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Short Report: Detection of Coliforms in Drinking Water Using Skin Patches: A Rapid, Reliable Method that Does Not Require an External Energy Source

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Abstract. The detection of coliforms requires incubation in a laboratory, generally powered using electricity. In many parts of the developing world, however, external energy sources such as electricity are not readily available. To develop a fast, reliable method for detecting coliforms in water without an external energy source, we assessed the efficacy of six test kits for the identification of coliforms in water samples. To assess the possibility of using body temperature as the sole source of heat for incubation, bacterial samples were then mixed with the enzymatic test kit reagent and attached to the human body surface using a patch system. The patches were attached to the bodies of volunteers for 24 hours and the practicality and accuracy of the patches were assessed. Coliforms were detected within 24 hours in all patches. This innovation will facilitate the testing of water quality by researchers and by economically disadvantaged people without electricity.

INTRODUCTION

To date, over a third of the world's population (i.e., 2.5 billion people) lack access to modern sanitation facilities, and 780 million individuals lack access to improved drinking water, according to the World Health Organization (WHO). Diarrhea caused by waterborne disease is the major cause of death for more than 2 million people annually in developing countries, including 760,000 children < 5 years of age.[1,2]

Pathogenic organisms originating from the intestinal tracts of warm-blooded animals, particularly humans, are responsible for the majority of waterborne diseases[2,3]; to prevent these diseases, identification of fecal contamination is vital. However, the test methods required to detect waterborne pathogenic organisms are time consuming and expensive, especially for people in developing countries, who make up most of those affected by contaminated drinking water.[4] Therefore, it is more practical to examine the water for non-pathogenic indicator organisms associated with fecal contamination.[3] The coliform group of bacteria has been used for more than 100 years as an indicator for microbes originating from feces.[5] The absence of coliform bacteria suggests that the water is microbiologically safe. Although not a perfect system (for example, coliform and *Escherichia coli* detection did not correlate with detection of noroviruses in a recent study of Korean water quality[6]), both the United States Environmental Protection Agency and WHO assert that coliform testing is generally sufficient to determine whether water is safe to drink.[7,8]

Several methods for detecting coliforms have been published and are in use around the world.[9,10] However, in vulnerable areas where there are not sufficient sanitation facilities, there is a tremendous need for a device that is easily manufactured, distributed, and used by individuals in developing world settings to detect contamination in water. We evaluated several existing methods as the basis for the development of such a device, and out of these, the enzyme substrate coliform test was regarded as the most economical and easiest to disseminate.[10] This method is simple; all that is required is to place reagents from a kit into a sample of unverified water and incubate at 35°C for 24 hours. However, areas with polluted water tend not to have the financial or technological requirements for such experiments (i.e., incubators and power sources such as electricity).

One novel idea is to use colorimetric tests carried out in an "eco-pouch" using body heat to incubate the samples. Using such an eco-pouch, an unverified water source could be tested by simply adding the chromogenic substances into the pouch along with a sample of the water in question, attaching the pouch to the body, and allowing the sample to incubate there over 24 hours. We investigated the feasibility of this novel idea through various experiments, with the objective of introducing a rapid and reliable method for coliform detection that does not require any external energy source.

MATERIALS AND METHODS

Enzyme substrate coliform test. The enzyme substrate coliform test used β-galactosidase, with ortho-nitrophenyl-β-galactoside as the substrate, which turns yellow upon decomposition. The standardized process has been previously described.[10,11] When an incubator was used, samples were incubated at 35 ± 0.5°C. Six commercially available test kits, each manufactured by a different company, were tested in the initial stage of the study: AquaCHROM, Colilert, Colitag, ColiTest, EC-Blue, and Readycult Coliforms 100 (CHROMagar, Paris, France; IDEXX Laboratories, Inc., Westbrook, ME; CPI International, Santa Rosa, CA; Humas Co., Daejeon, Republic of Korea; Nissui Pharmaceutical Co., Tokyo, Japan; and Merck KGaA, Darmstadt, Germany, respectively).

