

***E. coli* Monitoring – Effective Techniques and Test Kits for Volunteers**



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Why Monitor Bacteria?

Anything that adds fecal material to water can add bacteria and pathogens

- *Humans*

- swimming “accidents”
- malfunctioning septic systems
- sewage treatment plant discharges

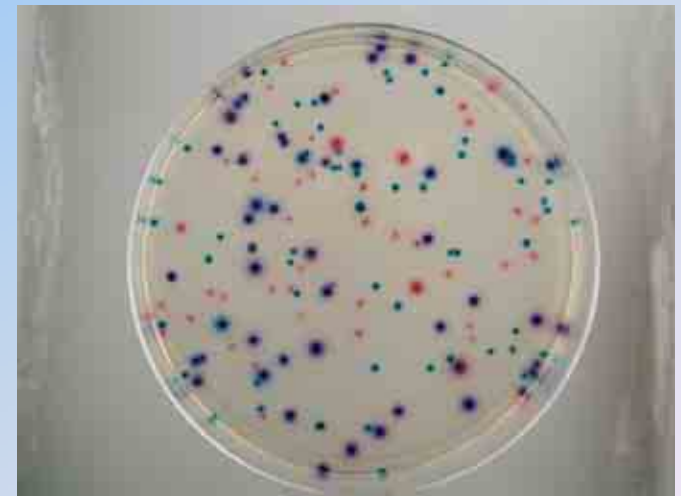
- *Animals* (warm blooded animals)

- direct contribution
- runoff from fields after manure application
- storm water runoff from lands with wildlife
- pet droppings



Using *E. coli* or Fecal Coliform Bacteria

- Bacteria can be used as indicator bacteria since they can suggest the presence of pathogens in water
 - Pathogens are disease-causing microorganisms which cause illnesses such as fever, sore throats, diarrhea, abdominal cramps, hepatitis, respiratory infections and chest pains. Symptoms may be mild and confused with other diseases, so people may not realize that water got them sick.
- Bacteria are easy to collect and analyze, relatively safe to handle and are usually present when pathogens are present
- Are generally harmless
- The more fecal coliform populations present in water, the greater likelihood that pathogens are present.

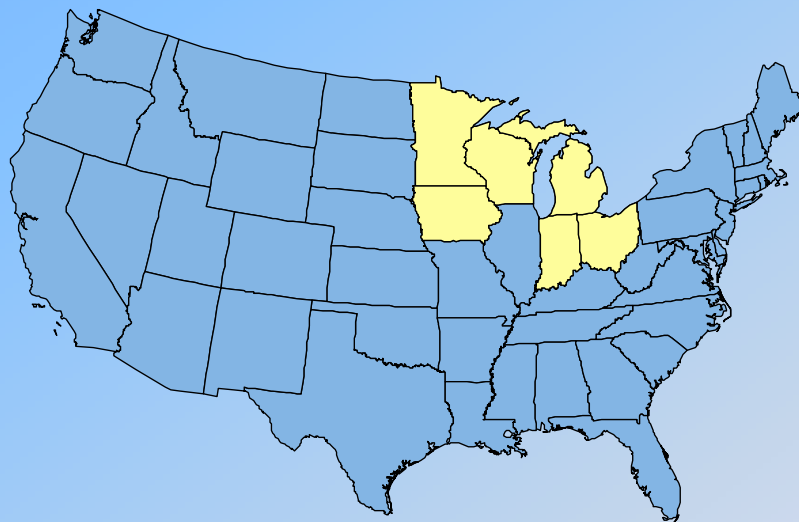


Why not sample for pathogens?

- Difficult to isolate and identify
- Few laboratories capable of analyzing for pathogens
- Cost
- Length of time to analyze
- Results for only one pathogen
- Number of pathogenic organisms present in polluted waters are generally very low



