Adopt-a-BeachTM Quality Assurance Project Study Plan

Alliance for the Great Lakes

July 17, 2009

Project Managers:	
Jamie Cross	s (Overall Program & MI Outreach)
Todd Brenn	nan (WI Outreach)
April Matho	er (OH Outreach)
Frances Car	nonizado/Abby Crisostomo (IL, IN Outreach)
Quality Assurance	Managers:
Stephanie S	smith
Lyman Wel	lch
Joel Bramn	neier

Table of Contents

Project Management

Title and Approval Sheet

Table of Contents

Distribution List

Project/Task Organization

Problem Definition/Background

Project Task/Description

Quality Objectives and Criteria

Special Training/Certifications

Documentation and Records

Measurement/Data Acquisition

Sampling Process Design (Experimental Design)

Sampling Methods

Sample Handling and Custody

Analytical Methods

Quality Control

Instrument/Equipment Test, Inspection and Maintenance

Instrument/Equipment Calibration and Frequency

Inspection/Acceptance for Supplies and Consumables

Non-direct Measurements

Data Management

Assessment and Oversight

Assessments and Response Actions

Reports to Management

Data Validation and Usability

Data Review, Verification and Validation

Verification and Validation Methods

Reconciliation with User Requirements

Relevant Documents

3. Distribution List

All Project Managers & Quality Assurance Managers will receive a copy of the QA Project Plan.

4. Project/Task Organization:

Lyman Welch, as the Manager of Water Quality Program, will maintain the official, approved QA Project Plan, as well as working on projects to reduce sewage overflows, protecting recreational waterways, eliminating mercury and addressing pharmaceutical pollution. Lyman is a former attorney with the Chicago law firm of Mayer, Brown & Platt. Most recently, he served as Associate Director & General Counsel of the Mid-Atlantic Environmental Law Center at Widener University Law School in Wilmington, Delaware.

Joel Brammeier, acting President for the Alliance for the Great Lakes, will continue to supervise this project. Since 2000, Joel has focused the Alliance's work on restoration of urban habitats and protection of the Great Lakes from the impacts of invasive species. Joel's current work includes implementing coastal habitat recovery along the Illinois shoreline and eliminating transfer of aquatic species between the Great Lakes and the Mississippi River. Joel received his Master of Science degree from the University of Michigan's School of Natural Resources & Environment in 1998 and his Bachelor of Science from Valparaiso University in 1996.

Stephanie Smith, Education Program Director at the Alliance for the Great Lakes, will oversee the integration of the public beach component of this project into the Adopt-a-Beach program. Stephanie received a master's degree in environmental science, with a concentration in environmental education, from Antioch New England Graduate School in Keene, N.H. She also earned a teaching certificate in middle school general science. After graduate school, Stephanie taught hands-on 8th grade science in Lowell, Mass.

Jamie Cross has been on staff with the Alliance since 1999, and serves as the acting manager of the Adopt-a-BeachTM program. In her role at the Alliance she supervises all aspects of the Adopt-a-BeachTM program – by which over 7,000 volunteers clean up the coastline along four out of the five Great Lakes. Jamie also manages outreach and training for volunteers in Michigan.

Alliance outreach staff will manage volunteer outreach, training and beach visit scheduling. Current Alliance outreach staff include: Todd Brennan (WI), April Mather (OH), Frances Canonizado/Abby Crisostomo (IL and IN). Contact information for outreach staff is available on our website here: http://www.greatlakes.org/Page.aspx?pid=590

Data collection and input in the database will be done by volunteers. This information will then be used by Alliance staff, partner organizations (such as the US EPA), state EPA and Departments of Natural Resources, local health departments, volunteers and local municipal beach managers to begin to address these sources of pollution.

5. Problem Definition/Background

The Great Lakes, the largest freshwater body in the world, comprise 90% of the United States' fresh surface water and provide enormous ecological, economic, and social assets to area residents and to the hundreds of species that depend on them.

Beyond the environmental damage and health risks posed by pathogenic pollution, beach closings send a signal to residents that the Great Lakes are not healthy. Beaches, for the general public, serve as a barometer to assess the overall health and usability of the Great Lakes. When residents are turned away from beaches due to pathogenic pollution or other types of pollution such as foul smelling algae that can be harmful to human health discourages public use and involvement in them — reinforcing a stereotype that the lake is dirty and something to be avoided rather than cared for.

The Alliance is committed to developing procedures to help clean up our beaches along the Great Lakes. The Great Lakes Regional Collaboration's published strategy for improving Great Lakes health specifically addresses beach water quality. To that end, the EPA is working with states to develop beach sanitary surveys to identify specific sources of contamination at Great Lakes beaches. The Alliance for the Great Lakes developed this Project Study Plan and Adopt-a-Beach TM Guide in accordance with EPA's Beach Sanitary Survey Tool so that beach managers can identify sources of contamination at their beaches, and address them accordingly. The EPA Beach Sanitary Survey Tool identifies technically sound and consistent approaches to identify pollution sources.

The Alliance for the Great Lakes Adopt-a-Beach TM Project Study Plan is organized under EPA's recommended structure for QA Project Plans. Where necessary, this document refers to the Alliance's Adopt-a-Beach TM Guide to further explicate data-gathering procedures. The structure falls into four major categories: project management, measurement/data acquisition, assessment and oversight, and data validation and usability.

6. Project/Task Description

The purpose of this study is to monitor beach and water quality through science-based testing and observations and data collection of water quality information such as bacteria, pH, temperature, longshore current, litter, etc. Sample collection

conditions including air temperature, wind direction, wind speed, time of day, sky conditions, wave conditions, current, and sample flow variations (during/post rain event vs. low flow) are considered in the evaluation of causal relationships. Volunteers are encouraged to use their data to evaluate their beach to determine beach needs to improve beach conditions. Visual assessments aid in determining local action for improvement of beach quality.

The year-round Adopt-a-Beach TM program invites groups and individuals to commit to two to five visits over a year. At each visit, adopters collect and record the litter they find, the conditions at the beach, and test the water for E. coli levels using materials provided by the Alliance. Adopters then enter their data into the Alliance's online database. Program data is compiled and reviewed annually and semi-annually by the Alliance. After two or three visits, adopters are encouraged to examine their data and develop an action project to improve conditions at their beach. The Alliance helps adopters with this process.

All samples will be collected at public beaches or private shoreline with owner's permission. Beaches will be selected based on volunteer interest or need. For more precise location data, some volunteers have the ability to use the Global Positioning System (GPS) to calculate where they take their water sample, record their location and include this location in their completed report.

Once volunteers collect data, they can either send completed forms to the Alliance for the Great Lakes headquarters to be entered into a database, or they can enter it themselves in the same secure online database. We encourage adopters to use the online data entry system. Each year, Alliance for the Great Lakes will release a report that highlights important trends in findings. Data will be submitted to state agencies annually as it becomes available in digital form.

7. Quality Objectives and Criteria

All Petrifilm will be used or disposed of prior to their expiration dates. Petrifilm, Whirl-Paks and pipettes will be provided by Alliance for the Great Lakes to ensure quality control. See further quality control measures for Petrifilm in the Routine Visit Form Guide, p. 5 (linked below).

Volunteers are given specific instructions on measuring wave height, longshore current, water quality, and other variables in the Routine Visit Form Guide based on EPA measuring standards. Site Coordinator instructions for quality control are also outlined in the Routine Visit Form Guide, p. 14.

8. Special Training/Certifications

The appropriate outreach staff is trained by the Alliance to oversee all volunteers.

The majority of volunteers go through in-person trainings which include a PowerPoint presentation and/or onsite training. Training includes testing trainee comprehension through demonstrating what they just learned. All procedures are outlined during the training sessions. During the training, expired Petrifilms are sometimes used or sampling is conducted in the field, which depends on location and weather conditions. All volunteers that register for the program receive a start-up kit which includes the Adopt-a-Beach TM guide with instructions on monitoring and collection of data. The guide is also downloadable on our web site.

Training also specifies prescribed safety procedures. These include the use of gloves while sampling, cleansing of hands with alcohol wipes, or antiseptic lotion before and after visits, inclusion of a first aid kit at all sites, and exercising extreme caution when children are near the water. Please see attached training presentation (PowerPoint).

Trained team leaders supervise a volunteer's first real sampling event in order to confirm they are doing it correctly. Finally, the Alliance provides annual refresher training for volunteers.

9. Documentation and Records

The reporting format can be found in the Routine Visit Form Guide. Volunteers enter data into the online database or submit their data via the post to Alliance offices. Project managers enter data into the data entry system. Data collected online is downloaded on a regular basis to be stored in the organizational computer filing system. This database is backed up on a regular basis through Alliance general office operations. Quality Assurance Managers are responsible for keeping the most current copy of the approved QA Project Plan which is also provided to Alliance outreach staff. Alliance outreach staff are responsible for sending that QAPP to volunteer team leaders.

Measurement/Data Acquisition

1. Sampling Process Design (Experimental Design)

All field methods and quality assurance steps are specified in the Routine Visit Form Guide before data collection. The data collection is largely based on EPA's beach sanitary survey protocol. Parameters to be sampled were chosen based on relevance to water quality in the Great Lakes system and ease of measurement. The data collection includes information on several parameters relevant to beach water quality, including wave height, longshore current speed & direction, pH, bacteria e. coli & coliform, temperature, odor, and turbidity. Information is also collected on the amount of litter on the beach, oily sheens, algae in the water and along the beach, wildlife and dead birds. Biological assessment is limited to

bacteria testing and counts of wildlife on the beach to align with the EPA beach survey procedure. Fish and macroinvertebrate data are not collected.

The total number of samples is based on the number of volunteers willing to survey the beaches. If volunteers schedule a time to sample and the beach is inaccessible due to weather or other purposes, volunteers will reschedule their visit. For examples of critical data versus data for informational purposes, please refer to the Routine Visit Form Guide.

In addition to data collected on the Routine Visit Form, Alliance volunteers collect data on litter found on the beach. Volunteers use a Litter Monitoring Form to collect information on 46 specific debris items such as cigarettes, tampons, condoms, beverage containers, firework debris, etc. Volunteers also have an option to write in additional items of concern found during their visit. All data is subject to review by project managers and outreach staff.

2. Sampling Methods

Volunteer samplers measure water temperature, pH, Coliform and *E. coli* in water. Coliform bacteria and *E. coli* will be monitored using a Petrifilm, a pipette, and a Whirl-Pak bag. The Alliance provides Petrifilm plates to monitor *E. coli* and coliform bacteria. We use the Petrifilm in a manner that is deemed reliable through a comparative Grand Valley State University study. (Attached). Whirl-Pak bags, small sterile plastic bags, are used to take the water sample, and sterile pipettes are also used to ensure the quality of the test. Expired Petrifilms are discarded. Collection methods and quality control measures are outlined in the Routine Visit Form Guide, pp 5-6.

Measurement methods for wave height, longshore current, bather load, pollution sources, wildlife, speed of current and algae and litter observations can be found in the Routine Visit Form Guide, pp. 3-4, 8-14.

3. Sample Handling and Custody

Water samples are collected using sampling pole or by wading out into the water, conscious of water flow and avoiding contamination. Two samples are taken using two separate Whirl-Pak bags to draw water for each Petrifilm. Samples are taken from where water is at least one meter deep. The Whirl-Pak is opened just before collecting the sample to avoid contamination. The water sample is placed on the Petrifilm while at the beach. A sterile pipette is used to withdraw 1 milliliter of water from the Whirl-Pak and is added onto the pink circle of the Petrifilm. Plates are stored in a sealable bag in a dark area and incubated (35°C or 95°F) in a horizontal position with the clear side up for 24+ hours. If temperature cannot be controlled, the Petrifilm is sealed in a bag at room temperature or warmer, out of direct sunlight, for 48 hours. After the sample is taken, the

Petrifilm is placed in a sealable bag. Used Petrifilms are sterilized using one ml of bleach and placed in a sealed plastic bag and disposed of properly in the garbage.

The Routine Visit Form Guide requires samples to be identified by date, time, location and number of volunteers. Volunteers enter this information directly and generally electronically into the Alliance's database.

4. Analytical Methods

Quality of the water is assessed using the Environmental Protection Agency's water quality standards for safe swimming (no more than 235 *E. coli* colonies per 100 ml of water).

See the Routine Visit Form Guide for other details of specific performance criteria for general beach conditions, pH and other water quality measures, bather load and pollution sources.

5. Quality Control

Petrifilm, Whirl-Paks and pipettes will be provided by Alliance for the Great Lakes to ensure quality control. Alliance staff may conduct reviews of data for outliers or extreme variability and follow-up with volunteers when unusual results are found. Missing data will be listed as "Blank" or "Not Entered" by the data reporting system.

6. Instrument/Equipment Test, Inspection and Maintenance

Alliance staff inspects Petrifilm, pipettes, and Whirl-Pak bags before they are sent to volunteers to ensure they are in good working condition and have not expired. Volunteer Team Leaders will check items upon receipt for visible damage.

7. Instrument/Equipment Calibration and Frequency

N/A

8. Inspection/Acceptance for Supplies and Consumables

Petrifilm, Whirl-Paks and pipettes will be provided by Alliance for the Great Lakes and inspected by staff before shipment to volunteers. Volunteer Team Leaders will check items upon receipt for visible damage.

9. Non-direct Measurements

Our volunteers gather most of their data directly; however it is possible to get information on temperature, rainfall, and bather volume through non-direct measurements using beach-based resources (lifeguard data, etc.). We use this data when it is difficult to measure directly due to volume issues (bathers) or a time-delay in gathering the data (measuring rainfall). See the Routine Visit Form Guide pp 1, 2 & 8 for further detail.

