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The Effects of Temperature on Indicator Species Tests for Water Quality

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THE EFFECTS OF TEMPERATURE ON INDICATOR SPECIES TESTS FOR WATER QUALITY

AN INVESTIGATION OF THE RESPONSE OF TESTS AT TEMPERATURES OUTSIDE THE RECOMMENDED RANGE

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ABSTRACT

This study sought to investigate the different response of two indicator species tests: Hach® PathoScreen™ Field Test and IDEXX Colilert® using the Quanti-Tray/2000® enumeration method. Both tests were carried out according to the instructions enclosed in the packages using diluted water samples taken from the secondary clarifier at the West Side Waste Water Treatment Plant in Fayetteville, AR. The tests were carried out at various temperatures in an attempt to reflect conditions that could be encountered in the field, where electricity, equipment, and expertise may not be available. The PathoScreen™ test responded adequately at a relatively wide range of temperatures, from approximately 22°C to 35°C, whereas the Quanti-Tray/2000® method was much more sensitive, producing erratic responses. In addition to PathoScreen™ yielding more consistent responses, the field kit and materials necessary for that test are much cheaper than those for using the Quanti-Tray/2000® method. These results suggest that the Hach® PathoScreen™ test, a hydrogen sulfide (H_2S) presence-absence (PA) test, is more reliable for use in the field.

INTRODUCTION

The presence and concentration of indicator bacteria in water can be a good indication of water quality. These species tend to be present when pathogens are present and absent when pathogens are absent. There are several tests which can be run to evaluate the concentration of indicator bacteria in a given water sample, among them the IDEXX Colilert® test and the membrane filter methods. However, useful though these tests are, they require expensive and cumbersome equipment such as incubators to be carried out, which makes them less practical in rural areas of developing countries where funds and reliable sources of electricity may not be available. Cheaper, more feasible tests exist which do not require incubators or other costly equipment; for example, tests that utilize the presence or absence of hydrogen sulfide-producing bacteria without requiring a specific incubation temperature seem promising, provided the ambient temperature of the location in question is not either too cold or too hot. However, the response times and accuracy of these tests may change with varying temperature. This research aims to gather data and ultimately provide information that may be used to determine the best test to use in different situations or to develop new efficient, low-cost, effective tests for indicator bacteria species for use in rural areas of developing countries. The indicator tests utilized in this study, Hach® PathoScreen™ and IDEXX Colilert® test using the Quanti-Tray/2000® enumeration method, were tested at various temperatures and monitored throughout a 48 hour period for the PathoScreen™ test and a 28 hour period for the Quanti-Tray/2000® method. The results of this research should be used to make informed decisions on the selection and development of indicator bacteria tests, particularly for applications in developing countries where other tests are impractical or impossible due to lack of funding or electricity to perform them.

LITERATURE REVIEW

Several other papers over similar or related subject have been published in the recent past. These papers reported similar findings and recommendations to this paper and to each other. Overwhelmingly, the results of these studies concluded that presence-absence H_2S tests were more accurate and practical for use in the field, specifically rural areas of developing countries. A few point out the need for further research to be done in this area, as well.

In their article "Comparison and verification of four field-based microbiological tests: H_2S test, Easygel®, Colilert®, Petrifilm™," which appeared in the Journal of Water, Sanitation and Hygiene for Development in 2011, Patty Chuang, Stephanie Trottier, and Susan Murcott found that a combination of the 20 mL H_2S test and Easygel® gave better results than any single test performed alone. In fact, they recommended that none of the field-based tests be utilized on their own and strongly recommended that the H_2S test and Easygel® combination be tested on a larger scale and with a greater sample size before the combination was recommended for general use in the field (Chuang et al, 2011).

Typically, testers look for the presence or absence of fecal coliform bacteria in drinking water samples to determine the water quality, as these bacteria are key indicators that dangerous pathogens could be present. However, these tests are expensive, highly dependent on temperature, and often quite intricate to carry out. In their paper "The Hydrogen Sulphide *(sic)* (H_2S) Paper-Strip Test," Luke Mosley and Donald Sharp discuss the use of the H_2S paper strip test in Pacific Islands. They argue that this particular test is well suited for use in the Pacific Islands as the results are visual, making interpretation of the results simple for people with minimum training (Mosley and Sharp, 2005). They go one to state that an indication of the risk that pathogens are present can be determined from this test because the time it takes for the test to change color appears to show a correlation with the levels of fecal coliform bacteria found in the sample (Mosley and Sharp, 2005).