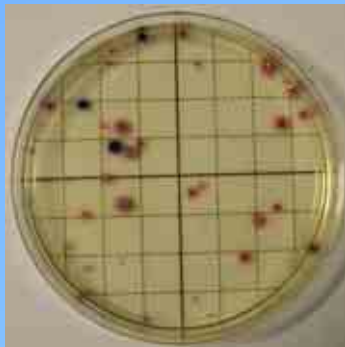
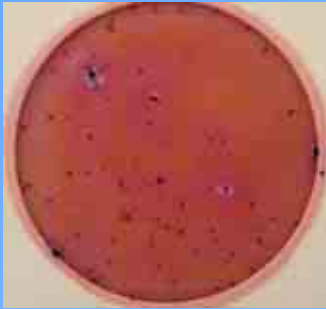
Volunteer Monitoring of *E. coli* in Upper Midwest Streams: A Comparison of Methods and Preferences



Project Goals

- Build the capacity of Volunteer Monitoring programs to understand and use the most appropriate *E.coli* testing protocols (test kits) and develop watershed based sampling strategies.
- Compare and test current “bacteria kits” and compare them with certified lab analyses
- Develop a comprehensive training program for volunteers on *E. coli* testing in targeted watersheds in six states.
- Share results of our work with other states across the country, primarily via the National Volunteer Monitoring Facilitation Project.

Why research *E. coli* test kits?



- Waters impaired by bacteria across U.S.
- Many different kits being used
- No comparative, independent study of how well kits work

Why Volunteer Monitoring?

- Monitoring provides educational opportunities for interested local residents and students.
- Citizen research data is needed to help the watershed project prioritize decisions.
- Volunteers extend limited agency resources
- Cost of lab analysis is high & access to certified labs is problematic.
- Citizens need an easy, reliable, inexpensive test.



Collection Techniques & Following Protocols



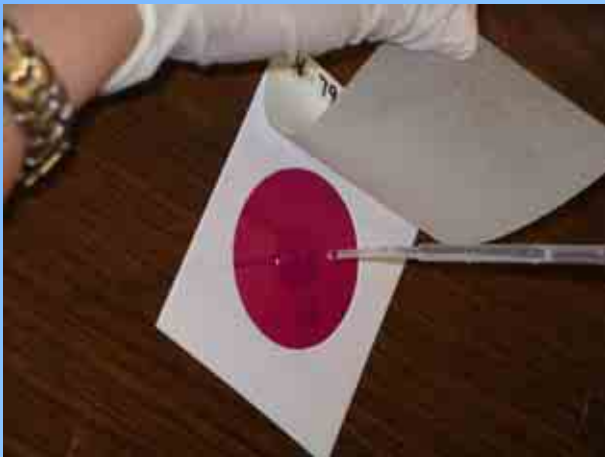
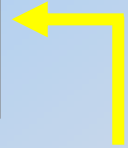
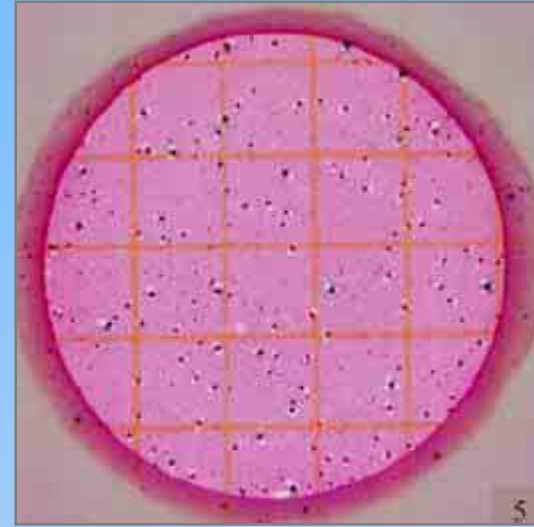
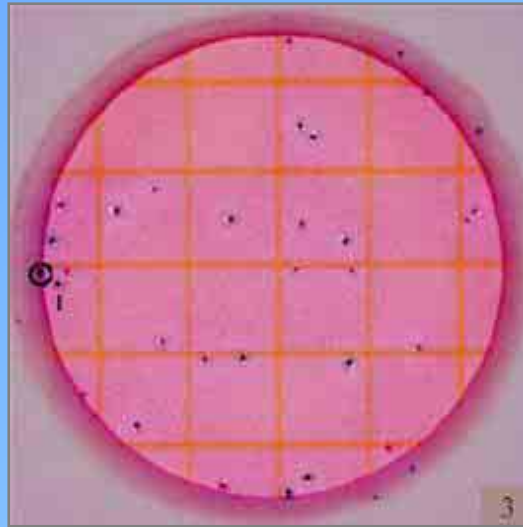
Testing Methods