10. Data Management

Each volunteer team will complete a Routine Visit Form and Litter Monitoring Form during every visit to a collection site. An example of these forms is attached. Once data is collected by volunteers, they enter data into the Alliance's database.

All collected data will be submitted to the appropriate state EPA. We would like to work with Great Lakes states to make the data in our database available for state EPA databases.

Assessment and Oversight

1. Assessments and Response Actions

Assessment scheduling is managed by Alliance outreach staff in each state. Volunteer Team Leaders conduct assessments in the field and document site visit results in online database. The number and frequency of assessment activities depends on volunteer availability.

2. Reports to Management

Volunteer Team leaders may include comments in data reports entered into the database system. Alliance staff reviews the data in preparing annual reports.

Data Validation and Usability

1. Data Review, Verification and Validation

Project Data is reviewed by Alliance staff. Staff reaches out to team leaders if data appears highly variable.

2. Verification and Validation Methods

The Alliance recommends volunteers conduct a follow-up visit and assessment if data shows unusual results. Interpretation of data should include analysis of sample results comparing differences over time at the same sample location.

3. Reconciliation with User Requirements

The Alliance tests for *E. coli* using a method reasonably certain to provide accurate validated data. See attached GVSU study for further information.

Relevant Documents

Adopt-a-BeachTM Routine Visit Form http://www.greatlakes.org/Document.Doc?id=379

Adopt-a-Beach TM Litter Monitoring Form http://www.greatlakes.org/Document.Doc?id=378

Adopt-a-BeachTM Guide http://www.greatlakes.org/Document.Doc?id=380

Adopt-a-BeachTM Training Presentation (PowerPoint) To be sent in separate file

Vail, J.H. R. Morgan, C.R. Merino, F. Gonzales, R. Miller, and J. L. Ram. Enumeration of waterborne Escherichia coli with petrifilm plates: comparison to standard methods. GVSU Water Resources. March 20, 2003.

QAQC Plan Attachments

Adopt-a-Beach™ Routine Visit Form

http://www.greatlakes.org/Document.Doc?id=379

Adopt-a-Beach ™ Litter Monitoring Form

http://www.greatlakes.org/Document.Doc?id=378

Adopt-a-Beach™ Guide

http://www.greatlakes.org/Document.Doc?id=380

Adopt-a-Beach™ Training Presentation (PowerPoint)

To be sent in separate file

Vail, J.H. R. Morgan, C.R. Merino, F. Gonzales, R. Miller, and J. L. Ram. *Enumeration of waterborne Escherichia coli with petrifilm plates: comparison to standard methods*. GVSU Water Resources. March 20, 2003.

Adopt-a-Beach™ Routine Visit Form

Answer these questions during your beach visit. Use our companion Adopt-a-Beach™ Guide for question by question instructions on how to complete the form.

If you have questions about Adopt-a-Beach™, contact adoptabeach@greatlakes.org.

٧	/hat	to	do	with	vour	data
٧١	/IIal	LU	uv	WILLI	YUUI	uata

- Enter your results online in the Adopt-a-Beach™ pages at *www.greatlakes.org/adoptabeach* by logging into your personal account.
- If you don't have an Adopt-a-Beach™ account, create an account by visiting Adopt-a-Beach™ at www.greatlakes.org/adoptabeach.
- If you don't have internet access, mail your results to Alliance for the Great Lakes, 700 Fulton St. Ste. A, Grand Haven, MI 49417 or fax to 616-850-0765

Beach name and I	location (city an	d state)							
dopt-a-Beach™ tea	am name				Team Leader			····	
isit date		15.00			Visit time of da	y (e.g. 11:00 a.	m.)		
umber of volunte	eers	Estimated time	spent completing	g the Routine Visi	t Form	-			
escribe the oints. Some	boundarie e groups ha	s of the bearave adopted	ch area you a portion o	have adopte f a beach are	ed using fixed ea and some	l objects, st groups hav	reet names e adopted a	or other fixed n entire beach	reference 1.
. General									
Air tempe	erature (Ro	und to the no	earest degr	ee.)	_				
Celsius [☐ Fahrenhe	elt (Check typ	e of measur	ement taken.)				•
. Wind dire	ction 🗆 No	wind \Box	S 🗆 SE	□sw □	N NE	\square NW \square	E 🗆 W (0	check the answ	er that applie
3. What is t Form Guid		eed? (Circle	one of the	options belo	w.) *See Bea	ufort Wind	Scale detail	ed in the Rou	tine Visit
Knots	Under 1.	1-3	4-6	7-10	11-16	17-21	22-27	28-27	34-40
Description	Calm	Light air	Light breeze	Gentle breeze	Moderate breeze	Fresh breeze	Strong breeze	Near gale	Gale
I. When wa	s the most that 24 ho	recent rain urs = 1 day,	event? (If i 48 hours =	t lasted mor 2 days and	e than one da 72 hours = 3	ay, check th 3 days.	e appropriat	e answer.)	
☐ Less than	24 hours	ago	☐ Less th	an 48 hours	ago	•			
☐ Less than	72 hours	ago	☐ More th	nan 72 hours	s ago	□ I don't	know		

5. Describe the rain	event, if one has oc	curred in the past 7	72 hours (3 days).		
☐ Misting	☐ Light rain	☐ Steady ra	in 🗆 Hea	vy rain	,
☐ No rain event in the	ne past 72 hours	□ I don't kn	ow Other	e.g. snow, hail) desc	ribe:
	ithin the past 72 ho eters. (If you do not l			each, measure the a gauge.)	mount of rain in
in/cm (r	ound to nearest 10th	n degree)	\square No rain gauge		
7. What are the cur	rent sky conditions?	(Check one of the c	ptions below.)		
Sky condition	Sunny	Mostly sunny	Partly sunny	Mostly cloudy	Cloudy
Amount of cloud coverage	No clouds	1/8 to 1/4	3/8 to 1/2	5/8 to 7/8 🔏	Total coverage
8. What is the curre the distance betwee where the waves are	n the crest (tallest p	eet? (Check one of the oint of the wave) to	ne options below.) \ the trough (the lowe	Wave height is detern st point of the wave)	nined by measuring just lakeward of
☐ No waves	☐ Waves less th	nan 1 foot	☐ 1-2 feet	☐ 3-4 feet	
☐ 5-6 feet	☐ 6-8 feet		☐ Over 8 feet		•
9. Describe the inte	ensity of the waves.	(Check one of the op	otions below.)		
☐ Calm ☐ Media	ım 🗌 Rough				
10. Longshore curre meters. See Routine	ent: What is the amo e Visit Form Guide fo	unt of time (in rinstructions on how	n seconds) that it ta v to measure the lor	kes your floatable ob ngshore current.	ject to travel 10
	eed of the longshore				
10 meters ÷ by	time in seconds	= speed in I	meters per second		
Example #1: 10 me Summary: Your float meters per second.	ters ÷ 30 seconds table object moves 1	= .33 meters per s 0 meters in 30 seco	second ands. Therefore, the	speed of the longsho	ore current is .33
Example #2: 10 me Summary: Your float meters per second.	ters ÷ 40 seconds table object moves 1	= .25 meters per s 0 meters in 40 seco	second onds, therefore the s	speed of the longsho	re current is 0.25
11. What is the dire	ection of the longsh	ore current?	(The longshore	current runs parallel	to the beach.)
□ No current □ S	S □ SE □ SW	□N □NE □N	W □E □W		
12. Did you measur	e the longshore cur	rent? 🗌 Yes	□ No		
13. Comments or re	esason you did not n	neasure the longesh	ore current:	A A SHARING TO SHARING THE SHA	· · · · · · · · · · · · · · · · · · ·
		· · · · · · · · · · · · · · · · · · ·			
14. General comme	ents and observation	s about general bea	ch conditions:		·
		NA 14 (14)			

II. Water Quality				
15. Some adopters ma		easure water pH. If	you are one of these adop	nters, please enter the pH
16. If a pH reading wa	s taken, please indicat	e the testing metho	d you used. (Check the ap	propriate answer.)
☐ pH paper	\square pH liquid solution	☐ pH meter		
Your water sample sho	uld be taken at the sam h is first encountered an lue dots) and Coliform (comments" section.	ne location in the mi nd at 6 inches belov	ddle of your adopted beacl v the surface. If you are us	nd to determine your results. n where 24-30 inches (2 - sing the Alliance's test kits, u do not conduct and include
Sample #1				
Test type	E. coli – water (blue dots)	Collform (red dots)	Enterococcus	E. coli – sand
Number of dots				
Sample #2				
Test type	E. coli – water (blue dots)	Coliform (red dots)	Enterococcus	E. coli – sand
Number of dots				
Some adopters have t	he ability to use the Gi	obal Positioning Sys		here they take their water
		'W (Longitude		note.
° 'N (Lati	temperature?			
	eit (Check type of meas		/	
			sits? (Check the appropria	te answer.)
_			ito: (oncon the appropria	,
☐ Yes ☐ No	☐ This is our first bea			
				-
21. Describe the odor	of the water. (Check or	ne or more of the or	tions below.)	٠
☐ No smell	☐ Sewage	☐ Algae (decayir	ıg plants) ☐ Fis	hy
\square Sulfur (rotten eggs)	☐ Musty (wet soil)	☐ Other If of	her, describe:	

	ibe the turbidity (cloud on you take your water s	iness) of the water. (Check one of the options below.) Observe turbidity at the same cample.	
☐ Clear	☐ Slightly cloudy	☐ Cloudy ☐ Opaque (solid)	
23. Additi	onal observations abou	t water quality:	_

III. Bather Load (Number of people at the beach	ı١
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24. For people IN or ON the water, not on the beach, describe the type of activity and number of people involved. (Use the table below to fill in the number of people involved in the activities listed below.)

Type of activity	Sailing/ power boating	Canoeing/ kayaking	Jet skiing	Fishing	Windsurfing/ kite boarding	wading	Other (in and on the water)
Number of people engaged in this activity							

If other, describe the type	of activity IN or (ON the water:				
25. What is the total num 26. General comments an			in the water, E	XCLUDING YOUR	GROUP?	
		·				
		112				

IV. Potential Pollution Sources

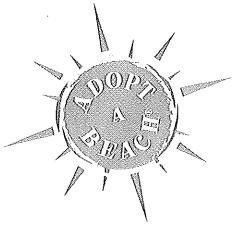
27. Identify any of these features up to 500 feet from the beach boundary that are visible. (See Adopt-a-Beach™ Guide to determine speed of current.)

☐ Yes ☐ No ☐ Gushing ☐ Steady stream ☐ Trickle ☐ Brown ☐ Green ☐ Black ☐ White ☐ Red ☐ Clear ☐ Foamy ☐ Algae ☐ Debris ater ☐ Oily sheen on	☐ Trickle ☐ Brown ☐ Green ☐ Black ☐ White ☐ Red ☐ Clear ☐ Foamy ☐ Algae ☐ Debris	☐ Trickle ☐ Brown ☐ Green ☐ Black ☐ White ☐ Red ☐ Clear ☐ Foamy ☐ Algae ☐ Debris water ☐ Oily sheen on water
☐ Steady stream ☐ Trickle ☐ Brown ☐ Green ☐ Black ☐ White ☐ Red ☐ Clear ☐ Foamy ☐ Algae ☐ Debris atter ☐ Oily sheen on	Brown Green Black White Red Clear Foamy Algae Debris water Steady stream	☐ Steady stream ☐ Trickle ☐ Brown ☐ Green ☐ Black ☐ White ☐ Red ☐ Clear ☐ Foamy ☐ Algae ☐ Debris water ☐ Oily sheen on water
☐ Green ☐ Black ☐ White ☐ Red ☐ Clear ☐ Foamy ☐ Algae ☐ Debris ater ☐ Oily sheen on	☐ Green ☐ Black ☐ White ☐ Red ☐ Clear ☐ Foamy ☐ Algae ☐ Debris water ☐ Oily sheen on	☐ Green ☐ Black ☐ White ☐ Red ☐ Clear ☐ Foamy ☐ Algae ☐ Debris water ☐ Oily sheen on water
☐ Green ☐ Black ☐ White ☐ Red ☐ Clear ☐ Foamy ☐ Algae ☐ Debris ater ☐ Oily sheen on	☐ Green ☐ Black ☐ White ☐ Red ☐ Clear ☐ Foamy ☐ Algae ☐ Debris water ☐ Oily sheen on	☐ Green ☐ Black ☐ White ☐ Red ☐ Clear ☐ Foamy ☐ Algae ☐ Debris water ☐ Oily sheen on water
☐ Algae ☐ Debris ater ☐ Oily sheen on	☐ Algae ☐ Debris water ☐ Oily sheen on	☐ Algae ☐ Debris water ☐ Oily sheen on water
ease describe the feat	ure identified:	·
in question 27, use or d above. Please refer Alliance's test kits, fill e an explanation in the	ne of your <i>E. coli</i> test kits to Adopt-a-Beach™ Guide in: <i>E. coli</i> – water and C e "comments" section.	coliform. Write in "not tested" fo
adopted beach.)		<u> </u>
(red dots)	Enterococcus	E. coli – sand
	d above. Please refer Alliance's test kits, fills an explanation in the allution sources listed adopted beach.) Coliform	d above. Please refer to Adopt-a-Beach™ Guide Alliance's test kits, fill in: <i>E. coli</i> – water and Collian explanation in the "comments" section. Industrian sources listed for question 27? Coliform (red dots) Enterococcus