Mark D. Sobsey and Frederic K Pfaender discuss the H_2S test and the validity of various versions of it in their 2002 paper "Evaluation of the H_2S Method for Detection of Fecal Contamination of Drinking Water." Hydrogen sulfide producing bacteria are associated with the presence of fecal contamination in a water supply. The H_2S test, used to detect the production of H_2S by enteric bacteria in a volume of water, indicates positive results by the formation of a black precipitate (the result of the reaction of the H_2S with iron in the test medium) (Sobsey and Pfaender, 2002). However, many versions of and modifications to the test have been made since the initial report of the test in 1982; there is not a worldwide standard for the H_2S test and only a few versions have been tested or compared with other indicator bacteria tests for fecal contamination (Sobsey and Pfaender, 2002). Sobsey and Pfaender recommend a standardization of the H_2S test before it is adopted for widespread use. They cite different forms of the test using a variety of different media (such as paper strips or powdered media) and different incubation times and temperatures (Sobsey and Pfaender, 2002). The test is quite simple and cost effective, although it does have some shortcomings. Sobsey and Pfaender point out the false positives are common with H_2S tests, as the test only reveals that there are H_2S producing organisms present, not what they are nor where they came from; they argue that false positives can make the test ultimately more expensive, as it may then require further testing or attempting to acquire alternate sources of water (Sobsey and Pfaender, 2002).

Although Sobsey and Pfaender described the detracting qualities of the H_2S test, it nevertheless remains widely accepted as a feasible, inexpensive alternative for use in villages or other field situations where laboratory tests are impossible. In the 2007 research paper "Evaluation of Hydrogen Sulphide *(sic)* Test for Detection of Fecal Coliform Contamination in Drinking Water from Various Sources," by D. H. Tambekar et al, published in the *African Journal of Biotechnology*, the assertion that the H_2S test is a valid recommendation for use in rural areas of developing countries is affirmed, as it may be performed at the village level without skilled personnel or sophisticated equipment. They found that the H_2S test performs at an acceptable level in the detection of fecal contamination in drinking water, particularly in instances where water is subject to contamination by improper, unhygienic collection, handling, or storage (Tambekar et al, 2007). It was further concluded that the H_2S test is a more useful alternative to Most Probable Number (MPN) tests in the detection of fecal contamination of drinking water, and therefore recommended for routine

monitoring of drinking water quality in rural or field situations where there is a lack of electricity, equipment, and technical expertise (Tambekar et al, 2007).

Another widely used water quality indicator species test, the fecal coliform test, may have outlived its usefulness, according to Michael P. Doyle and Marilyn C. Erickson in their article, "Closing the Door on the Fecal Coliform Assay," which appeared in *Microbe* magazine in 2006. Doyle and Erickson state that limitations and complications associated with the fecal coliform bacteria test make its continued appropriateness questionable for food and water testing. They point out that, although there are many studies that correlate levels of fecal coliform bacteria with the presence of *E. coli*, the value of the fecal coliform test as an indicator of fecal contamination is rendered null when bacteria of non-fecal origin are the main organisms detected (Doyle and Erickson, 2006). One major reason Doyle and Erickson feel that the fecal coliform test has outlived its usefulness is the fact that the results of the tests may be, and often are, misinterpreted by physicians, public health officials, and the media, resulting in the sensationalizing of situations that are not necessarily worth the hype they create, usually when applied to food, beverage, or water sample testing (Doyle and Erickson, 2006). Misunderstanding of the results of fecal coliform tests may be attributed to information found on government and academic sites, which provide information which fail to point out the possibility that positive fecal coliform tests may be caused by bacteria from non-fecal origins (in other words, false positives may arise from the presence of certain types of non-fecal bacteria) (Doyle and Erickson, 2006). Doyle and Erickson suggest that in the future, the fecal coliform test should specifically state that it is not a reliable indicator of either *E. coli* or fecal contamination, at the very least, and preferably eliminate the test as an indicator of fecal contamination all together (Doyle and Erickson, 2006). For a more reliable test, they suggest the *E. coli* test (Doyle and Erickson, 2006).