Comparison of the six test kits at various temperatures. To determine the best test kit for use with the eco-pouch, the accuracy of results at a range of temperatures (25°C, 30°C, 35°C, and 40°C) that might be found in different locations on the surface of the human body was evaluated. An overview of the experimental process is shown in Table 1.

Preparation of coliform suspensions. For the preparation of the initial *Enterobacter* (TC) and *E. coli* (EC) suspensions, *Enterobacter* BioBalls and *E. coli* BioBalls were suspended in phosphate buffered saline (PBS) (Gibco BRL, Grand Island, NY). A set of four sample bottles of coliform suspension from this initial step, labeled "TC," "EC," "TC+EC," and

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TABLE 1
Overview of experimental procedures

Procedure	Description
Preparation of *Enterobacter* (TC), *E. coli* (EC), and mixed (TC + EC) suspensions.	Suspensions of *Enterobacter* and *E. coli* in phosphate buffered saline (PBS) were prepared from BioBalls (BTF) initially containing 10,000 colony-forming units (CFU) each, as described in the Methods.
Preparation of coliform suspension bottles	Four bottles of coliform suspension from the previous step were prepared, and tagged, "TC," "EC," "TC + EC," or "control," for *Enterobacter* suspension, *E. coli.* suspension, mixed *Enterobacter* and *E. coli.* suspension, and PBS only, respectively. A set of these four suspensions was prepared for each of four different temperatures (25°C, 30°C, 35°C, 40°C), for a total of 16 bottles. A set of these 16 bottles was in turn prepared for each of the 6 different test kits.
Addition of reagents to the coliform suspension	Each of the six reagents, Colilert, Colitag, ColiTest, Readycult coliforms 100, EC-Blue, and AquaCHROM, was added to each set of 16 bottles and mixed vigorously.
Culturing in incubators	Samples were incubated at 25°C, 30°C, 35°C, and 40°C. Color changes were observed at 24, 48, and 72 hours.

"control," were prepared for four temperature conditions. To each of the "TC" bottles, 100 mL *Enterobacter* suspension (100 colony-forming units [CFU]/100 mL) was added. To each of the "EC" bottles, 100 mL *E. coli.* suspension (100 CFU/100 mL) was added. To each of the "TC+EC" bottles, 100 mL *Enterobacter* and *E. coli* suspension (100 CFU/100 mL) was added. The "control" bottles were filled with 100 mL 1X PBS. The resulting set of 16 sample bottles was prepared for use with each of the six different test kits being evaluated.

Evaluation of test reagents in the laboratory using incubators. The reagents from the six kits were each added to a set of 16 bottles prepared as described previously, and mixed vigorously by repeated inversion. The samples were incubated at 25°C, 30°C, 35°C, and 40°C in laboratory incubators, and the color change associated with each test was recorded at 24, 48, and 72 hours.

Evaluation of the enzymatic method using body temperature for incubation. Experiments were conducted to evaluate the feasibility and practicality of detecting coliforms using an enzymatic method with human body heat for incubation. One of the practical features of the enzymatic method kit is that it already uses sealed pouch-like containers, for example IDEXX Quanti-Trays. We attached these Quanti-Trays to the bodies of volunteers to create an "eco-pouch" system with minimal modification from the original test kit, to facilitate ease of use. Several factors were considered: the efficacy of the product, time required for the color changes, comfort of the patches, and the most effective locations for placement on the body. A 100 mL sample of *E. coli* suspension was mixed with an enzymatic test kit, and shaken vigorously to ensure that no bubbles were present. The Quanti-Trays were used according to the experimental instructions provided by IDEXX, with the *E. coli.* suspension solution described previously as the reagent. The multi-well Quanti-Trays were cut into smaller, 5-well sample trays (width 58 mm, depth 12 mm, height 5 mm) for better usability on the human body. The experimental samples, incubated using body temperature, were tested on 16 volunteers (3 male and 13 female). Twelve of the volunteers were 18 years of age, three were in their 30s, and one was in his 40s. We chose body parts that were relatively less influenced by outside temperature changes, and on which it would be most comfortable to attach the samples. It was assumed that different locations on the human body would vary somewhat in temperature, therefore 12 of the volunteers were divided into three groups, each with four people, whereas the remaining four volunteers attached the patches at random locations. The volunteers were instructed to keep the samples on their bodies at all times, to immediately report when they observed the color change to yellow, to minimize external temperature change as much as possible, to take pictures of the patches, and to take them off after the color changed. To attach the sample wells to the body, a large-sized patch was first attached to the body surface, a 5-well sample strip was then placed on the patch, and, finally, this was covered with a small-sized patch (width 58 mm, depth 12 mm, and height 5 mm) to fix the samples in place (Figure 1). The positive control samples consisted of the same *E. coli* suspension and the enzymatic test kit reagent, in the same kind of prepared Quanti-Tray sample wells, but incubated in an incubator at 35 ± 0.5°C.