•	'N (L	atitude)	<u> </u>	<u> </u>	'W (Lon	gitude)					
30. Are the f yes, pleas	re floata se descri	bles (ite	ems floating floatables p	in the wresent. (C	rater) prese Circle one o	n t? r more	☐ Ye of the option				
Туре	Street litter		Food- related litter	Medical items	Resin		Sewage- related	Building materials	Fishii relate		Household waste
Example	Cigare filters	ette	Food packing, beverage containers	Syringes	Tiny p		Condoms, tampons	Pieces of wood, siding		ng line, lures	Household trash, plas tic bags
	e the an		f debris/litte			1	e of the opt	ons below.)		High	
Amount Percentage		No Litte	er	Very Lo	w 	Low	· · · · · · · · · · · · · · · · · · ·			1 -	
rercentage beach	3 011	0%		1-10%		11-20)%	21-50%		51%	and up
b. Can y	s, descrik you ident	tify the s	source?	,	Addition to			th of your ad	opted a	-	
b. Can y 33. Describ Amount	, descrik you ident	tify the s	source? f algae in th	,	near the sh		ng the leng	th of your ad	opted a	ea of b	each.
b. Can y 33. Describ Amount Percentage	, descrik you ident e the an	tify the s	source? f algae in the Algae	e water i	near the sh Low 1-20%	ore ald	Med	th of your ad	opted ai	ea of b	each.
b. Can y 33. Describ Amount Percentage	, descrik you ident e the an	nount of	source? f algae in the Algae	e water i	near the sh Low 1-20%	ore ald	Med	th of your ad lum 0% a of adopted	opted an Hi	ea of b	each.
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1	Geese		Gulls	Dogs	Othe	r
Number						
		the shoreline, fill for identification.)	ll in the number fou	and in the appropa	iate box below.	
Туре	Common Ioon	Herring gull	Ring-billed gull	Double crested cormorant	Horned grebe	Other
Number found dead		t				
If other, describe):					
39. How many d	lead fish are on	the beach?				
40. If there are	other dead anin	nals on the beac	h (not including fisl	h and birds) list t	hem here.	
	Type of a	nimal			How many	
				L MANAGEMENT IN .		

			s are there within 5	00 feet of your a	dopted beach bo	undary?
		ers on your beach			dopted beach bo	undary?
	garbage containe	ers on your beach				undary?
(If there are no g	garbage contain Garbage Co	ers on your beach	n enter 0.)	Recyc	cling Containers	
(If there are no g	garbage containe Garbage Co	ers on your beach	ainers at this locat	Recyclion. (Check one o	cling Containers	tions below.)
(If there are no g	Garbage Contained Garbage Contained e and condition ans	on your beach entainers of garbage cont	ainers at this locat	Recyclion. (Check one o	cling Containers r more of the opt	tions below.) h no lids
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42. Describe us No garbage cans Garbage cans 43. Please add 44. Dld you take park authority to	e and condition ans Design present with lice any additional condition as ask them to end	of garbage cont gnated carry in casts Garbage comments or note a result of your lengty trash cans in	ainers at this locat arry out policy cans well maintaine es about your visit beach visit? For examore frequently.	Recyclion. (Check one o Garbage d Garbage here:	cling Containers r more of the opt cans present wit cans overflowing	tions below.) h no lids or knocked over



Alliance for the Great Lakes Adopt-a-Beach™ 700 Fulton Ave., Suite A Grand Hayen, MI 49417

Adopt-a-Beach™ Litter Monitoring Form

Please pick up everything you find. Record only the items found on the Litter Monitoring Form.

Pick up "pieces" of items, but do not record these fragments on the data card as this can distort the data totals.

What to do with your data:

- Enter your results online in the Adopt-a-Beach™ pages at www.greatlakes.org/adoptabeach by logging into your personal account.
- If you don't have an Adopt-a-Beach™ account, create an account by visiting Adopt-a-Beach™ at www.greatlakes.org/adoptabeach.
- If you don't have internet access, mail us your results to the address at left.
- Mail all completed sign-in sheets to the Alliance.

Team Leader:	Team Name:	Date:	·····
Cleanup Site Name (beach, park, etc.):		State:	
Category of Cleanup (choose one):	☐ Great Lakes Coast ☐ Inland (river	, lake, stream, tributa	ary, lake)
Type of cleanup: ☐ Shoreline/Beac	ch ☐ River/Stream/Tributary ☐ Lake		
Distance cleaned: in miles or k	Total weight of trash collected:	pounds of	or kilograms
•	Number of volunteers:		
	ing the cleanup. Tell us what they were entangle	d in (fishing line, rope	e, net, etc.).
Type of Animal	Entangled in		
		Dead	☐ Alive
		Dead	☐ Alive
	•	Dead	□,Alive
What were the most peculiar items you co	ollected?		



17 N. State Street Suite 1390 Chicago, IL 60602 Phone: 312-939-0838 ext. 228

Fax: 312-939-2708

adoptabeach@greatlakes.org

ITEMS COLLECTED: Please pick up ALL debris that yo Keep a count of your items using tick marks and ente Example: 8 Beverage Cans	ou find. Only record information for the items listed below. If the item total in the box.
Shoreline and Recreational Activities	Clothing, shoes
Bags (paper)	Cups, plates, forks, knives, spoons
Bags (plastic)	Food wrappers/containers
Balloons	Pull tabs
Beverage bottles (plastic) 2 liters or less	6-pack holders
Beverage bottles (glass)	Shotgun shells/wadding
Beverage cans	Straws, stirrers
Caps, Lids	Toys
Waterway Activities	
Bait containers	Fishing nets
Bleach/cleaner bottles	Light bulbs/tubes
Buoys/floats	Oil/lube bottles
Fish traps	Pallets
Crates	Plastic sheeting/tarps
Fishing line	Rope
Fishing lures/light sticks	Strapping bans
Smoking-Related Activities	Dumping Activities
Cigarettes/cigarette filters	Appliances (refrigerators, washers, etc.)
	Batteries
	Building materials
Cigarette lighters	Cars/car parts
Cigar tips	55-Gal. Drums
Tobacco packaging/wrappers	Tires
Medical/Personal Hygiene	Other Debris/Items of Local Concern
Condoms	Discarded food
Diapers	Firework debris
Syringes	Drug paraphernalia (crack pipes, bags, etc.)
Tampons/tampon applicators	Misc. items, describe
ALLIANCE FOR THE GREAT LAKES 17 North State St. Ste. 139 Chicago, IL 60602 Phone: 312-939-0838 ext.	

A DOPT A BEACH
Visit www.greatlakes.org

Chicago, IL. 60602 Phone: 312-939-0838 ext. 228 Fax: 312-939-2708 adoptabeach@greatlakes.org

Note to team leaders: Please tally your results onto a final data card and enter your data online at www.greatlakes.org/adoptabeach





Photo: Lloyd DeGrane, Alliance for the Great Lakes

Alliance for the Great Lakes Adopt-a-Beach™ Guide

Question by question instructions to assist adopters in completing the Adopt-a-Beach™ Routine Visit Form and Litter Monitoring Form.



Alliance for the Great Lakes Routine Visit Guide

Index

I. General Beach Conditions
II. Water Quality
III. Bather Load
IV. Potential Pollution Sources
V. Litter Monitoring Instructions

Adopt-a-Beach™ Program Contact information:

Michigan: Jamie Cross at 866-850-0745 ext. 12 or jcross@greatlakes.org

Indiana and Illinois: Frances Canonizado at 312-939-0838 ext. 228 or fcanonizado@greatlakes.org

Wisconsin: Todd Brennan at 414-559-0317 or tbrennan@greatlakes.org

Ohio: April Mather at 216-630-8140

or amather@greatlakes.org

Adopt-a-Beach™ program headquarters: 312-939-0838 ext. 228 or adoptabeach@greatlakes.org.

Additional information: There is additional information available on our website at *www.greatlakes.org* and follow the Adopt-a-Beach™ sun.

Adopt-a-Beach™ Routine Visit Guide

Use this guide to help you answer the Adopt-a-Beach™ Routine Visit Form questions. Do not write directly on the guide. Put your responses on the Routine Visit Form and enter them online after your beach visit. This guide also includes instructions for completing the Litter Monitoring Form.

If you have questions about Adopt-a-Beach™, contact adoptabeach@greatlakes.org or your state coordinator.

Sandy S	t. Beach	V			Pebbl	le, MI			
Beach name and	ocation (city and	state)	·				2000		
Pebble 7	tigh Sch	ool 10th	grade		Joe B	eachy		1,700	
Adopt-a-Beach™ tea	am name				Team Leader				
7/30/200	28				11:00			******	
Visit date					Visit time of day	(e.g. 11:00 a.m.)		
12		2 hours							
Number of volunte	ers	Estimated time s	pent completing t	he Routine Visit I	Form				
Describe the points. Some	e groups hav	ve adopted a	portion of b	each area a	nd some gro	oups have a	dopted an er	ntire beach.	
1/2 mile	of eithe	er side o	f the bed	ich paví	líon - oi	n the no	orth side	, to the I	<u>barking</u>
								· · · · · · · · · · · · · · · · · · ·	
lot and	on the s	outh sid	e, to the	e bike po	uth.				
I. General	Beach Co	nditions							
				7.0					
1. Air tempe	rature (Rou	nd to the nea	arest degree	.)					
					,				
🗌 Celsius 🏻	☑ Fahrenhei	i t (Check typ	e of measure	ement taken	.)			•	
grassy surfa liquid in the	ces. All air t thermomete check for te	nperature: Ail demperature in der due to the mperature ch	readings are absorption o	done in the of sun, which	shade. This could give	s is necessa you an incor	ry to avoid e rect reading.	xcessive war . If you don't	rming of the ; have a ther-
Report air te available, Ce	mperature i Isius is pre	n Fahrenheit ferred becau	or Celsius t se this scale	emperature was develo	scales, spec ped for and	cifying which is most con	one was use nmonly used	ed. If both so for scientific	cales are purposes.
2. Wind dire	ction 🗆 No	wind 🗆 S	□ SE 〔	∃sw □n	NE [□ NW □ E	E 🗆 W (Che	eck the answe	r that applies.)
How to mean other words, many weather wind for dire other light of 3. What is the	a north win er stations a ction, while ojects (such	nd pushes air and airports t the propeller as a piece o	from the not to measure b is measure t of string held	orth to the sooth wind did he wind spe up into the	outh. Weath rection and s ed. Use sur air) affected	ervanes or a speed. The t rounding obj d by wind to	erovanes are ail orients the ects such as determine w	e commonly ne instrumen s the grass, i rind direction	used at t into the trees, or
Knots	Under 1	1-3	4-6	7-10	11-16	17-21	22-27	28-27	34-40
			Light /	Gentle	Moderate	Fresh	Strong	Near gale	Gale
Description	Calm	Light air	breeze	breeze	breeze	breeze	breeze	Hear gale	duic
			. 5		.1 - 4 1	مأممميت حالا	f the wind M	Mind anaad a	on ho

How to complete the wind speed chart: First you will have to determine the speed of the wind. Wind speed can be measured through simple observations using the Beaufort Wind Scale on the next page.

Beaufort Wind Scale

Wind in Knots	Description	Appearance of Wind Effects
Less than 1	Calm	Calm, smoke rises vertically
1-3	Light Air	Smoke drift indicates wind direction, still wind vanes
4-6	Light Breeze	Wind felt on face, leaves rustle, vanes begin to move
7-10	Gentle Breeze	Leaves and small twigs constantly moving, light flags extended
11-16	Moderate Breeze	Dust, leaves, and loose paper lifted, small tree branches move
17-21	Fresh Breeze	Small trees in leaf begin to sway
22-27	Strong Breeze	Larger tree branches moving, whistling in wires
28-33	Near Gale	Whole trees moving, resistance felt walking against wind
34-40	Gale	Whole trees in motion, resistance felt walking against wind

		event? (If it lasted more to 48 hours = 2 days and 72)		the appropriate ans	wer.)	
Less than 24 ho	urs ago	\square Less than 48 hours a	go			
Less than 72 ho	urs ago	☐ More than 72 hours a	ago □ I dor	□ I don't know		
5. Describe the rai	n event, if one h	as occurred in the past 7	72 hours (3 days).			
		art: The answer to this que the constant of the appropriate answer.		your general observa	ation and knowledge	
☐Misting	☐ Light rai	n ☑ Steady ra	in 🗆 Heav	y rain		
☐ No rain event in	the past 72 hou	rs □l'don't kr	now			
Other (e.g. snow, h	ail) describe:		<u> </u>	1.01	- MARTON	
6. If it has rained vinches or centimet	within the past 7 ters. (If you do no	72 hours and you have a not have a rain gauge pleas	rain gauge at the be se select no rain gau	ach, measure the a	mount of rain in	
in/cm ((round to neares	t 10th degree)	No rain gauge			
en in the 24 hours you do not have ac	prior to your bea cess to this info	estion: The rain gauge shach visit. Some lifeguard s rmation, do not answer th se select no rain gauge.	stations have informa	ation on rainfall amo	unts at the beach. If	
7. What are the cu	ırrent sky condit	ions? (Circle one of the o	ptions below.)	· · · · · ·		
Sky condition	Sunny	Mostly sunny	Partly sunny	Mostly cloudy	Cloudy	
Amount of	No clouds	1/8 to 1/4	3/8 to 1/2	5/8 to 7/8	Total coverage	

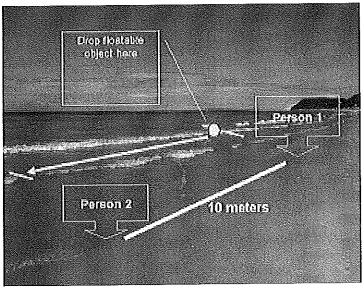
How to complete the sky conditions chart: The answer to this question is based on your general observations of the presence of clouds in the sky. Circle the sky condition that best describes your observations.