More closely relating to the topic of this paper is the article "Evaluation of Simple Microbiol *(sic)* Tests for Detection of Fecal Coliforms Directly at 44.5°C," by Suman Tewari, P. W. Ramteke, and S. K. Garg, which appeared in *Environmental Monitoring and Assessment* in 2006. The authors tested three different simple microbial tests: the H_2S paper strip test, the presence-absence test, and a fluorogenic brila broth test (BB test); all three tests were incubated at 44.5°C (112.1°F). The study found that the BB and PA test produced similar results to that of the standard MPN method, although the results of the H_2S test compared poorly (Tewari et al, 2006). The BB test proved to be incredibly sensitive, and was also noted as agreeing most closely with the results of the MPN test, with the results being in disagreement only 7.8% of the time (Tewari et al, 2006). The BB test was also found to be a highly specific indicator test for *E. coli*, with 84.4% of the organisms isolated by the BB test being identified as *E. coli*; those percentages were significantly lower in the results of the PA and H_2S test (43.4% and 33.3%, respectively) (Tewari et al, 2006). Along this line, the study does not recommend the MPN method for use in tropical areas; the paper cites the low return of *E. coli* by the MPN method (only 18.1%) brings the validity of the test into question for purposes of its application in tropical climes (Tewari et al, 2006).

HACH® PATHOSCREEN™ FIELD KIT

The Hach® PathoScreen® Field Kit tests for the presence of hydrogen sulfide-producing bacteria, such as *Salmonella* and *Citrobacter*, among others. The test consists of a powder growth medium which reacts with the hydrogen sulfide-producing bacteria, producing a black precipitate or simply an overall color change in the sample from yellow to black. The media can go bad if improperly stored. During the course of this research, a group of media packets were found which had been stored for a prolonged period of time in a humid, warm environment. It was noticed that the media in question were no longer in powder form, but had nearly solidified, creating a sticky, pasty texture, similar to vanilla beans scraped from the pod. These media were tested to investigate the effect of the changed texture on the results. Occasionally, positive results were yielded, but it was very hit-and-miss. Thus, it is important to only use unexpired, powdery media packets for reliable testing purposes.

PROCEDURE

The PathoScreen™ tests were conducted at 20°C (68°F), 22°C (71.6°F), 32.5°C (90.5°F), 35°C (95°F), 40°C (104°F), and 44.5°C (112.1°F). All tests were from the same water sample: a diluted sample of water taken from the secondary clarifier at the West Side Wastewater Treatment Plant in Fayetteville, AR. For each temperature, a pipette was used to fill five (5) sterile 20-mL scintillation vials with water from the sample. Then, one (1) packet of the PathoScreen™ powder medium was put into each vial. The vials were capped and shaken until the powder medium had completely dissolved. Then five scintillation vials were placed in incubators set at each temperature and left to incubate for 24 hours. After 24 hours of incubation, the samples were checked, the results recorded, and left to incubate for another 24 hours. After a total of 48 hours of incubations, the samples were checked once again, the final results recorded, and the samples properly disposed.

RESULTS

In the analytical procedure for the PathoScreen™ Field Kit, the range of incubation temperature is listed as falling between 25°C and 35°C (75°F and 95°F), with an incubation period between 24 and 48 hours. This range of incubation times and temperatures are supported by the findings of this study, with the lower temperature yielding results at an earlier time and which suggest a higher concentration of hydrogen sulfide-producing bacteria, according to the MPN table given by the Hach® Company in the analytical procedure booklet (*Table 1.1)*. Temperatures outside of this range were investigated as well, and with interesting results. Samples incubated at 22°C (71.6°F) yielded positive results slightly before 24 hours, and indicated a higher concentration of indicator species than the higher temperatures. However, below 22°C (71.6°F), the tests yielded no results, even after 48 hours. Samples incubated at temperatures of 40°C (104°F) and above yielded no results as well.