- **6 E. coli Testing Kits:**
 - Coliscan® Easy Gel (incubated)
 - Coliscan® Easy Gel (not incubated)
 - 3M™ Petrifilm™
 - Coliscan® MF Method Kit (*IN only*)
 - Colisure® Method with IDEXX Quanti-Tray/2000™ (*IA only*)
 - Colilert® Method with IDEXX Quanti-Tray/2000™ (*IA only*)
- **Followed Specified Protocol for Quality Control**
 - Testing spring, summer, & fall
 - Split water sample sent to certified lab for analysis
 - Completed Data Sheet

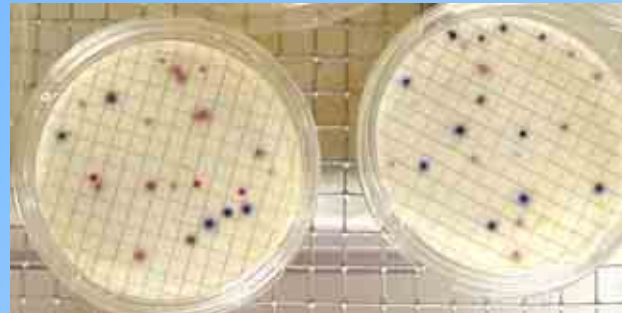
Coliscan® Easy Gel (incubated and not incubated)



3M™ Petrifilm™



Coliscan® Membrane Filtration



Colisure™ & Colilert with the IDEXX Quanti-Tray®/2000



Test 1

Ranking of the % of time the test kit & lab values were both either above or below the 235 cfu value (EPA's cutoff value for total body contact recreation)

Volunteer	NO	YES
	YES	NO
	Lab	

235 cfu?