8. What is the current wave height in feet? the distance between the crest (tallest purples where the waves are breaking.			Wave height is determined by measuring e lowest point of the wave) just lakeward of
☐ No waves ☐ Waves less than	1 foot	☑ 1-2 feet	☐ 3-4 feet
☐ 5-6 feet ☐ 6-8 feet		☐ Over 8 feet	
How to complete the wave height chart: Find measuring the distance between the crest (toward of where the waves are breaking. See	allest point of t		
wave length crest ställwider level trough	1. While feet. Ha then av five sep 2. If con measur just lak record v	ave two observers in erage their results. Parate waves and the ditions are safe and e wave height by ca eward of where the where the wave cres	ht — 2 options ore, you can estimate the wave height in independently estimate wave height and Measure or estimate the height of at least en take the average. If the temperature is comfortable, you can arrying a graduated stick into the water to waves are breaking. Use the stick to set and the following wave trough hit the een the two is the wave height.
Alternately, you can visit the National Ocean http://www.crh.noaa.gov/grr/marine/index.punder "nearshore forecasts". However, this	ohp, which inclu	des data on wave h	neights and click on a location near you
9. Describe the intensity of the waves. (Che	eck one of the o	options below.)	
☑ Calm ☐ Medium ☐ Rough			
How to describe waves: To answer this quedescribes the waves.	stion observe th	ne waves at your be	each and check the answer that best
To answer questions 10, 11 & 12 see instrudirection of longshore current".	ıctions listed ur	nder question 14 in	this guide "About measuring speed and
10. Longshore current: What is the amount meters.	of time <u>120</u>	(in seconds) that it	takes your floatable object to travel 10
To determine the speed of the longshore cur	rent use the fo	llowing equation:	
10 meters \div by $\underline{120}$ time in seconds =	. <u>083</u> speed in	meters per second	i
Example #1: 10 meters ÷ 30 seconds = Summary: Your floatable object moves 10 m meters per second.			e speed of the longshore current is .33
Example #2: 10 meters ÷ 40 seconds = Summary: Your floatable object moves 10 m meters per second.			speed of the longshore current is 0.25
11. What is the direction of the the longsh	ore current? (Ti	ne longshore curren	t runs parallel to the beach.)
□ No current 🗹 S □ SE □ SW □ I	N	W □E □W	
12. Did you measure the longshore current	? [V Yes	□No	
13. Comments or resason you did not meas	oure the longes	hore current:	
	alandonia de la companio de la comp		

About measuring speed and direction of longshore current: Longshore current moves in a direction parallel to the shore. The measurement for longshore current speed is in meters per second. The current direction is reported by which way the water is flowing, for example, a westward current flows to the west.

To determine speed of a longshore current you will need the following materials:

- Meter stick (or other measuring device)
- A floatable object like an orange, tennis ball, baseball or driftwood of similar size and weight. The object you select should be relatively flat so that it has very little wind resistance and can flow freely in the current. Make sure to retrieve your object (if it is not part of the natural beach environment) before leaving the beach.
- Watch with a second hand or digital watch that records seconds



Procedure to measure speed of longshore current:

- A. Measure off and draw a 10-meter line in the sand parallel to the water.
- B. Position one person at each end of the line you have drawn. The person at the "beginning" of the line should assume the role of time keeper and have a watch with a second hand.
- C. Throw your floatable object, just behind the line of breakers, approximately 2 meters outside of your measuring area away from your beginning line. Note: The longshore current is closer to the shore than you might expect! Both people should watch the object as it moves.
- D. When the object passes the beginning of the line, the timekeeper starts timing.
- E. When the object passes the person stationed at the end of the line, that person tells the timekeeper to stop timing. Record the time.
- F. If time permits, repeat this process so you can calculate the average of multiple trials. You can repeat it in a different area along the beach as well.
- G. Use the formula for speed = distance in meters ÷ time in seconds, to calculate the speed of the longshore current for all trials, and then calculate the average of the longshore current.
- H. This procedure is not foolproof. If your floatable object does not move after a few minutes, try again. If you cannot get this to work at all, it could be because of weather conditions, or there might not be a longshore current at all.

How to measure the direction of the longshore current: Observe the direction that the floatable object (or driftwood) flows in the above procedure. If a current is going from north to south, the current direction is recorded as south or south-going; similarly, a current going from east to west is recorded as west or west-going. (This is the opposite of wind direction, which is recorded as the direction from which the wind is blowing).

14. General comments and observations about general beach conditions:	1147 H	_

II. Water Quality				
15. Some adopters may level of the water.		easure water pH. If yo	ou are one of these adopte	rs, please enter the pH
How to complete pH qu your site, do not answer		to you, enter the pH re	esult. If you are not already	determining pH levels at
16. If a pH reading was	taken, please indicat	e the testing method	you used. (Check the appro	opriate answer.)
☑ pH paper	☐ pH liquid solution	☐ pH meter		
How to complete pH te	sting method question	: Check the appropriat	te answer if you are determ	ining pH levels at your site
Your water sample shou 2.5 feet) of water depth	ald be taken at the sam is first encountered and ue dots) and Coliform (comments" section.	ne location in the midd and at 6 inches below t	de for specific protocol and dle of your adopted beach w the surface. If you are using ot tested" for any test you o	here 24-30 inches (2 – g the Alliance's test kits,
Sample #1 (from the mi		each area)		
Test type	E. coli – water (blue dots)	Coliform (red dots)	Enterococcus	E. coli – sand
Number of dots	1	5	Not tested	Not tested
Sample #2 (from the mi	iddle of your adopted b	each area)		·
Test type	E. coli – water (blue dots)	Coliform (red dots)	Enterococcus	E. coll – sand
Number of dots	1	5	Not tested	Not tested
18. Comments or resas	•		each area	
Some adopters have th	e ability to use the Gl	obal Positioning Syste	em (GPS) to calculate whe ion, provide your results he	re they take their water re:
° 'N (Latit	ude)° _	'W (Longitude)		
How to monitor for <i>E. o</i> . The Alliance will provide for your beach visit pleas	adopters with an E. col	li water sampling kit pr	ior to your beach visit. To req your beach visit schedule at l	ceive an <i>E. coli</i> kit in time least two weeks in advance

The Alliance provides:

- Whirl-Pak bags: small sterile plastic bag used to take the water sample
- Sterile pipettes: used to extract the 1 ml of water from the Whirl-Pak bag
- E. coli plates (Petrifilm for E. coli and coliform bacteria): Use to place 1 ml of water from your pipette in order to grow bacteria. DO NOT refrigerate your Petrifilm after it arrives in the mail. Store it at room temperate until you take your sample. PETRIFILM EXPIRES. Look for the written expiration date on the film. Discard expired Petrifilms.

You will need to provide:

- Gloves
- Sampling pole to use to take the water sample. This can be made from a fishing pole or an extension rod with a mop handle and alligator clips to hold the sampling bag. A PVC pipe with clips can also be used. You can also take a sample by wading out into the water without a sampling pole.
- Sealable bag to place the Petrifilm in after you have taken the sample

Health and Safety:

- · Wear gloves while sampling!
- Cleanse hands with alcohol wipes, or antiseptic lotion.
- · Bring a first aid kit with you to your site.
- Exercise extreme caution with students who are near the water.

Taking your water sample:

General: Take two samples using two separate Whirl-Pak bags to draw water for each Petrifilm.

- Wade into the water or put your sampling pole at least 20 feet away from swimmers and animals. If you wade into the water, face the horizon so the waves are less likely to wash bacteria from your body into your water sample.
- Avoid kicking up the bottom sediment of the sampling site. Pathogens can stick to solids, and excessive re-suspension might produce results that exceed local advisory limits.
- Avoid the "swash" zone, the area of low wave or water near the shore.
- Take samples from where water is at least one meter deep.
- To open the Whirl-Pak bag carefully tear off the top just before collecting the sample. Be careful not to touch the top or inside of the bag as bacteria from your hands can alter test results.
- Fill the sampling bag half full with water by sweeping down through the water in a U-shaped motion, 6 inches below the surface, moving away from your body. (Samples can be collected from a pier or bridge with a sampling pole.)

Placing your water sample on the Petrifilm: (This should be done while you are at the beach.)

- Open the packaging on the pipette by rolling back the plastic around the bulb side. Be careful not to touch the narrow tip of the pipette as your hands can contain bacteria that can alter test results.
- Use the sterile pipette, to withdraw 1 milliliter of water from the sample. To withdraw water, squeeze the bulb of the pipette and place the narrow end of the pipette in the water sample. Slowly release the bulb until you get 1 milliliter of water. Take the pipette out of the water without releasing the bulb. The water sample will remain in the pipette. Tip the pipette up so the water sample rests in the bulb.
- Carefully peel back the top film of the Petrifilm. Be careful not to contaminate the film by touching it with your fingers.
- Release the 1 milliliter sample onto the center of the pink circle of the Petrifilm.
- Slowly roll the cover of the Petrifilm over the water sample on the pink circle. Do not rub or touch the top of the Petrifilm after you have placed the film on top of the sample as it will alter your results.
- Store the plates in a sealable bag in a dark area and incubate them (35°C or 95°F) in a horizontal position with the clear side up for 24+ hours.

Preparation:

- Prepare a warm, dark place (35°C or 95°F) to incubate (see below) your sample. If you are not able to incubate your Petrifilm at the recommended temperature, keep the Petrifilm in a sealed bag at room temperature or warmer for 48 hours (as opposed to 24 hours). Keep the Petrifilm in a dark place as direct sunlight can kill the bacteria colonies. Without incubation, it is not as likely that you will see the gas bubbles that form, which confirm that the blue dots are E. coli colonies. However, you should still see blue and red dots appear on the Petrifilm which should indicate coliform and E. coli bacteria colonies.
- Write the date and sample number in the corner of the Petrifilm plate.
- Observe consistent labeling and recording protocol for samples. On the bag, include the date, time, site, and collector's initials.

Incubation:

Use an incubator or prepare a warm place (35°C or 95°F) to incubate your sample If you are not able to incubate your Petrifilm at the recommended temperature, keep the it in a sealed bag at room temperature or warmer, out of direct sunlight, for 48 hours (as opposed to 24 hours).

Reading your E. coli test results:

- Bacteria colonies will appear on the Petrifilm as red and blue dots. Blue dots represent *E. coli*. If you don't have any blue dots on your film, *E. coli* is not present in your water sample. Red dots indicate coliform bacteria. The Environmental Protection Agency's water quality standards for safe swimming require that no more than 235 *E. coli* colonies per 100 ml of water be found.
- Each dot represents one bacterial colony. Since only 1 ml of sample was used and colony counts are usually given per 100 ml, multiply each dot by 100 to get the number of colonies per 100 ml.
- Note that water quality tests such as this method are a screening method. High E. coli counts suggest further sampling should be done.
- Disposal: After counting the bacteria, use a dropper to place one milliliter of bleach on the pink circle. Place in a sealed plastic bag and dispose of it properly.

19. What is the water t	emperature? <u>68</u>	_ (Round to the nearest degree.)
☐ Celsius	it (Check type of measur	urement taken.)
mometer temperature st	tabilizes. To do this with	thermometer in the water for approximately two minutes, or when the ther- thout entering the water, tie a string onto the thermometer and hold onto Record your results and check the type of measurement taken.
20. Have you noted any	changes in water colo	or from previous visits? (Check the appropriate answer.)
☐ Yes ☐ No	This is our first beach	ach visit
If you have noted a char	nge in color, describe it.	t
How to note changes in beach more than one till priate answer.	n water color: If this is yome, note any changes yo	your first Adopt-a-Beach [™] visit skip this question. If you have visited your you have observed in regard to the color of the water and check the appro-
21. Describe the odor of	of the water. (Check one	ne or more of the options below.)
V No smell	☐ Sewage	☐ Algae (decaying plants) ☐ Fishy
☐ Sulfur (rotten eggs)	☐ Musty (wet soil)	☐ Other
with smells that may be	associated with water b	w minutes to determine how the water smells to you. We have provided you r bodies. Check one or more of the options that apply to your observations. slow the chart to describe the odor.
22. Describe the turbid location you take yo	lity (cloudiness) of the vour water sample.	water. (Check one of the options below.) Observe turbidity at the same
☐ Clear Slightly c	loudy 🗆 Cloudy	y ☐ Opaque (solid)
		How to complete the turbidity chart: First you must determine the turbidity (cloudiness) of the water to do this stand on the water's edge of your beach and observe the clarity of the water. Use the images below to help you determine turbidity.
Slightly cloudy Cloud	dy Opaque (solid)	
23. Additional observa	tlons about water qualit	lity: <u>foamy at shoreline</u>
How to complete addit		st any additional comments you would like to make about your observations

III. Bather Load (Number of people at the beach)

24. For people IN or ON the water, not on the beach, describe the type of activity and number of people involved. (Use the table below to fill in the number of people involved in the activities listed below.)

Type of activity	Sailing/ power boating	Canoeing/ kayaking	Jet skiing	Fishing	I Surting	Windsurfing/ kite boarding	Swimming/ wading	Other (in and on the water)
Number of people engaged in this activity	2	1					18	

If other, describe the type of activity IN or ON the water:
How to complete the activity and number of people involved in the activity chart: Count the number of people engaged in a particular activity and enter the number in the table provided under the correct type of activity. If there is an activity that people are engaged in that is not included in the list, use the section titled "other" and describe the type of activity in the space provided below the chart.
25. What is the total number of people ON the beach, not in the water?
How to record total number of people at the beach: Count the number of people at the beach. In your count, include the number of people in and on the water. If the beach is large, choose a representative area to use to count the number of people as a base to estimate the rest of the beach. Lifeguards often maintain records of bather density throughout the day. If available, you can also use gate or visitor numbers for that beach.
26. General comments and observations at the beach.
How to list general comments: List any additional observations about the number of people at the beach.
A busy day at the beach for it being 11 am.

