Q-161

Efficacy of Standard Methods for Water Testing at a Range of Nonstandard Temperature


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Background

Standard, reliable testing for fecal contamination of water remains inaccessible to the majority of the world's population. Evaluation of water sources in developing countries (and following emergencies or natural disasters) cannot rely on electricity, skilled personnel, or a controlled laboratory environment. A simple, specific, and reliable field test that can be used in a variety of conditions is urgently needed.


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Materials and Methods

In an effort to test the viability of currently available *Escherichia coli* detection assays at an assortment of temperatures, 15 strains of *E. coli* from various geographical areas, animal, and soil sources were used. Each was inoculated onto 5 standard and nonstandard tests and incubated at 5 temperatures: 23, 30, 37, 40, and 45˚C. Tests studied included Petrifilm™, Colilert®, EPA method 1604 (MI Medium), MacConkey II (MAC II) Agar and EC Medium multiple-well fermentation.

Assays Tested

- Two standard ß-glucuronidase assays—Colilert® (IDEXX, Westbrook, ME) and membrane filtration with MI Agar (BD-BBL, Sparks, MD).
- Two nonstandard (and less expensive) assays using 4-methylumbelliferyl-p-glucuronide (MUG) as the indicator nutrient. EC Medium with MUG (Difco, Sparks, MD) was chosen for comparison with Colilert®, and Mac II Agar with MUG (BD, Sparks, MD) was used in contrast with MI Agar.
- A Petrifilm™ (3M™ Microbiology, St. Paul, MN) assay was also included.

Protocols

All test protocols were adapted from the standard methods recommended by either the manufacturer or the EPA.The Colilert® assay consisted of three snap packs (pre-measured for 100 mL samples) combined within 300-mL of *E. coli*-inoculated water, and divided by 1 mL samples into five 48-well culture plates. Similarly, 11.1 g of dry EC Medium were added to a 300-mL sample and transferred to 48-well plates. Prior to experimentation, the EC Medium was tested repeatedly for sterility by incubation of uninoculated medium at 37°C for 24 hours. Petrifilm™ consisted of 1-mL water samples transferred onto five Coliform Count Plates. For the agar assays, a total of ten 100-mL samples were run through a 0.45-µm filter and transferred to agar medium. MI Agar and MAC II Agar were purchased preplated, and alcohol-sterilized forceps were used in handling of the filters.

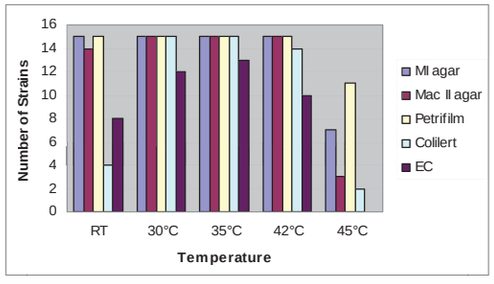
***E. Coli* Strains Tested**

Accession #	Strain Name	O	H	K	Class	Host	Locale	Date	Clinical
TW02282	MT-512	2				chicken	France	1972	trachea
TW02283	MT-513	2				chicken	France	1972	salpbinx
TW02284	MT-515	78				chicken	France	1972	lung
TW06930	52-1232	141	12			goose	USA (NY)		feces
TW03284	ECOR-21	121				steer	Bali		healthy
TW03289	ECOR-29	150	21			k-rat	USA (NV)		healthy
TW02053	ECOR-33	7	21		ECOR#5	sheep	USA (CA)		healthy
TW03292	ECOR-34	88	NM		ECOR#5	dog	USA (MA)		healthy
TW02060	ECOR-45	ON	M			pig	Indonesia		healthy
TW03298	ECOR-47	OM	18			sheep	New Guinea		healthy
TW02066	ECOR-67	4	43			goat	Indonesia		healthy
TW11566	MT2a					environment (soil)	Puerto Rico		
TW11569	MT4a					environment (soil)	Puerto Rico		
TW11573	MT7c					environment (soil)	Puerto Rico		
ATCC 25922									

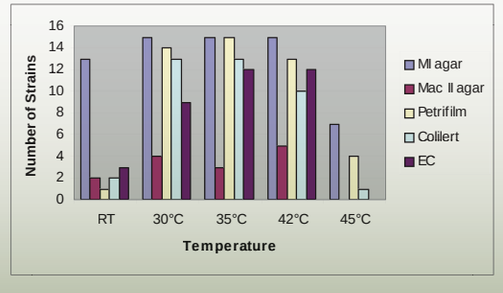
Results

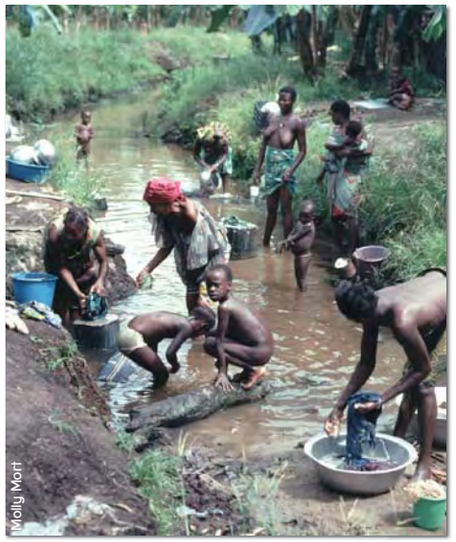
- *E. coli* can be cultured at temperatures ranging from approximately 22°C to 40°C. Some strains could be cultured at 45°C, but not all. Preliminary testing at 4°C demonstrated no visible growth at 24 hours.
- The commercial tests and media used have been optimized for use at 35°C ± 2°C. However, growth was observed at temperatures from 22°C to 42°C using these tests.
- Of the tests studied, membrane filtration with MI Agar gave the best performance for ß-glucuronidase detection, ß-galactosidase detection, and *E. coli* specificity across all temperatures. Petrifilm™ produced the best growth of *E. coli* at all temperatures but lacked specificity. Colilert® did not perform well outside its optimized temperature range so would be of limited usefulness in low-resource settings. The nonstandard tests, Mac II Agar and EC Medium multiple-well fermentation, gave inconsistent results and would not be useful for a reliable test in uncontrolled conditions.

Number of strains exhibiting growth at 5 temperatures in each test system



Number of strains that exhibited *E. coli*-positive results in each test system at various temperatures

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Conclusions

- *E. coli* can be cultured at temperatures from 22°C to 40°C. However, 45°C gave inconsistent results strain to strain.
- This range would allow testing using some standard methods without use of an incubator to test for fecal contamination of drinking water supplies.
- MI agar gave the best specificity for *E. coli*, but Petrifilm gave comparable results and is more easily transportable to the field, easy to use and cost-effective.

Acknowledgements

This study was made possible by the generous support of the American people through the United States Agency for International Development (USAID) under the HealthTech program, Cooperative Agreement #GPH-A-00-01-0005-00, managed by PATH. The contents are the responsibility of PATH and do not necessarily reflect the views of USAID or the United States Government. The authors wish to thank Dr. Mark Sobsey of the Department of Environmental Sciences and Engineering at the University of North Carolina for valuable discussions and input in selecting the research topic and for providing the *E. coli* test strains.

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