey, volunteers were asked which *one* method they preferred for enumerating *E. coli* colonies. This summary provides results of responses received from the volunteers.

I. Volunteers' ease of learning assessments from end of training surveys

Immediately following training sessions volunteers were asked to rank how easy it was to learn how to perform the methods to assess *E. coli* using both of the test kits in their state. According to the results, of those that responded 16 volunteers preferred 3M Petrifilm™ as compared to 6 volunteers who indicated a preference for Coliscan Easygel® - Incubated.

Tables 5a & 5b. Summary of post-training rankings of confidence of use by Michigan, Minnesota, Ohio and Wisconsin volunteers during 2005 and Frequency for post-training responses to rankings of confidence of use.

5a. Rank

Rank	Coliscan Easygel®		3M Petrifilm™	
	Count	%	Count	%
1	6	27.3%	16	72.7%
2	16	72.7%	6	27.3%

5b. Frequency

	Frequency	Percent	Valid Percent
Valid	22	57.9	100.0
Missing	8	13.2	
	9	28.9	
Total	16	42.1	
Total	38	100.0	

Combination of MI, MN, OH and WI Responses

NOTE: 8 = Both ranked at '1'
9 = Non response

II. Volunteers' end of season confidence rankings

The following tables indicate the results of the end of season confidence rankings as derived from the volunteer assessments.

Tables 6a & 6b. Summary of end of season rankings of confidence of use by Michigan, Minnesota, Ohio and Wisconsin volunteers during 2005 and Frequency for post-training responses to rankings of confidence of use.

6a. Rank

Rank	Coliscan Easygel®		3M Petrifilm™	
	Count	%	Count	%
1	13	44.8%	16	55.2%
2	16	55.2%	13	44.8%

6b. Frequency

	Frequency	Percent	Valid Percent
Valid	29	76.3	100.0
Missing 9	9	23.7	
Total	38	100.0	

Combination of MI, MN, OH and WI Responses

NOTE: 9 = Non response

III. Volunteers' end of season reliability rankings

The following tables indicate the results of the end of season reliability rankings as derived from the volunteer assessments.

Tables 6a & 6b. Summary of end of season rankings of reliability of use by Michigan, Minnesota, Ohio and Wisconsin volunteers during 2005 and Frequency for post-training responses to rankings of reliability of use.

7a. Rank

Rank	Coliscan Easygel®		3M Petrifilm™	
	Count	%	Count	%
1	17	63.0%	10	37.0%
2	10	37.0%	17	63.0%

7b. Frequency

	Frequency	Percent	Valid Percent
Valid	27	71.1	100.0
Missing 8	1	2.6	
9	10	26.3	
Total	11	28.9	
Total	38	100.0	

Combination of MI, MN, OH and WI Responses

NOTE: 8 = Both ranked at '1'
9 = Non response

IV. Results of Single Question Survey – Which one method was preferred by volunteers at the end of the season

At the close of the 2005 monitoring season, project staff in Michigan, Minnesota, Ohio and Wisconsin asked their volunteers to identify the *one* method they preferred for enumerating *E. coli* colonies. From the four states 15 volunteers indicated they preferred 3M Petrifilm™ while 13 volunteers indicated a preference for Coliscan Easygel® (Incubated). It is important to note that 10 of the 38 volunteers were non-responses.

Lessons learned/Difficulties Identified Coliscan Easygel® (Incubated) (Michigan, Minnesota, Ohio and Wisconsin)

Pros

- Set-up was very simple once the process was learned.
- Easy to use.
- The incubator works quite well; the temp holds @ 35°C with minimal adjustments.
- The time to conduct analysis was very little.
- Counts seemed more varied and higher

- Summary of Pros**
- Showed good results
 - Easy to use
 - Process was simple
 - Less time consuming

Cons

- Hard to tell the difference between teal and blue.
- These also took a long time to gel.
- I had to wait quite a while before I was able to put them into the incubator.
- Mixing can be inconsistent when same technique is being used.
- Counting can be variable as well with grid provided..
- It takes so long to see results.
- Results varied a little.
- Temperature will be an issue.
- Hard to ensure a constant temperature with this method.
- Gel setting time varied according to ambient temps.
- Prone to uneven distribution if slightly tilted.
- It's not as simple as it sounds.
- Hard to tell the difference between teal and blue.
- Isn't consistent with results with some dishes only having few colonies while other dishes from the same sample will have numerous colonies
- Is at times tougher to get an accurate count of E. coli colonies
- Biggest problem is becoming comfortable with identifying E. coli colonies (teal vs. blue).
- Differences in number of colonies which develop in two Petri dishes from the sample is puzzling
- On several occasions, the count after 48 hours was much higher than the 24 hours observation.
- The variety of colors and numbers of colonies changed during the season.
- Some two-color patterns were quite hard to interpret – count or don't count.
- Temperature and rainfall had significant impacts.
- It was more difficult to pick out the correct colors
- Problems early on with getting the order of the steps wrong, which resulted in bad samples.
- Wastes time and materials
- Slightly more difficult to set up and differentiate color
- I had some difficulty deciphering between E. coli and other types of coliform that was present on the plates
- This kit requires more judgment in determining which colonies are actually e. coli
- Requires slightly more time and planning because the material has to be removed from the freezer ahead of time and it must set for about an hour after inoculation before it can be put into the incubator