RESULTS

Accuracy of six test kits at various temperatures, incubated in the laboratory. Test results for each of the six commercial kits are shown in Table 2; of the six kits, Kit 2 showed clear, accurate color changes after only 24 hours. Kit 2 was therefore chosen as the basis for the patches, and, ultimately, the eco-pouch. Although Kit 4 and Kit 5 also showed appropriate results after 24 hours, the color changes were somewhat inconsistent.

Efficacy of the enzymatic method using body heat for incubation. Table 3 lists the locations of the samples on the volunteers' bodies. Volunteer 11 (V11) was the only volunteer who did not take a hot shower. The color change seen with all 15 of the other volunteers may have been accelerated because of the external influence of their hot showers. The color of all of the samples changed within 24 hours (Table 4). Moreover, the volunteers did not report any difficulty while the experiment was carried out.

This experiment indicates that the test sample, and thus the pouch, with its robust packaging and small size, is safe and comfortable for the human body unless the sample bursts. Furthermore, the observation of a color change in all of the samples within 24 hours indicates efficacy for detection of *E. coli*. Photographs of the test wells were taken when the color changed (Figure 1).

DISCUSSION

Economic feasibility and environmental benefits: appropriate technology. Appropriate technology (AT), initially suggested by economist E. F. Schumacher,[12] has traditionally been