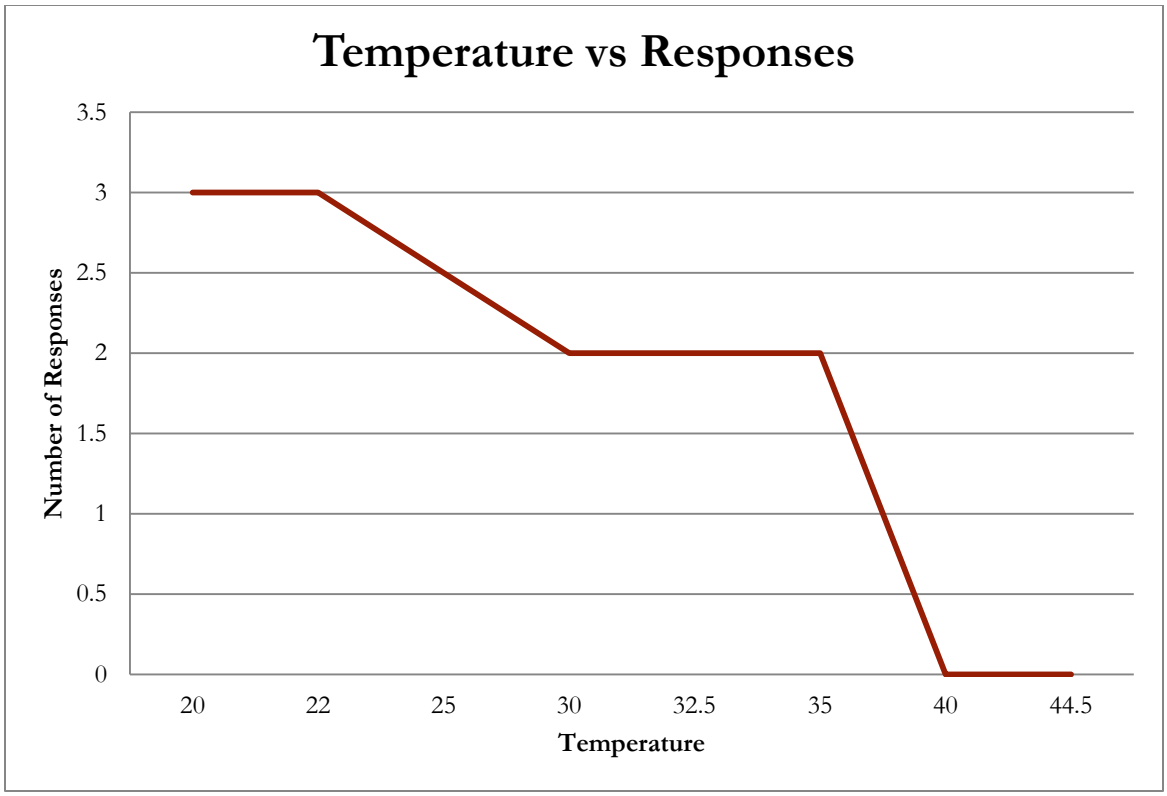

Figure 1.1: Temperature vs. Number of Responses for PathoScreen™ Field Kit

MPN Indicated by Results	
Positive Tubes	**MPN/100mL**
0	<1.1
1	1.1
2	2.6
3	4.6
4	8.0
5	>8.0

Table 1.1: Most Probable Number of Indicator Species, based on number of vials which returned a positive result.[1]

After incubation, most samples showed either a pronounced overall color change or a granular black precipitate. However, at certain temperatures, specifically those in the upper range of acceptable temperatures, a gray, gel-like precipitate was produced which either sat at the bottom of the scintillation vial or was present throughout the entire vial. This was taken as a positive result, but was noted as distinctly different from the results at lower temperatures. The gel-like precipitate occurred only at temperatures above 30°C (86°F).

[1] Hach Company. *Analytical Procedure: PathoScreen™ Field Kit.*

Figure 1.2: Sample after 24 hours of incubation at 22°C (71.6°F). The vial on the far right exhibits essentially no color change. The color change which indicates positive results is quite obvious by comparison.

Figure 1.3: Samples after 24 hours of incubation at 32.5°C (90.5°F).