IV. Potential Pollution Sources

27. Identify any of these features up to 500 feet from the beach boundary that are visible. (See Adopt-a-Beach™ Guide to determine speed of current.)

(Check the answer that	lushing	<u> </u>		
applies)	teady stream	□ Gushing □ Steady stream □ Trickle	☐ Gushing ☐ Steady stream ☐ Trickle	☐ Gushing ☐ Steady stream ☐ Trickle
Speed of Current (in seconds)	.2 M/sec			
☐ G ☐ B Water Color ☐ W	Brown Green Black Vhite Red Clear	☐ Brown ☐ Green ☐ Black ☐ White ☐ Red ☐ Clear	☐ Brown ☐ Green ☐ Black ☐ White ☐ Red ☐ Clear	☐ Brown ☐ Green ☐ Black ☐ White ☐ Red ☐ Clear
Characteristics (Check all	Foamy Algae Debris Dily sheen on water	☐ Foamy ☐ Algae ☐ Debris ☐ Oily sheen on water	☐ Foamy ☐ Algae ☐ Debris ☐ Oily sheen on water	☐ Foamy ☐ Algae ☐ Debris ☐ Oily sheen on water

Dom man rem omer a poterio

How to complete this chart:

- 1. Observe your beach area within 500 feet of the beach boundary to determine if there are any of the features listed (rivers, streams, ponds,). If there is not a water source, in the column marked "other" write "none." Note: features like those listed are not always associated with potential sources of pollution but it is good practice to examine any of these features as they can carry pollution to your beach.
- 2. Estimate the amount of water in the source as either: gushing, steady stream or a trickle. Check the appropriate response.

3. Determine the speed of current. See method below.

4. Check the visual characteristics that describe the water source that you have identified.

These visible sources can provide valuable information about the magnitude of the potential pollutant carried by these sources to the beach.

How to measure speed of current: If possible, measure flow in a straight section of the stream or another source that has a stable bottom that is 10 meters in length.

To determine speed of current you will need the following materials:

Meter stick (or other measuring device)

- A floatable object like an orange peel or other floatable object similar in size and weight. The object you select should be relatively flat so that it has very little wind resistance and can flow freely in the current. Make sure to retrieve your object (if it is not part of the natural beach environment) before leaving the beach.
- Watch with a second hand or digital watch that records seconds.

Procedure	to	measure	speed	of	current:
-----------	----	---------	-------	----	----------

- 1. Measure off and draw a 10-meter line in the sand parallel to the water.
- 2. Position one person at each end of the line you have drawn. The person at the "beginning" of the line should assume the role of timekeeper and have a watch with a second hand.
- 3. Throw your floatable object, approximately 2 meters upstream of your measuring area.
- 4. When the object passes the beginning of your 10-meter line, the timekeeper starts timing.
- 5. When the object passes the person stationed at the end of the 10-meter line, that person tells the timekeeper to stop timing. Record the time.
- 6. If time permits, repeat this process so you can calculate the average of multiple trials. You can repeat it in a different area along the beach as well.
- 7. Use the formula for speed = distance in meters ÷ time in seconds, to calculate the speed of current for all trials, and then calculate the average.

To determine the speed of the current use the following equation:

10 meters ÷ by time in seconds = speed in meters per second

Sample equation #1: 10 meters ÷ 30 seconds = .33 meters per second Summary: Your floatable object moves 10 meters in 30 seconds, therefore, the speed of the current is .33 meters per second.

Sample equation #2: 10 meters \div 40 seconds = .25 meters per second Summary: Your floatable object moves 10 meters in 40 seconds, therefore the speed of the current is 0.25 meters per second.

This procedure is not foolproof. If your floatable object does not move after a few minutes, try again.

28. Bacteria sample results. If you did not have any features as outlined in question 27, you can skip this question. If you did have any of the features listed in question 27, use one of your *E. coli* test kits provided by the Alliance to test for bacteria in water at the feature listed above. Please refer to Adopt-a Beach™ Guide #17 for specific protocol and to determine results. If you are using the Alliance's test kits, fill in: *E. coli* – water and Coliform. Write in "not tested" for any test you do not conduct and include an explanation in the "comments" section.

Feature Sample (from the water feature identified at your adopted beach.)

Test type	E. coli – water (blue dots)	Coliform (red dots)	Enterococcus	E. coll – sand	
Number of dots	2	14	Not tested	Not tested	

How to monitor for *E. coli* using the Alliance's *E. coli* test kit: See instructions for Question 17.

Did your team do a bacteria test for pollution sources listed for question 27?	Yes	□ No						
9. General comments about your water test:								
· · · · · · · · · · · · · · · · · · ·								
Some adopters have the ability to use the Global Positioning System (GPS) to calc sample. If you can use a GPS device to measure your sample location, provide your	ulate wher results her	e they take their water e:						
° 'N (Latitude) ° 'W (Longitude)								

30. Are there floatables (items floating in the water) present? ✓ Yes □ No If yes, please describe the floatables present. (Circle one or more of the options below.)

Туре	Street litter	Food- related litter	Medical items	Resin	Sewage- related	Building materials	Fishing related	Household waste
Example	Cigarette filters	Food packing, beverage containers	Syringes	Tiny plastic peliets	Condoms, tampons	Pieces of wood, siding	Highing line	Household trash, plas- tic bags

How to complete the floatables chart: "Floatables" are items found floating in the water that are not natural to the environment. To complete the chart, observe any debris floating in the water during your visit and circle all of the types of debris that apply.

Note: Floatable debris causes problems at beaches because the objects can cause harm to aquatic animals, people, boats, fishing nets, and other objects. Communities also lose money when beaches must be closed or cleaned up, and the fishing industry and recreational and commercial boaters must spend thousands of dollars every year to repair vessels damaged by floatable debris (USEPA, 2002). Floatable debris also can be a source of bacterial contamination to bathing beaches.

31. Describe the amount of debris/litter on the beach. (Circle one of the options below.)

Amount	Litter	Very Low	Low	Medium	High
Percentage on beach	0%	1-10%	11-20%	21-50%	51% and up

How to complete the debris on the beach chart: Because your team will be conducting a litter cleanup at the beach you do not need to list or describe the debris at your beach in this section. However, you should observe the overall litter conditions at your beach and estimate the amount of debris on your beach. Circle the appropriate amount of litter that is on your beach.

Note: Beach debris or litter can cause problems similar to those caused by floatable debris (described above). The debris can be washed onto the bathing beach and affect wildlife. In addition, the presence of certain materials on the beach, such as medical waste and sewage-related items, can pose an immediate health hazard to beachgoers and can be a source of bacterial contamination to the bathing beach.

32. Do you see an oily sheen on the water and/or along the beach? (Check the appropriate answer.) \Box Yes $lacksquare$ No							
a. If yes, describe.							
b. Can you identify the source?							

How to answer presence of oil in the water and along the beach: Through general observations, determine if the water has an oily sheen. If an oily sheen is present, try to identify the source, such as a boat or jet ski, bottle in the water, etc.

33. Describe the amount of algae in the water near the shore along the length of your adopted area of beach.

Amount	Algae	Low	Medium	High
Percentage	0%	1-20%	21-50%	51% and up

How to complete the algae along the water's edge chart: Walk along the water's edge of your visit area and observe the presence of algae in the water. Circle the percentage of algae that is at the water's edge.

Note: Algae can be a nuisance along Great Lakes beaches and shorelines. Decaying algae can produce a foul odor that can deter people from visiting our beaches. Algae have also been suspected of harboring *E. coli*, which can lead to beach closures.

34. Describe the amount of algae on the beach along the length of your area of adopted beach.

Amount Algae		Low	Medlum	High
Percentage	0%	1-20%	21-50%	51% and up

How to complete the algae on the beach chart: Stand near the water's edge of your visit area and observe the presence

				beach. (Check one or n	
□ No algae		№ Attac	hed to rocks, stringy	⊮ Blobs of floatin	ng materials
☐ No obvious ma	☐ No obvious mass of materials		ed	☐ Other	
If other, describe	other, describe:				
How to complete apply to the phys ed below the cha	ical characteristic	e at your s of the a	beach location: Obser Igae. If you have selec	ve the algae at your bea ted "other," describe wh	ch location and check all tha at you see in the space provi
36. Describe the	color of the alga	e along t	he water's edge and o	n the beach. (Check one	or more options below.)
□ No algae	☐ Light gr	een	. □ Blue green	Dark green	☐ Yellow
Red	 Brown		☐ Other if oth	er, describe:	and the second s
apply to the colo	r of the algae. If y	ou have s	elected other describe	what you see in the spa	ach location and check all thance provide below the chart.
37. Please desc	ribe and count the Geese	s present	Gulls	Dogs	Other
		O O			

38. If you find dead birds along the shoreline, fill in the number found in the appropriate box below.

Туре	Common Ioon	Herring gull	Ring-billed gull	Double crested cormorant	Horned grebe	Other
Number found dead	0	1	0	0	0 .	0

tify sources of bacterial contamination and potential best management practices (e.g., pet owner education, better trash management to reduce available food sources at the beach) that could be used to reduce the amount of animal waste

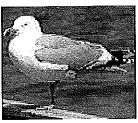
If other, d	escribe: _		w-	

How to complete this dead birds chart: Use the images below to determine the type (species) of bird that you have found on the beach. Count the number of dead birds and fill in the number in each category. If you have selected "other" and know the species of the bird found, use the space below the chart to describe.

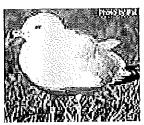
reaching the beach.







Herring gull



Ring-billed gull Has a distinct ring around its beak



Double crested cormorant



Horned grebe

Health and safety: Don't remove dead animals from shorelines unless you are prompted to do so by local officials using the proper removal methods.

Note: Decaying birds can contribute to water quality issues at your beach. Some areas along Great Lakes shorelines are experiencing *E. botulism* outbreaks that are killing large numbers of birds at certain times of the year (generally late summer through fall). If you find a large number of dead birds at your beach, the location may be affected by these outbreaks. It is important to determine the number and type of birds that are affected so professionals can track the numbers of species being lost.

39. I	How many	dead	fish	are	on the	beach?	1
-------	----------	------	------	-----	--------	--------	---

40. If there are other dead animals on the beach (not including fish and birds) list them here.

Type of animal	How many
turtle	1

How to determine number of dead fish on the beach: Count the number of dead fish that you find along the beach and fill in the number of dead fish found.

Note: Decaying fish can contribute to water quality issues at your beach.

41. How many garbage and recycling containers are there within 500 feet of your adopted beach boundary? (If there are no garbage containers on your beach enter 0.)

Garbage Containers	Recycling Containers
4	2

	4			
42. Describe use and o	condition of	garbage containers at this locatio	on. (Check one or more of the options below.)	
☐ No garbage cans	☐ Designa	ted carry in carry out policy	☐ Garbage cans present with no lids	
☑ Garbage cans prese	nt with lids	☑Garbage cans well maintained	\square Garbage cans overflowing or knocked (over
How to complete the gappropriate condition for			cans on your beach and their conditions. Che	ck th
ship between the amou	unt of trash t	present or if containers don't have hat you find on your beach and the have an effect on the amount of	e lids or are in bad condition you may see a re e trash cans. Observe the general conditions of trash on your beach.	elation of the

43. Please add any additional comments or notes about your visit here:

Beach looked pretty clean. Our group had a great time and can't wait for our next visit!

44. Did yo	u take any ad	ction as a result o	of your beach visit? For example: educate others about pollution, contact your
park autho	rity to ask the	em to empty trash	cans more frequently.
☑ Yes	□ No	If yes, describe:	Students wrote the park district to add recycling bins

Thank you for your time and dedication to keeping our beaches and shorelines healthy!

Important Additional Information

Responding to potential pollution sources found at the beach:

In most cases when you find a potential pollution source at the beach (e.g. overflowing garbage bin or an outfall pipe discharging water with a high e. coli count), we recommend that you conduct a follow-up visit a few days or a week later to see if the problem has been corrected. You may request additional water quality test kits for follow-up sampling through your state Adopt-a-Beach[™] coordinator.

However, if you are witnessing an environmental event that may lead to imminent threat to human health or the environment (e.g. an oil or chemical spill), please report the situation using EPA's toll free National Response Center hotline at 800-424-8802 and also notify your state Adopt-a-Beach™ coordinator.

Contact us: adoptabeach@greatlakes.org

Litter Monitoring Instructions Instructions for Site Coordinators

Follow the directions below for a successful cleanup! Use the orange Adopt-a-Beach™ Litter Monitoring Form found in your startup kit and available for download online to record your litter data. To find the Litter Monitoring Form, visit www.great-lakes.org and follow the Adopt-a-Beach™ sun to the Adopt-a-Beach™ homepage and look for a link to "forms and instructions".