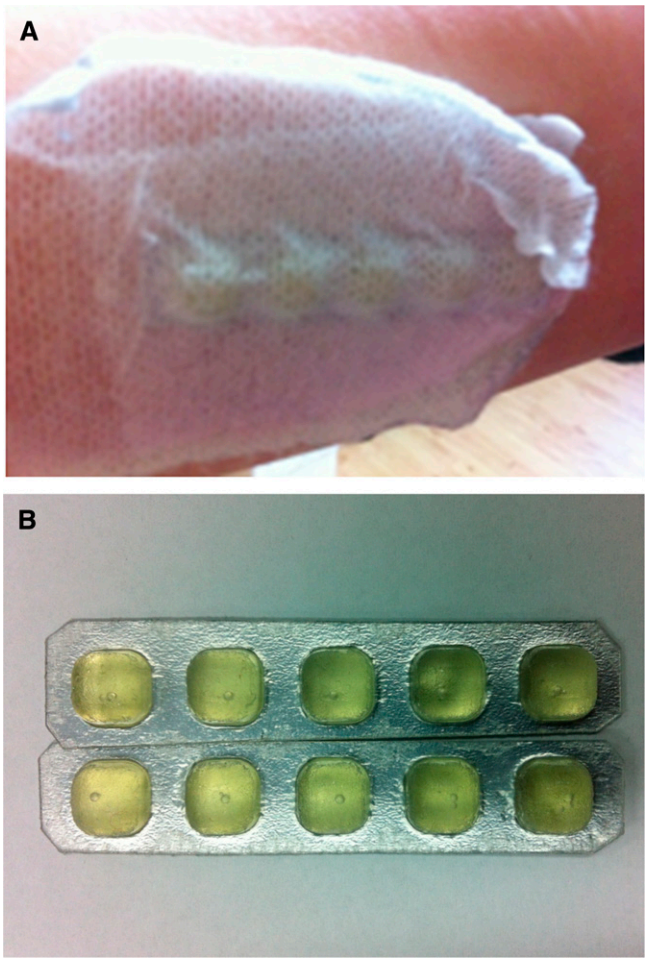

FIGURE 1. Photographs of samples incubated using body temperature (example from the inner arm). (**A**) Sample tray attached to the inner upper arm. To incubate using body temperature, 5-well sample trays were attached to body parts that were relatively less influenced by outside temperature changes. Seven locations were investigated: abdomen, back of the neck, thigh, upper chest, outer upper arms, inner upper arms, and inner lower arms. To attach the sample wells to the body, a large-sized patch was first attached to the body surface, the 5-well sample strip was then placed on the patch, and this was covered with a small-sized patch (width 58 mm, depth 12 mm, and height 5 mm) to fix the samples in place. The samples remained attached to the body at all times until the color changed. (**B**) Sample bubbles after incubation on the inner upper arm, detached.

reserved for use in a developing world context.[13] In general, AT methods require fewer resources, are easier to maintain, and have less of an impact on the environment compared with techniques developed from mainstream technology.[14,15] In practice, AT can be described as using the simplest level of technology that can effectively achieve the intended purpose in a particular location. Our eco-patch using Kit 2, described in this study, is a good example of AT. Although there are some commercially available *E. coli* test kits that can be used at room temperature, the results may be compromised by variability in room temperature. This may be especially problematic in developing world settings which, unlike the air-conditioned laboratories common in industrialized settings, may not be able to supply the continuous and appropriate temperature for consistent culturing of *E. coli*. Another alternative is the phase-change incubator, which can also be used to test for *E. coli* in drinking water[16,17]. However, although the phase-change incubator is a good example of AT and a low cost incubator, it still requires phase change materials and external insulation cases. Using the eco-patch method in this study, the simple, affordable, and widely available kits can be used without electricity by making use of human body heat for incubation. It is therefore well-suited for widespread use

TABLE 2
Results from six commercial test kits for coliforms in drinking water

Test kit	Time (hrs)	Temp. (°C)	TC suspension		EC suspension		TC + EC suspension		Control group	
			TC	EC	TC	EC	TC	EC	TC	EC
Kit 1	24	25	–	–	–	–	–	–	–	–
		30	+	–	+	+	+	+	–	–
		35	+	–	+	+	+	+	–	–
		40	+	–	+	+	+	+	–	–
	48, 72	25	+	–	+	+	+	+	–	–
		30	+	–	+	+	+	+	–	–
		35	+	–	+	+	+	+	–	–
		40	+	–	+	+	+	+	–	–
Kit 2	24, 48, 72	25	+	–	+	+	+	+	–	–
		30	+	–	+	+	+	+	–	–
		35	+	–	+	+	+	+	–	–
		40	+	–	+	+	+	+	–	–
Kit 3	24	25	–	–	+*	+†	+*	+†	–	–
		30	+	–	+	+	+	+	–	–
		35	+	–	+	+	+	+	–	–
		40	–	–	+	+	+	+	–	–
	48, 72	25	+	–	+*	+†	+	+	–	–
		30	+	–	+	+	+	+	–	–
		35	+	–	+	+	+	+	–	–
		40	+	–	+	+	+	+	–	–
Kit 4‡	24, 48, 72	25	+	–	+	+	+	+	–	–
		30	+	–	+	+	+	+	–	–
		35	+	–	+	+	+	+	–	–
		40	+	–	+	+	+	+	–	–
Kit 5	24, 48, 72	25	+	–	+	+	+	+	–	–
		30	+	–	+	+	+	+	–	–
		35	+	–	+	+	+	+	–	–
		40	+	–	+	+	+	+	–	–
Kit 6	24	25	–	–	–	–	–	–	–	–
		30	–	–	+	+	+	+	–	–
		35	–	–	+	+	+	+	–	–
		40	–	–	+	+	+	+	–	–
	48, 72	25	–	–	+	+	+	+	–	–
		30	–	–	+	+	+	+	–	–
		35	–	–	+	+	+	+	–	–
		40	–	–	+	+	+	+	–	–

*Color was opaque.
†Exact color was hard to recognize.
‡After 48 hours, some glutinous-appearing blue-green precipitate began to form in the Kit 4 and TC + EC bottles, especially in the 25°C and 30°C bottles.