- Summary of Cons**
- Hard to count (Blue vs. teal hard to distinguish)
 - Time consuming
 - Condensation a problem
 - Regulating Temperature a problem
 - Wastes time/materials
 - Complicated stages

Suggestions/Observations

- Use a strong magnification and a bright light.
- I kept wanting to count the teal colonies as E. coli.
- You need to be consistent with the process and what you count.
- Allow enough time to set before incubating.
- Just really liked this method better.
- Learned to make sure the Petri dish was turned over before incubation period to ensure condensation did not affect readings.
- Really good identification reference photographs showing what is counted and which ones are not counted would be helpful.
- Also, if possible, it would be nice to know what other types of bacteria grown in this medium.
- This method seems more scientific and likely to produce better results.
- The larger volumes used (up to 5 ml) would seem to provide a better chance of seeing E. coli colonies in the incubated gels.
- Need to swirl the water sample each time that the pipette removes a given volume (settling of debris and attached bacteria may be a factor).
- As a side note, since I also do Citizen's Stream Monitoring for the MPCA and Pine County SWCD, I have a record of daily rainfall to the 0.00 inch for the same period; that kind of information might be useful in a future project.
- I learned that I could not rush the process that it paid to be very slow and deliberate.
- Remember to remove from freezer before leaving to sample. For Muskingum River, I used 200 and perhaps
- Reading the results, More examples of positive and negative readings are needed.
- I can't say that I encountered any difficulties with this kit.

Lessons learned/Difficulties Identified 3M Petrifilm™ (Michigan, Minnesota, Ohio and Wisconsin)

Pros

- No problems, this test was easy to use.
- It's easy to use.
- Easy to use and count
- Easier to read color shades and numbers.
- Grows better in Agar mixture.
- Easier to identify.
- Very easy to use and very easy to read
- This method was fairly easy to use and interpret
- This method seems to be a quick.
- Easy screening of the bacteria content of a stream or lake.
- Fastest to process
- : Seemed consistent and easy to use
- This test kit was pretty simple.
- I did not encounter any difficulty reading the results
- This kit is very simple in that it can be inoculated and then put directly into the incubator
- Almost no judgment is required
- Easier to set up and read, especially with "the bubble factor" counts seemed stable after 24 hours

Summary of Pros

- Simple to do/set-up
- Easy to read colors and numbers
- Material easy to use
- Identification not a problem
- Fast test to conduct

Cons

- Some difficulty in getting water sample to spread evenly.
- All gas bubbles are not equal. Some gas bubbles disappear after 24 hrs. along with the purple center.
- Trouble getting water to cover plate without air bubbles
- Just didn't seem to grow many colonies
- Hard to tell color variance
- Had to learn to adjust to right amount of water to cover plate. (not too much; not enough)
- Evenly spreading sample was difficult
- Also difficult to count colonies when a lot of gas bubbles was associated with the developing colonies.
- The only difficulty I had was some 48 hr. E.coli colonies mixed with another and wasn't as easy to spot as the 24 hr. sample.
- The 48 hour reading was sometimes less reliable due to merging of colonies during the second 24 hours – they often became very large.
- It sometimes took a few tries to get the sample spread evenly across the film
- Sometimes the liquid would seep out of the pink circle and the films got kind of sticky when they were in the incubator.
- Hard to count because of the density of red, blue and teal colonies.

Summary of Cons

- Colonies too small/close together
- Gas bubbles cause problems counting colonies
- Temperature can impact results easily
- Poor consistency
- Challenging steps to process

Suggestions/Observations

- Place the water drop in the middle of the circle, and spread from there to avoid bubbling
- I preferred counting colonies with a color (coliscan), rather than looking for colonies with a gas bubble.
- . If it were possible to use 10 Petrifilm test per sample rather than 3, and if it was statistically proper to combine the results of all the test to extrapolate to the cfu/100 ml standard, I would feel more comfortable with the results.
- One I would recommend to other lake associations.
- I did learn that the places we did testing from proved very tricky when the water was low, the mud made the spots difficult (and eventually unsafe) to enter. If I were to do testing again I would choose a different location to ensure consistency throughout the test.
- Limited to 100 ml sample which wasn't large enough to get a reading for Muskingum River THIS YEAR – may not be the same other years with higher flow.
- Reading the results, more examples of positive and negative readings are needed.
- This test kit did not seem as accurate as the Coliscan Easy Gel kit.