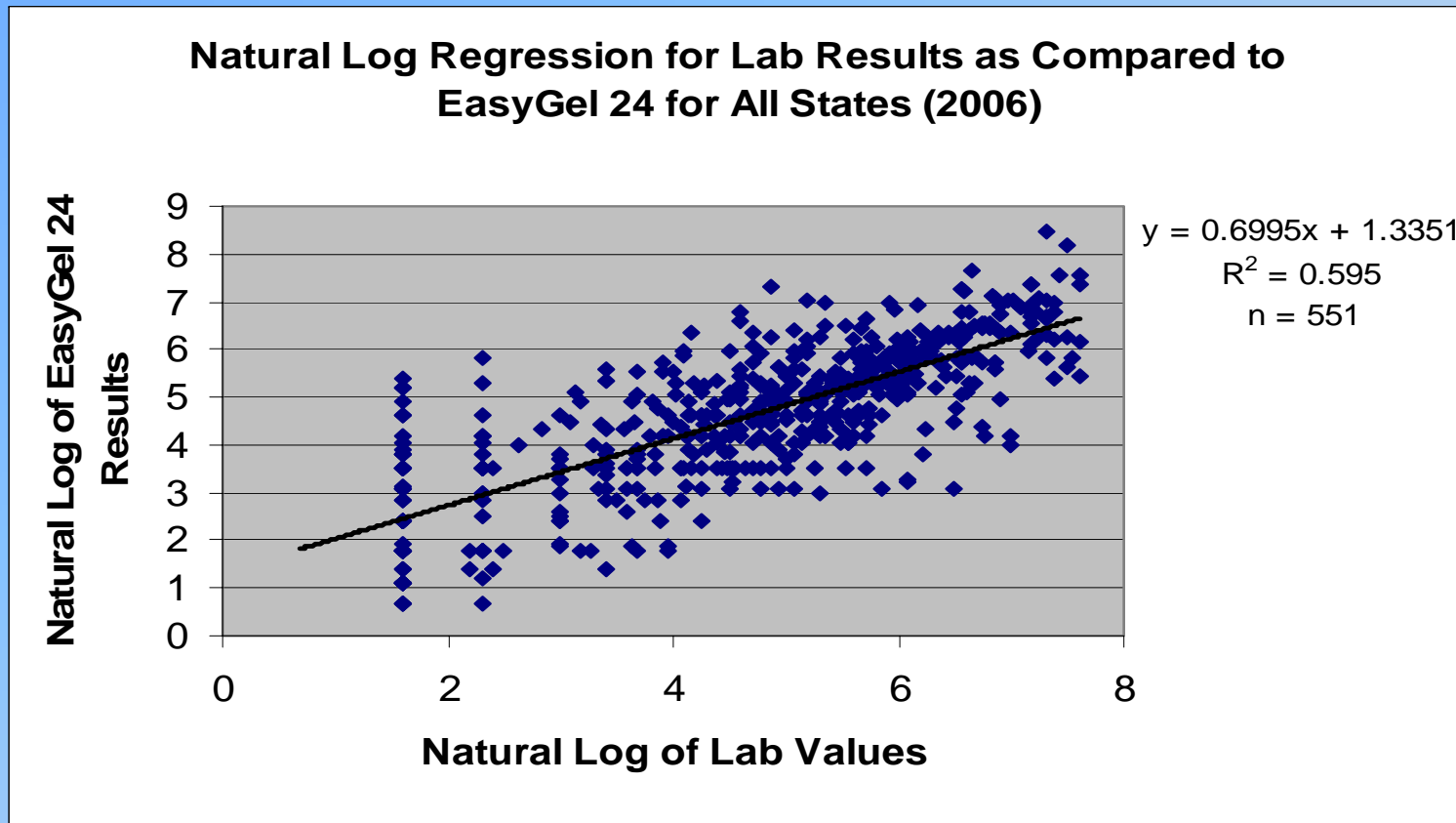
Results for 2005 Combined Data

Percent of samples with test kit and lab values both either above or below the “cutoff” value.

		235 cfu cutoff	1000 cfu cutoff
Test	n	% Agreement	% Agreement
Colisure (IDEXX)	174	87%	96%
Petrifilm (3M)	504	85%	94%
Colilert (IDEXX)	163	83%	92%
Easygel - 35°C	504	81%	92%
Coliscan MF	95	79%	92%
Easygel - Room Temp	250	63%	85%

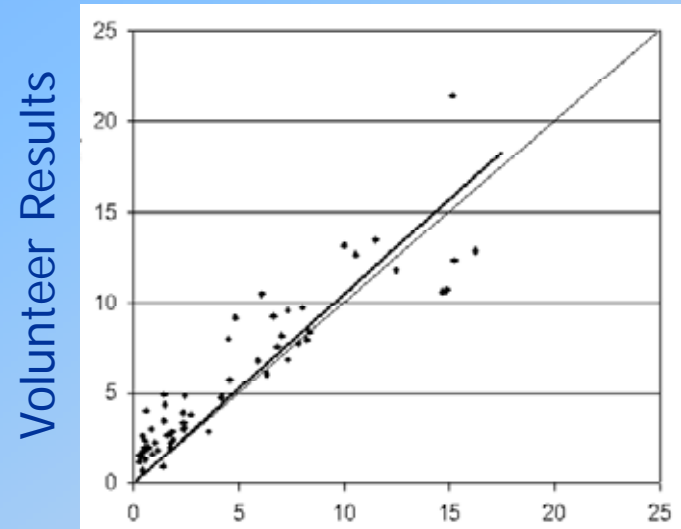
Test 2

Relating Volunteer Values with Certified Lab Values for each test kit.

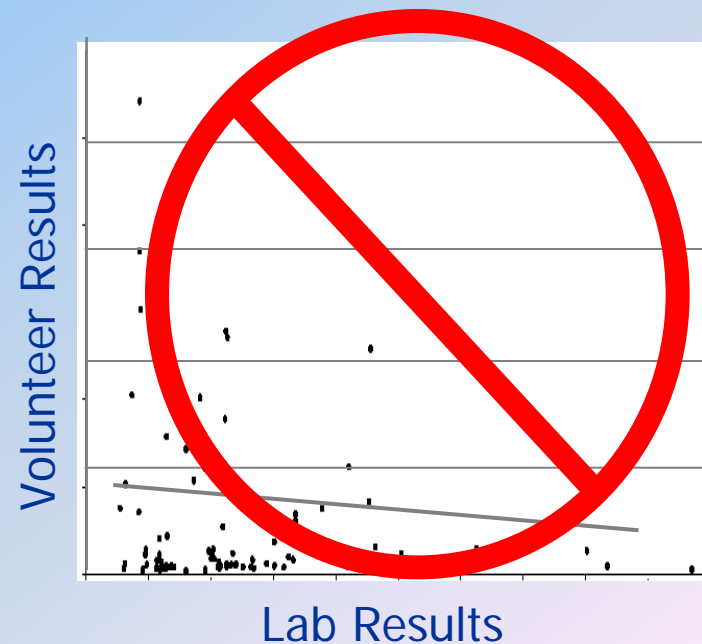
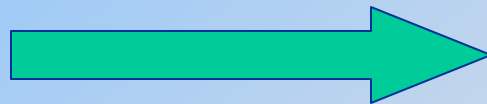