TABLE 3
Grouping of volunteers and locations on the body of the samples

Group	Location on the body	Volunteer
Group 1	Outer upper arm, thigh	V1,V2, V3,V4
Group 2	Inner upper arm, abdomen	V5,V6, V7,V8
Group 3	Outer upper arm, upper chest	V9,V10, V11,V12
Group 4–1 (random)	Outer upper arm, inner upper arm	V13
Group 4–2	Left and right inner lower arms	V14
Group 4–3	Inner lower arm, back of the neck	V15
Group 4–4	Left and right outer upper arms	V16

TABLE 4
Recorded attachment and detachment times of the patches

Groups	Volunteer	Attachment time (Feb. 6th)	Detachment time (Feb. 7th)	Incubation time
Group 1	V1	17:30	8:16	14 hrs 46 min
	V2	18:15	7:37	13 hrs 22 min
	V3	18:32	7:34	13 hrs 2 min
	V4	18:42	8:21	13 hrs 39 min
Group 2	V5	18:25	7:35	13 hrs 10 min
	V6	18:19	7:33	13 hrs 14 min
	V7	18:20	6:45	12 hrs 25 min
	V8	18:26	7:38	13 hrs 12 min
Group 3	V9	19:04	7:40	12 hrs 36 min
	V10	18:53	9:31	14 hrs 38 min
	V11	18:55	13:09	18 hrs 14 min
	V12	19:02	8:33	13 hrs 31 min
Group 4–1	V13	10:00	10:00	24 hrs
Group 4–2	V14	10:00	10:00	24 hrs
Group 4–3	V15	10:00	10:00	24 hrs
Group 4–4	V16	10:00	10:00	24 hrs

in developing countries. In addition to direct use in public health applications, this system could also be useful to biologists and health researchers working in the field, both in developing countries and in other settings lacking the convenience of a power source such as electricity (wilderness settings, for example).

Drawbacks and future improvements. Kit 2 was the best among six kits tested in this study, but a few improvements are warranted. One of the drawbacks of this kit is that the sample wells could rupture. Therefore, the durability of the kit could be reinforced. Cultural factors may pose a problem as well, in that people might hesitate to attach the wells to their bodies. If this is the case for a given population, livestock could be evaluated as an alternative incubation source. Furthermore, armband-type test kits may be considered more acceptable, and could also be tried. In addition, pouches made of biodegradable materials could be tried.

CONCLUSION

This research affirmed the usability of the enzymatic method at various temperatures, including 25°C, 30°C, 35°C, and 40°C, by incubating the test kit with coliforms at these temperatures. The observation that this test kit works in a full range of temperatures, from 25°C to 40°C, suggested that it could function when incubated at temperatures commonly found on various human body surfaces. In the second part of the study, attachment of the samples, in sealed Quanti-Trays, on various locations on 16 volunteers' body surfaces, confirmed the feasibility of this system. The enzymatic method using Kit 2 accurately indicated an *E. coli* suspension as coliform-contaminated water, using only body surface temperature for incubation.

The application of this eco-friendly incubation method represents an AT method for testing environmental water for the presence of pathogens. This method is especially applicable in developing countries because advanced technology, equipment, and facilities are not affordable. The only information required by users is how much water to put in the pouch, how long it should be attached to the body, and what color indicates contamination.

This research represents a preliminary method for water quality testing that uses no external energy, is inexpensive, and is safe. We hope that these patches will next be developed into inexpensive pouches that are mass-produced and distributed to persons in need.

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