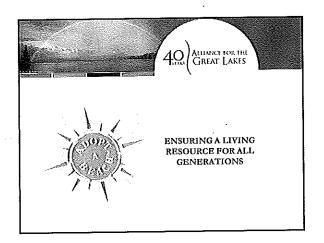
- Divide the participants into small groups of 3 4 people (or maintain groups that have been established to collect Routine Visit Form data) to clean up and record litter data.
- Assign one person to record the litter collected in each small group. Request that groups complete both sides of the Litter Monitoring Form.
- Give each group: an Adopt-a-Beach™ Litter Monitoring Form, gloves (one per volunteer or two gloves if they request it), one (or more) bag(s) for litter and one (or more) bag(s) for recyclable items (i.e. plastic bottles, aluminum cans, etc.). Ideally the bags should be different colors to minimize confusion. Find out about recycling practices in your community so you can explain what participants should collect and understand how to dispose of the recyclable items properly.
- As volunteers remove litter, ask them to record their findings by making tally marks next to the debris item on the Litter Monitoring Form. Follow the example on the Form. At the end of the cleanup, make sure each small group records the total number of items their group has found at the end of the cleanup. This number goes in the small box to the left of each item name.
- You (or an individual or group you choose) should be in charge of a **final** Litter Monitoring Form. Note "Final Data" on one of the Forms. At the end of the cleanup, make sure this card has a number total of ALL items picked up by the other small groups.

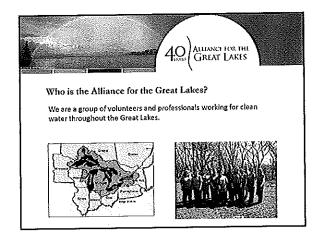
Additional instructions for volunteers when picking up and recording litter:

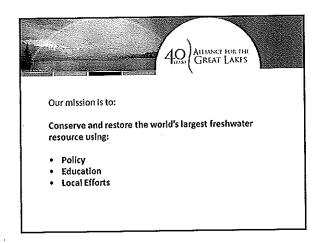
- Pick up everything you find that is not a natural part of the environment. If you find animals entangled in debris (fish, birds or other wildlife) record them on the Summary Card but do not touch them or try to dispose of them yourself. Notify your local park district or beach authority about the location of the dead wildlife you have found.
- · Record only the items found on the data card.
- Pick up "pieces" of items, but do not record these fragments on the data card as this can distort the data totals. You may make note if you feel you have found an exceptionally high number of fragments, especially if they might pose a danger to humans or wildlife.

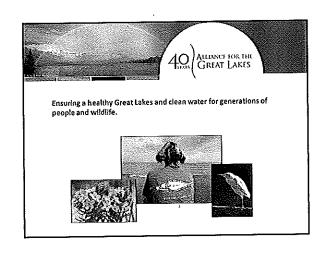
• Stress personal safety such as not touching suspicious looking materials, barrels, needles, etc. All barrels, except those that are empty and completely clean, should be reported to your local authorities. Mark down all codes on barrels to assist in identifying the contents. To report an oil or chemical spill, contact the U.S. Coast Guard's National Response Center at (800) 424-8802. If a volunteer finds a medical syringe ask them not to pick it up. Site coordinators can safely dispose of syringes by placing them in an empty bottle with a secure top.

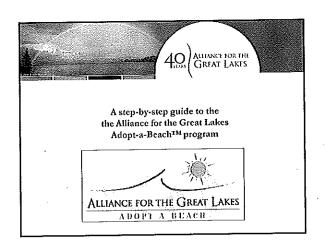
If you do not have access to the internet send completed forms to: Alliance for the Great Lakes, 700 Fulton St. Ste. A, Grand Haven, MI 49417 or fax to 616-850-0765

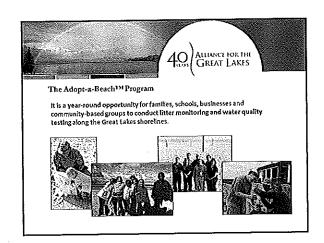


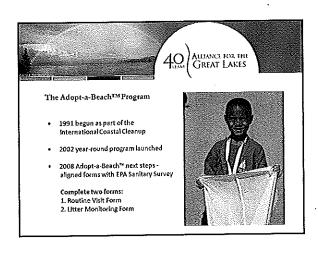


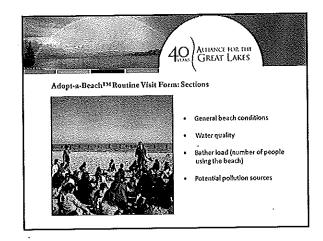


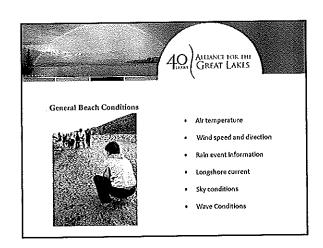


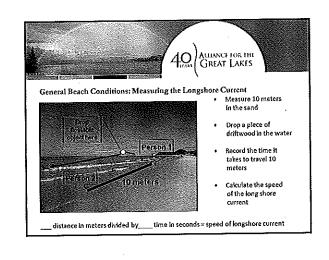


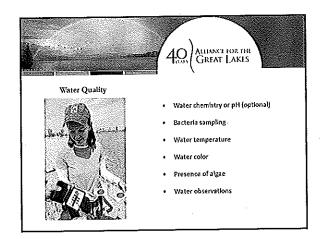


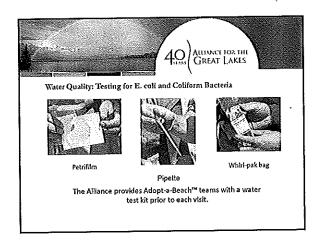


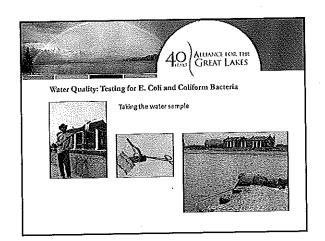


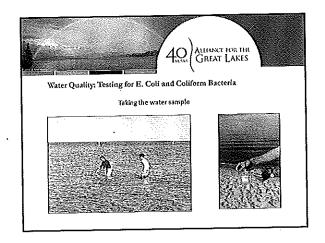


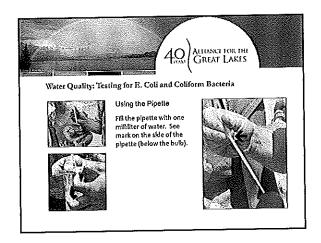


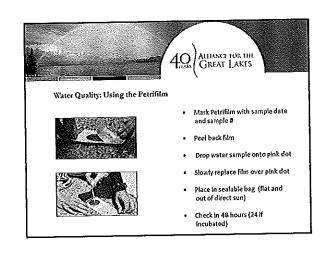


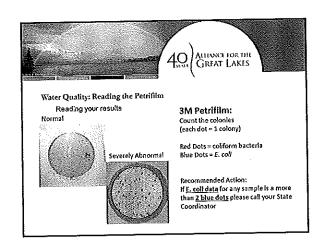


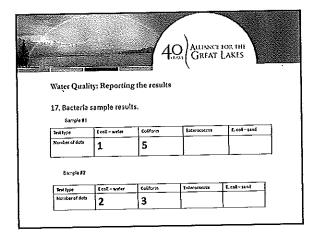


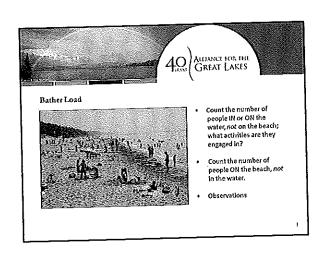


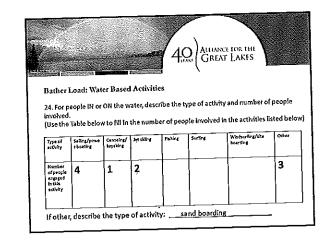


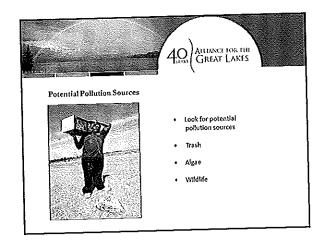


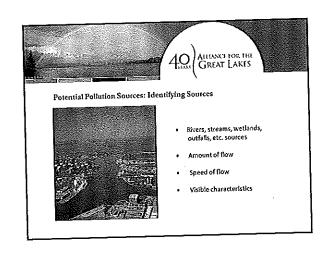


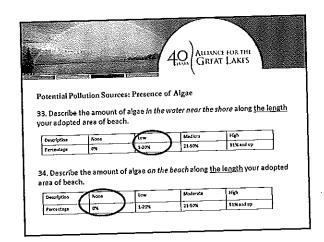


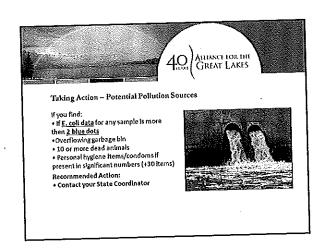


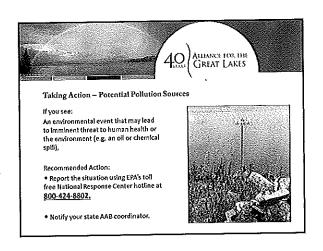


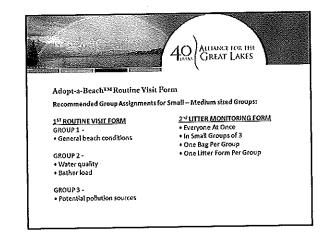


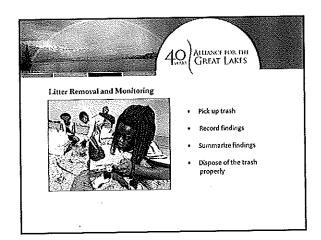


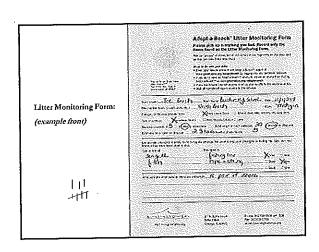


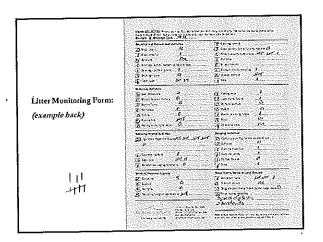


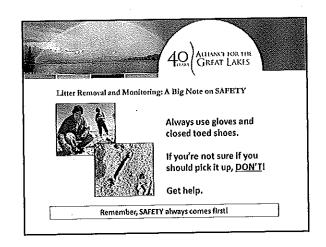


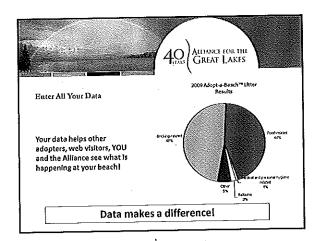


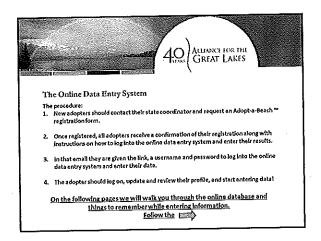


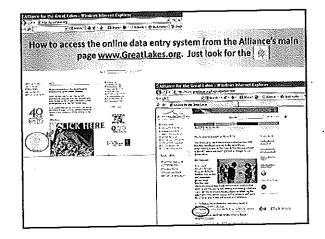


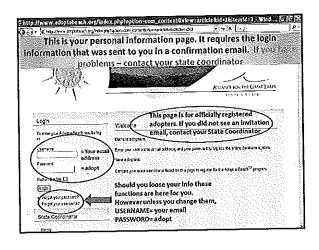


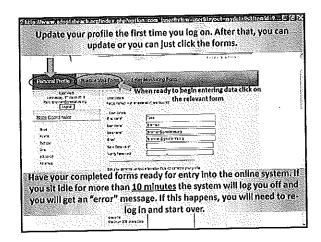


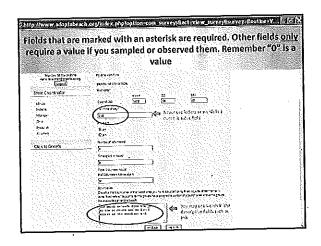


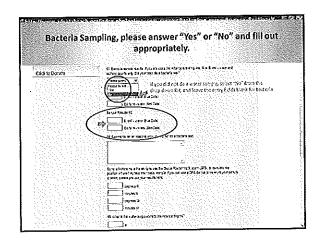


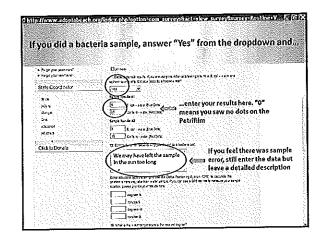


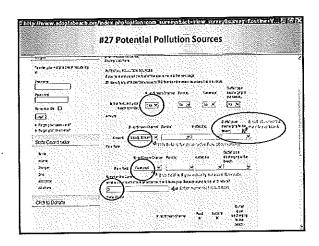


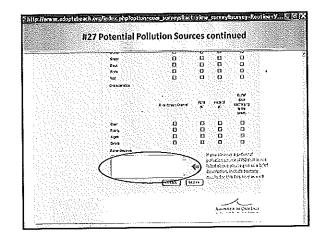


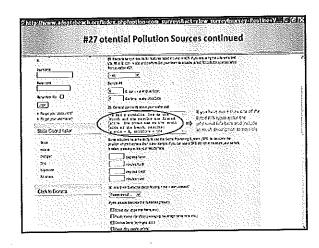


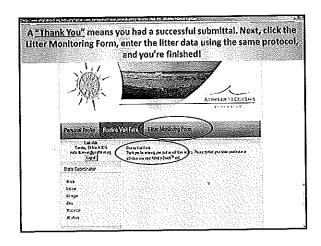


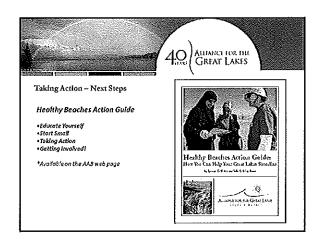


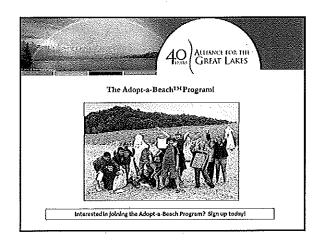


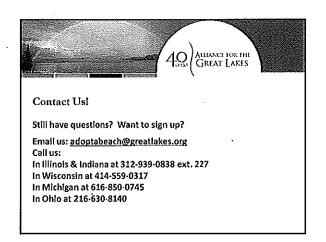




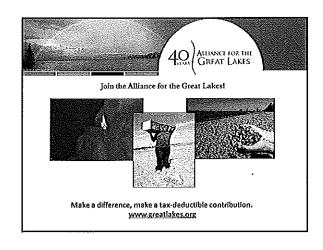












Enumeration of Waterborne Escherichia coli with Petrifilm Plates: Comparison to Standard Methods