- Kits showing best relationship between volunteer and lab values are:

- IDEXX Colisure
- IDEXX Colilert
- 3M™ Petrifilm™
- Coliscan Easygel – Incubated



- Those with poor correlation
 - Coliscan Easygel – non-incubated
 - Coliscan MF (Membrane filtration)



Test 3

Cost Considerations

Method	Cost/Sample	Additional Costs
Coliscan® Easygel	\$1.85	Incubator (varies ~\$50)
3M™ Petrifilm™	\$1.06	Incubator (varies ~\$50)
Coliscan® MF	\$1.70	Incubator (varies) Filter apparatus (\$7.00) Syringe & hose (\$2.50)
Colisure™ and Colilert Methods with IDEXX Quanti-Tray®/2000	\$5.45	Incubator (varies) Sealer (\$4,000) UV light & box (\$240)

Test 4.

Volunteer Perceptions & Attitudes

Volunteer confidence in IN:

- (1) Coliscan Easygel ® -
Incubated
- (2) 3M Petrifilm

IA volunteers chose:

- (1) Colisure® with IDEXX Quanti-Tray /2000
- (2) 3M Petrifilm
- (3) Colilert® with IDEXX Quanti-Tray /2000

No selection for Membrane Filtration; Easygel Non-incubated

Volunteers in MI, MN, OH, WI:

- Ease of use immediately following “spring training”
 - (1) 3M Petrifilm
 - (2) Coliscan Easygel Incubated

- End of season confidence
 - (1) Coliscan Easygel
 - (2) 3M Petrifilm Incubated

Conclusions based on previous factors

- IDEXX, Coliscan Easygel ® (incubated) and 3M Petrifilm perform well in describing when bacteria counts are above and below 235 cfu/100 mL (EPA standard)
- Strongest correlations with lab results are IDEXX, Petrifilm, and Easygel Incubated
- Volunteers nearly equally split in their assessment of two methods (EasyGel & Petrifilm)
- IDEXX performs as well as Easygel and Petrifilm, but the cost of IDEXX methods might be too expensive for volunteer groups

Performing Bacteria Analysis using one of the selected test kits

Analyzing *E. coli* bacteria using test kits

Coliscan Easygel

Contains 2 enzymes specific chemicals (chromogens)

- “Red Gal” (galactosidase)
 - Red or pink color produced
- “X-Gluc” (glucuronidase)
 - Teal color produced
- Only *E. coli* produces both enzymes



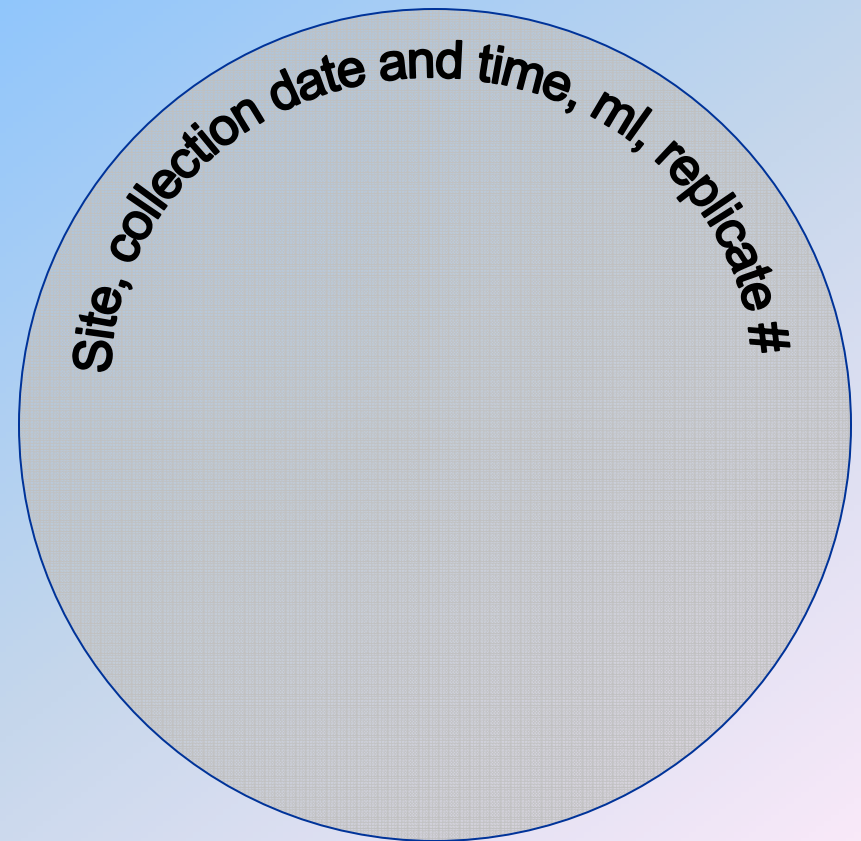
Red + Teal = Dark Blue/ purple

Preparation for Kit Use

- Avoid contamination of all sampling equipment (inside of Petri dish, tip of pipet, bottles, caps, etc.)
- Let all media come to room temperature
- Turn on incubator (fill well w/ distilled water)

Preparation continued

- Label the bottom Petri dishes using a permanent marker
 - Label should include:
 - site name
 - date and time of collection
 - volume of water plated
 - replicate #, if any
- Label bottom of dish
(WHY the BOTTOM?)
- Label along edges!!!



Coliscan Easygel

- Carefully remove pipet from sterile paper (keep sterile). You are using 1 ml pipets
- Transfer 1 ml to 3 ml of water from collection container to media bottle
 - Label your Easygel media bottle w/ amount used
- Swirl the media bottles to mix and then pour each into a labeled Petri dish (use the lid as a shield from the outside environment)

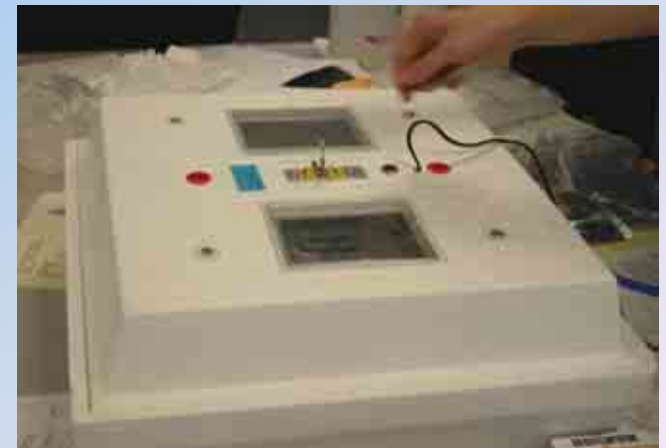


Coliscan Easygel

- Swirl the dish (figure-8) gently to evenly distribute the media (careful not to splash over the side or get any on the lid)
- Solidify plates
 - let plates sit undisturbed at room temperature (out of direct sunlight) for 45 minutes or until solid

STOP HERE UNTIL MEDIA IS SOLID

- Make sure water has been added to incubator
- Incubator should read between 35°C and 37°C
- Incubate plates for 24 and/or 48 hours
 - place plates (upside down to reduce condensation on cover) at 35-37° C (can stack them)
- Read plates and record data



Disposal of Sample

- Place about a teaspoon of household bleach onto each plate (inside)
- Allow it to sit for 5 minutes
- Place in a watertight bag and discard in normal trash
- Remove water from incubator, let dry, store

Where to Review/Purchase Kits?

- Coliscan EasyGel:
 - Micrology Labs – <http://www.micrologylabs.com>
- 3M Petrifilm:
 - 3M – http://solutions.3m.com/wps/portal/3M/en_US/food-safety/microbiology/products
- IDEXX
 - <http://www.idexx.com/water/quantitray/>



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