J. H. Vail, R. Morgan, C. R. Merino, F. Gonzales, R. Miller, and J. L. Rami*

Abstract

Escherichia coll is often monitored in environmental waters as an indicator of the possible presente of human pathogens associated with isces. Pourities B. collicoliform count plates (3M, Mianespolis, MN), previously validated for enumerating E. colt in food, were tarted for monitoring R. cell in environmental water. Escherichia coli comatt in environmental mater camples enumerated with Petrifiba were sigofficiantly correlated (R > 0.9) stops = 0.9-1.0; p < 0.001) with counts obtained with three commonly used methods, mTEC (Sector Dickinson, Sparks, MD), m-Colibius (Hach, Loveland, CO), and Colliert-18/IDEXX Quanti-Tray 2000 (IDEXX, Wastbrook, ME). Blue colories on Retriftin plates were most reliably identified as B. coll when accompanied by gas formation, as determined by characterization of the colonies on MacCoules ugar plates CPML Microbiologicals, Mississungs, ON, Canada) and by polymerase chair reaction (PCR) with E. colf-specific primers. The ranks disadvantage of Patrillian places for environmental water testing is the small volume (1 mL per sample) that can be tasted; however, the pistes appear to be suitable for streening and locating sitts that erroted criterio for total body and partial body contact. Simplicity of use and storage, reliability, and relatively low cost make Petrifilm plates suitable for volunteer-based and educational water quality monitoring applications, particularly when used as a preliminary coversing resthod to identify problem sites.

Tion Levels of bacteria are a concern for many ma-rine, brackish, and freshwater environments. Elevated levels of bacteria in coastal waters are associated with increased risk of gastrointestinal symptoms for recreational swimmers (Cabelli, 1977; Dufour, 1984; Priles, 1998; USEPA, 1986). Because of known association with fecal matter, levels of E. coli bacteria are a key regulatory measure of the healthfulness of recreational waters (USPPA, 1986, 1999a). For fresh waters, the USEPA recommends criteria of 126 E. coll colony forming units (cfu)/100 mL for the geometric mean of five samples over a 30-day period and 235 E. coll ofu/100 mL for a single sample, but states set their own standards (USEPA, 1986). For example, in Michigan, rivers, lakes, or streams measuring greater than 300 E. coli cfu/100 mL on a single day or more than 130 E. coll cfu/100 mL for a 30-day geometric mean are considered out of compliance for total body contact (e.g., beaches); 1000 E. coll cfu/100 mL is out of compliance for partial body contact (e.g., fishing, boating) (Rule 62; Michigan Department of Bavironmental Quality, 1999).

Despite the general awareness of the need for monitoring, many places that are suspected to be out of compli-

I.H. Vall, Robert B. Annis Water Resources Institute, Grand Valley State University, Muskegon, MI 49441. R. Morgan and R. Miller, Department of Biology, Grand Valley State University, Allendale, MI 49401. CR. Marino and J.L. Run, Department of Physiology, Wayne State University, Detroit, MI 48201. Received 1 Mar. 2002. *Corresponding author (Jeffram@med.wayne.edu).

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ance are not monitored due to perceived high cost and complexity of equipment involved in a local monitoring program. Citizen based volunteer monitoring programs have been developed in several states, such as Iowa (Seigley, 2001), and have been used for purposes of preliminary screening of local waters for identifying problem areas. However, since E. coll enumeration methods generally require expensive media or equipment not generally available to volunteers, a convenient, inexpensive method of E. coll enumeration is needed for such programs.

This paper describes the testing of Petrifilm E. colif coliform count plates as a new, convenient method for enumerating E. coll in environmental waters. Petrifilm plates have previously been described for use in enumerating E. coll in food and dairy products (Curiale et al., 1991; Priego et al., 2000; Russell, 2000; AOAC, 2000a,b), and it therefore seemed reasonable to evaluate whether they may also be useful in water testing. Petrifilm plates consist of plastic films with grids that are coated with Violet Red Bile nutrients, a tetrazolium indicator, and gelling agents. The gel contains a β-glucuronidase indi-cator for confirmed detection of E. coli. The present study is a multilaboratory investigation comparing E. coli enumeration of environmental water samples with Petrifilm technology with E. coll enumeration by methods frequently used by each participating laboratory. The methods to which Petrifilm enumerations were compared were the mTEC, m-ColiBlue, and Colilort-18/ IDEXX Quanti-Tray methods.

Materials and Methods

Water Sample Sources

Bach participating laboratory collected environmental water samples near its location. The source sites were chosen to have a range of bacteria levels ranging from near zero up to relatively high noncompliant levels, based on previous experience at the same sites. Water from Ruddiman Lagoon and tributaries, in the city of Muslegon, MI (43°13' N, 86°17' W) was enumerated by Petrifilm and m-Colibius methods at the Annis Water Resources Institute. Water from a small tributary of the Grand River in Ottawa County, MI in a rural area near the intersection of 68th Avanue and Leonard Street in Coopersville, MI (43°1' N, 85°57' W) was enumerated by Petrifilm and mTEC methods at Grand Valley State University. The tributary is located in a rural mea mostly occupied by cattle pastures and was sampled just upstream from its confuence with the Grand River. Water from various sites in the middle Rouge River subwatershed in the Rouge River watershed, in several suburbs west of Detroit, MI (42°22' N, 83°25' W) were entimerated by Petrifilm and IDEXX Quanti-Tray/Collect-18 methods at Wayne State University. In addition,

Abbieviations cfu, colony forming units; PCR, polymerase chain re-

vail et al.: enumeration of a coli with petrifilm plates

E. coll colonies on Petrifile plates from water obtained from various sites in the Clinton River watershed (42°35' N, 82°55' W) were used for further characterization of Petrifilm colonies.

Sampling and Enumeration Procedures

All water samples were obtained with sterile bottles or sterile Whiripak bags (Nasco, Fort Atkinson, WI), transported on ice, and analyzed within 4 h. Samples were tested in various dilutions, as indicated below, to assure that bacted a concentrations were within the appropriate range for each technique (American Public Health Association, 1998).

Petrillin Procedure. The methodology for the Petrillin

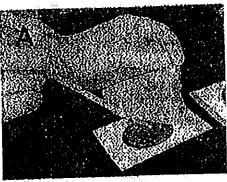
Petrifilm Procedure. The methodology for the Petrifilm plates was to (i) incoulate and spread 1 mL of water on the get (see Fig. 1A), (ii) incubate the plate at a temperature of 35 ± 1°C for 24 ± 2 h, and (iii) count the number of blue colonies associated with a small gas bubble. Coliform colonies appear red surrounded by a bubble, due to an indicator dye and the trapping of gas produced by the coliforms by the upper film of the Petrifilm plate. Escherichia coll colonies are charecterized by a blue precipitate surrounded by a gas bubble; blue colonies with gas are counted as K. coli, while blue colonies without gas are not (AOAC Official Methods, as described by the 3M interpretation guide). An example of the results obtained with one such plate is illustrated in Fig. 1B.

results obtained with one such plate it manufacts in Fig. 105.

m.ColiBlue24 Analysis. The m.ColiBlue24 mombine filtration broth is USEPA approved for analysis of total coliforms and E. coll in drinking water (USEPA, 1999b) for enumerating total coliforms and E. coll in a proposed rule for ambient waters (USEPA, 2001). Eschetichis coli colonies are characterized by a blue color due to a reaction between the enzyme \$\textit{\textit{Blueuronidase}}\$ and \$\textit{\textit{browno}}\$-b-glucuronidase and \$\textit{\textit{browno}}\$-b-glucuronidase and \$\textit{\textit{browno}}\$-b-glucuronidase and \$\textit{\textit{browno}}\$-b-glucuronidas. One-milliliter ambient water samples were diluted with 99 mL of sterile buffered dilution water and 100 mL was filtered through a sterile 47-mm nitrocellulos filter with a pore size of 0.45-\textit{\textit{mm}}\$ (Millipare, Bedford, MA). The filter was then placed on an absorbent pad pre-soaked with in CollBlue nutrient broth in a Potri plate and incubated at 35 \textit{\textit{mm}}\$ 0.7°C for 24 h. Blue colonies were counted as \$E\$ coli. One-milliliter portions of the undiluted water samples were assayed on Petrifilm plates.

mtRC Technique. The USBPA-approved original E. colimothed was used (Method 1103.1; USBPA, 2000). Nutrient plates were prepated with dehydrated mTPC agar powder. Environmental water samples were serially diluted 10-fold. One-milliliter samples of each dilution were filtered through a sterile 47-ram nitrocellulose filter with a pore size of 0.45 µm (Millipore) and aseptically placed on mTEC agar plates. Similarly, 1-mL samples of the same diludous were assayed on Petrifilm plates. The mTEC plates were incubated for two hours at 35 ± 0.5°C and then incubated at 44.5 ± 0.2°C for 22 to 24 h. After incubation, the filter membranes were placed on Whatman (Maidstone, UK) filter paper that had been saturated (1.0 mL) with trea substrate media containing 2.0% urea (w/v) and 0.01% phenol red (w/v). Colombs that remained yellow, yellow-brown, or yellow-green were considered E coli.

Colliert-IS/IDEXX Quanti-Tray Method. Use of Colliert-18 with Quanti-Tray 2000 trays to enumerate E. coll is described in USEPA (2001). Fifty-millilliter ambient water samples from the sampling sites were diluted fivefold. Then, our of 103 mL of the diluted sample, three 1-mL samples were assayed on Petrifilm (1 mL on each plate out of the 105 mL diluted sample), and the remaining 100 mL was added to Colliert-18 and assayed in IDEXX Quanti-Tray 2000 trays, according to the manufacturer's instructions. Quanti-Tray



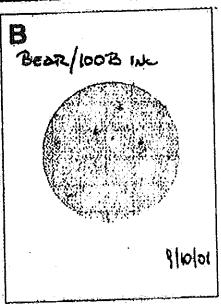


Fig. 1. Application of water to a Fetrilium plate and resultant bacterial growth. (A) The top film is lifted while a Loul water sample is applied with a sterile transfer pipet. (B) Fetrilium plate efter a 24-h incubation with a 1-rul environmental water sample. Dark spots in this grayscale image were blue in the original.

samples were incubated 20 h at 35.5°C, after which fluorescent (B-glucuronidese positive) wells on the Quanti-Tray were counted, to calculate the most probable number (MPN) of E. coli chi/100 mL in the diluted sample, according to a chart supplied by the manufacturer.

Further Characterization of Petrifilm Colonies

Blue colonies were picked from Petrifixa plates with sterile inconleting loops and streaked onto MacConkey agar plates. MacConkey plates were incubated for 24 h at 35.5°C, the presence of plak (i.e., lactose-formenting, as expected for E. coli) colonies was noted, and then well-isolated pink colonies were inoculated into Coilier-18 medium. After culturing for 20 h at 35.5°C, the presence of yollow color and fluorescence was noted, sterile glycerol was added to a final concentration of 14%, and then the culture was frozen until further analysis. For the polymerate chain reaction (PCR), 1 µL of the thawed culture was subjected to thermocycling (anneal, 60°C; synthe-

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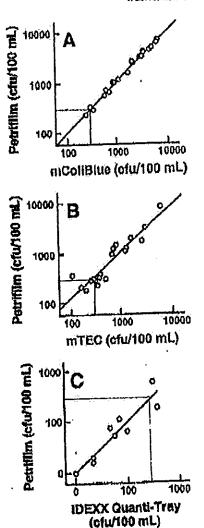


Fig. 2. Comparisons of Fetrillian to three standard methods for E-coll connectation of cavironmental water samples. Unto were normalized to 100 mL and transformed with logicin/100 + 101 pilot to linear regression. Vertical and hoticoiral lines in each graph indicate 300 cfulfed nd., the blickigan maintains whole body contact limit. (A) Comparison with the in-Collilus mathod, with 20 water samples from Ruddiman Legoen and teleprinaries. The regression line has stope = 1.01, R = 0.995, and p < 0.001. (B) Comparison with the mTEC mathod, with 19 water samples from a teleprina to the Grand River. The regression line has stope = 0.91, R = 0.93, and p < 0.001. (C) Comparison with the Collect-18/IDEXX Quanti-Tray method, with nine water samples from the Middle Range River. The regression line has stope = 0.908, R = 0.935, and p < 0.001.

sis, 72°C; malt, 94°C; 30 cycles) with the following E. collspecific primers: 298F, 5'-AATAATCAGGAAGTGATGG AGCA-3'; and 884R, 5'-CGACCAAAGCCAGTAAAGTA GAA-3', which amplify a sogment of the \$\text{p}\$ gluouronidase gene. Identity of the PCR products was confirmed by sequencing.

Statistic

Regression analysis was done on log-transformed that after multiplication by appropriate factors to take into account the

amount of dilution, so that counts in 100-mL volumes were being compared, and adjusted upward by a small constant to prevent taking the logarithm of zero. The log transformation used was logarithm of zero. The log transformation used was logarithm of zero. The log transformation mTBC, and m. ColiBlue assays, in which 1 mL was the volume assayed, the transform was therefore log(counts × 100 + 10). For IDEXX Quanti-Tray assays, in which the assayed volume was 100 mL, the transform was log(most probable number + 10). The means of log-transformed values of duplicate or triplicate assays or the individual log-transformed values when measured without replicates were used in subsequent correlations, and in calculations of method means, standard deviations, and statistical significance with palzed i tests. Statistical tests were performed with Sigmastat 2.0 (Jandal Scientific, 1995) software. For linear regression, Patrifilm data was used as the dependent variable and the "standard" measurement method as the independent variable. A repeat of the analysis with a simple log transform and adjusting only the zero count samples to 10 cfu/100 mL to avoid log(0) yielded essentially identical conclusions.

Regults

Comparison of Petrifilm Results with Other Engineration Methods

Comparison with m-Coliblue. Samples from Ruddiman Lagoon and tributaries were enumerated in triplicate with Petrillim and in duplicate with m-Coliblue. Water samples were collected from four sites on five occasions, for a total of 20 water samples. Correlation of the counts obtained with the two methods is illustrated in Fig. 2A. Counts ranged from as low as 200 cfu/100 mL to as high as 7000 cfu/100 mL. Linear regression of counts determined from Petrillim assays versus counts determined with m-Coliblue, after log transform, gave a slope of 1.01, R = 0.995, and p < 0.001.

The variability of data obtained with the Petrifilm method was assessed by determining the 95% confidence intervals of each triplicate measurement. The 95% confidence intervals averaged 17% of their corresponding mean values.

To test for bias one way or the other for the values determined by Petrilim versus the m-ColiBlue method, the mean values obtained by each method for each water sample were compared with a paired t test. The overall method means and standard deviations of the log-transformed counts were 3.207 ± 0.451 for Petrilim and 3.202 ± 0.458 for m-ColiBlue. The mean difference of paired measurements was -0.0054 ± 0.0455 , indicating no significant difference between the results obtained with the two tests (p = 0.60, paired t test).

Comparison with mTEC. Escherichia coli in water

comparison with MTEC. Escriencial con in water samples from a tributary to the Grend River were enumerated in triplicate with both Perrifilm and mTEC methods. Two water samples were collected on each of nine occasions, and one water sample was collected on a tenth occasion, for a total of 19 samples. Linear regression of the Petrifilm data against the mTEC data, illustrated in Fig. 2B, gave a slope of 0.911, R = 0.933, and p < 0.001.

The 95% confidence intervals of Petrililm triplicates averaged 39% of their corresponding mean values. Similarly, the 95% confidence intervals for the mTEC triplicates averaged 53% of their corresponding means. A

paired t test indicated no consistent differences between paired measurements made with the Petrifilm and mTEC methods on the same water samples (p = 0.38: method means: Petrifilm, 2.84 = 0.46; mTEC, 2.81 \pm 0.47; difference between paired samples, 0.035 \pm 0.169).

Comparison with Colliert 18/IDEXX Quanti-Tray. Samples collected from nine sites on the Middle Rouge River were diluted fivefold and then enumerated in triplicate by Petrifilm and in one 100-mL sample, by Collier-18/IDEXX Quanti-Tray, After transformation for comparison of equivalent 100-mL volumes, the regression of the Petrifilm results versus IDEXX results gave a slope of 0.908, R = 0.935, and p < 0.001 (Fig. 2C). Paired tests by the two methods were not significantly different from one another (p = 0.80; method means: Petrifilm, 1.81 \pm 0.55; IDEXX, 1.79 \pm 0.56; difference between paired samples, 0.018 ± 0.200); however, the average 95% confidence interval for the Petrifilm triplicates was quite large, equal to 101% of the corresponding means. The large 95% confidence intervals, compared with their corresponding means, reflect the fact that several of the water samples had low counts (0-2 colonies per plate) and therefore a variation of one or two colonies per Petrifilm plate produced a large percentage change of this measure.

Gas Formation as a Criterion for Identifying E. coll Petrifilm Colonies

As noted in the Materials and Methods, the AOAC Prescribed Method, recommended by 3M, requires that only blue colonies with gas bubble formation be counted as E. coli. To determine the importance of this criterion in accurately determining the correct number of E. coli colonies, the proportions of gas forming and non-gas-forming blue colonies were counted in several experiments. In addition, gas-forming and non-gas-forming colonies were picked from Petrifilm plates and characterized further.

Proportions of gas-forming and non-gas-forming colonies were determined in four experiments. In one experiment, five water samples collected from Ruddinan Lagoon and tributaries were assayed in triplicate on Petrifilm plates. On the resultant IS Petrifilm plates a total of 149 blue colonies were present, of which 107 were blue with gas (72%) and 42 blue colonies exhibited no gas formation (28%). For samples from the Middle Rouge River, blue colonies with gas accounted for 64% of a total of 64 blue colonies counted on 27 plates. For water samples from the Clinton River watershed, blue colonies with gas accounted for 86% of 190 blue colonies observed on 27 Petrifilm plates in one study and for 89% of 355 blue colonies observed on 30 plates in another study. On individual plates the proportion of nongas-forming blue colonies ranged between 0 and 50%.

Blue colonies from Petrifilm plates of two of the above experiments were streaked onto MacConkey plates and the proportion of Petrifilm colonies producing pink colonies on the MacConkey plates was determined (Fig. 3A). For both experiments 100% of the blue colonies with gas produced pink colonies on MacConkey plates. In fact, in most cases, only pink colonies

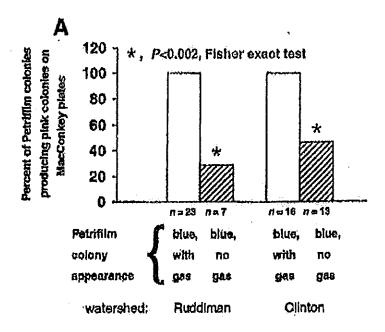
were present on the MacConkey plates. In contrast, for blue colonies without gas, in one experiment (water from Ruddiman Lagoon and tributaries), only 2 of 7 blue colonies without gas produced pink colonies on the MacConkey plates, and in the other experiment (Clinton River samples), only 6 of 13 no-gas blue colonies produced pink colonies of normal morphology. In both experiments, blue colonies without gas yielded a significantly lower proportion of MacConkey plates with pink colonies than observed for blue colonies with gas (Pisher exact test, p < 0.002).

Finally, from the Clinton River samples, bacterial clones from Petrifilm blue colonies with gas that were subsequently isolated on MacConkey plates were subjected to PCR with E. coli-specific primers. All 16 isolates produced the expected amplified product for E. coli, of which 12 are illustrated in Fig. 3B, and subsequently confirmed as coding for the E. coli B glucuronidase gene in comparison with a reference sequence (ABO0257, Bases 6765 to 7351) in Genbank (data not shown).

Discussion'

Although Petrifilm plates have previously been validated for use in detesting E. coll contamination of food (Curiale et al., 1991; Priego et al., 2000; Russell, 2000), they have not been tested extensively for use in detecting E. coli in environmental waters. The present study provides a comparison of E. coll enumeration in environmental water obtained with Petrifilm plates with three commonly used commercially available tests. Petrifilm results were highly correlated (R > 0.9) and equivalent (slope approximately = 1.0, no differences on paired r test) to mCollBlue, mTEC, and Collert/IDEXX Quanti-Tray tests, Analysis of differences between blue colonies with and without gas on the Petrillim plates suggest that due care in evaluating the presence of gas bubbles is necessary in counting colonies. More extensive testing of the Petrifilm method to determine rates of false positives, false negatives, efficacy in additional types of water samples, etc. could provide further validation of the use of Petrifilm plates, Nevertheless, the simplicity of using Petrifilm plates indicates that it may be a suitable method for citizen-based testing and environmental education programs.

Several characteristics of Petrifilm that make it suitable for volunteer-based monltoring of E. coli include ease of use, reasonable accuracy, sensitivity in an appropriate range, safety, low cost, ease of storage, and long shelf life. With three simple steps, as outlined in Materials and Methods, the Petrifilm method is easy to perform in both the laboratory and the field. Although the Petrifilm plates in this study were all inoculated in the laboratory, comparable results inoculating Petrifilm plates in the field have been found in our other studies and by volunteers (Ram, 2001). The dry gel on the plates sets up quickly with the addition of water, enabling the plate to be handled without spillage within a minute or two of inoculation. In other experiments with a range of incubation times (24-48 h) and temperatures, we have also found a good correlation with professional tests,



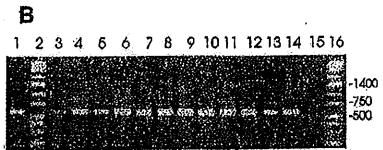


Fig. 3. Characterization of Petrifium colorides. (A.) Proportions of the Petrifilm colorides that produced plate colorides when grains on MacConkay plates, as a function of minites the blue colorides also produced gas. The number of colorides (a) streaked onto MacConkay plates from Petrifilm plates used to summers to Colorides (a) streaked onto MacConkay plates from Petrifilm plates used to summers to Colorides from the Colorides and Chinon materialed) is shown below each bar, ", p < 0.003, Fisher exact test. (B.) Polymeters chain reaction (PCR) products obtained from plate colorides grown on MacConkay plates from the "blue, with gain" Clinica waterished samples in (A.), supplified with Princers 203F and 884R. Lags 1, E. colf positive control; Lance 3 durough is, amplified on the total plates and 12 of the bolates, Lance 13, negative control (water instead of bacturial kolate). Sixts of selected bands on the DNA calibration ladders (Lance 2 and 16) are indicated.

giving needed versatility for volunteer use (C.R. Merino and J.L. Ram, unpublished data, 2001). The overall film packet is compact (7.7 × 10.1 cm), thin (about 1 mm), and stackable, so many plates fit easily into an incubator. The plastic cover sheet readily protects the user from the growing bacterial colonies. The cost, at approximately \$1.10 per plate in lots of 500, is less than Colliert but not as inexpensive as mTEC media; however, since Petrifilm does not require filtration apparatus, vacuum source, and space for pouring plates, its convenience may make it preferred in volunteer-based or aducational testing situations.

The correlations illustrated in Fig. 2 indicate that Petrifilm plates give a reasonably accurate E. coll count. The variability observed with Petrifilm can be considered in relation to compliant levels of E. coll. For example, in the high count range (>300 clu/100 mL), such as those samples analyzed in Fig. 2A and 2B, the 95% confidence interval averaged <40% of the mean, indicating that triplicate measurements having a mean > 500 clu/100 mL are significantly greater than the Michigan total body contact allowable limit of 300 clu/100 mL (e.g., a mean of 501 clu/100 mL would have 95% confidence limits approximately 200 clu/100 mL greater than and smaller than the mean of 501 clu/100 mL, placing 300 cfu/100 mL outside the 95% confidence limit, i.e., significantly different). Conversely, in the low count range (<300 cfu/100 mL), such as the samples analyzed in Fig. 2C, the 95% confidence intervals averaged 101% of the mean, suggesting that triplicate measurements averaging <145

VAIL ET AL: ENIMERATION OF & COLI WITH PETRIFILM PLATES

chi/100 mL, are significantly less than 300 chi/100 mL. For preliminary screening of water samples, consistent observations of Petrifilm plates having zero or one colony (corresponding to 0 or 100 cfu/100 mL) would be good indicators that the actual E. coll level (as measured by standard enumeration tests) is <300 cfu/100 mL.

These 95% confidence intervals, while substantial, can be compared with the variability inherent in other methods. Por example, a membrane filtration measurement of water having 300 cfu/100 mL would typically use a 10-fold dilution to yield 30 cfu on the filter (to be in the count range of the method). As noted in American Public Health Association (1998; Method 9222) "membrane counts really are not absolute" and are assumed to follow a Poisson distribution. For a count on the filter of 30 colonies, the 95% confidence interval would be ±10.9, or 36% of the number counted (American Public Health Association, 1998; Method 9222). For the IDEXX Quanti-Tray, the manufacturer provides a table of 95% confidence intervals. These vary over a broad range of counts; however, a representative comparison for this paper would be the average 95% confidence interval for the samples measured with Quanti-Trays in Fig. 2C. which averaged approximately 55% of their corresponding means, as calculated from the manufacturers' table. Thus, the 95% confidence intervals for the counts obtained with triplicate Petrifilm anumerations were comparable with that obtained with membrane filtration methods and the IDEXX Quanti-Tray in the high count range but were more variable than other methods when E. coli densities were <300 cfu/100 mL.

Petrifilm plates appear to be useful as a first step in obtaining environmental E. coll isolates. In the present study, blue coloules with gas were easily removed from Petrifilm plates and streaked on other nutrient media to isolate individual clones. The isolates obtained from 16 different Petrifilm colonies in this manner all produced PCR products consistent with their being E. coll. In this and other studies (J.L. Ram, unpublished data, 2001). Potrifilm plates have been a convenient first step in obtaining environmental E. coll isolates for sequencing.

The main disadvantage of the Petrifilm system is that only 1 mL of water can be used directly, giving loss precise measurements in samples containing low numbers of E. coll. It may be possible to combine a preliminary concentration step on a filter with the Petrifilm technique; however, this would somewhat negate the simplicity desired for a citizen-based testing method. For improved precision in anumerating water samples with low numbers of E. coli, replicates can be used as in the present study. Overall, the simplicity, reliability, and relatively low cost of the Petrifilm plates make them suitable for citizen-based and educational monitoring of E. coll, particularly when used as a preliminary screening method to identify problem sites at which more extensive testing can be done by professional water-testing laboratories.

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