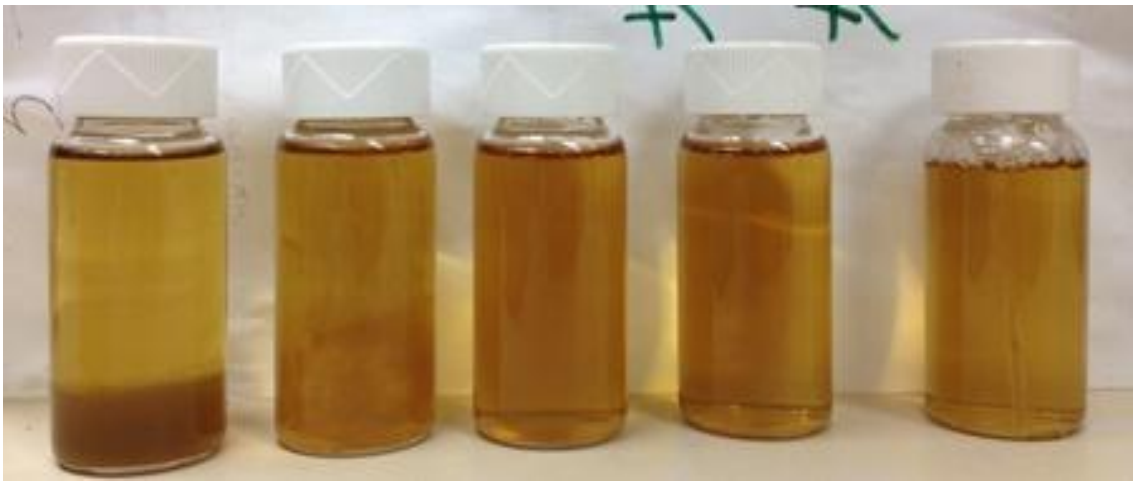

Figure 1.4: Samples after 24 hours of incubation at 35°C (95°F). The gel-like precipitate is present in two of these scintillation vials.

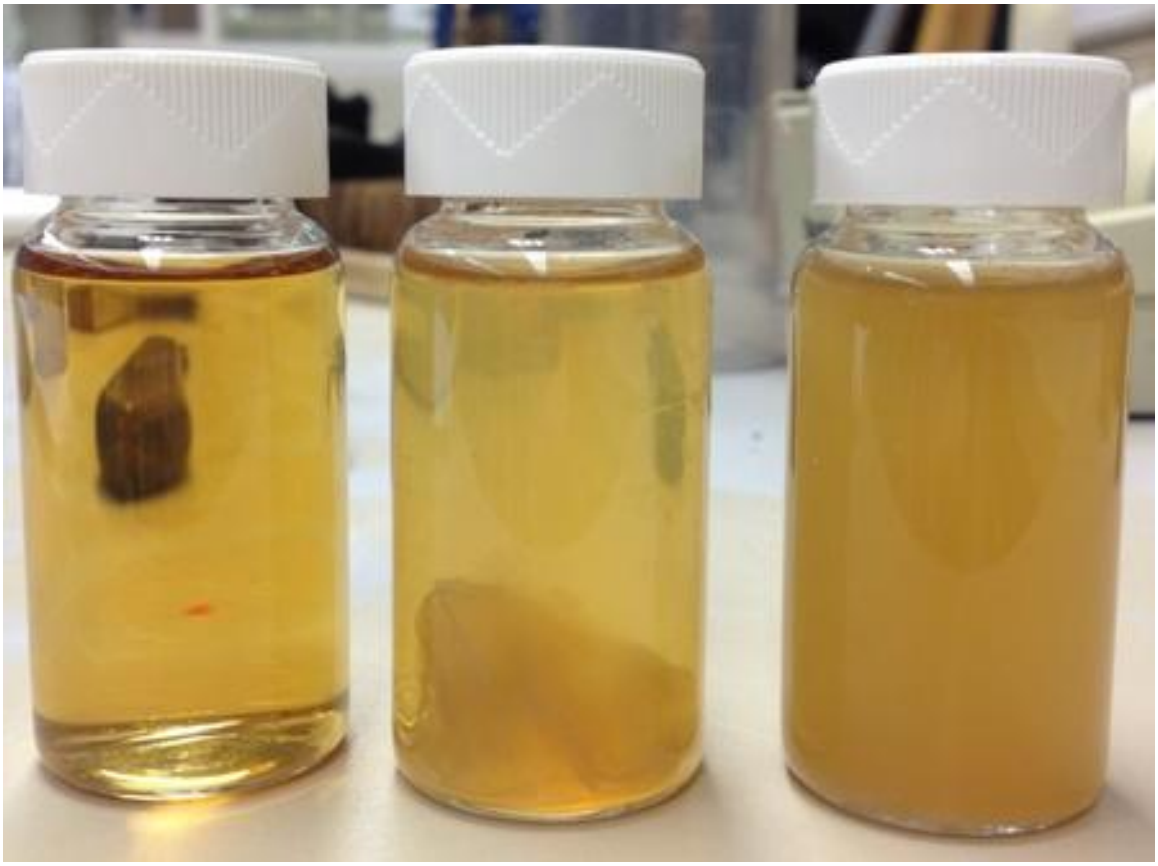

Figure 1.5: Vial with no change compared to vials with gel-like precipitate (middle) and overall gel presence (right).

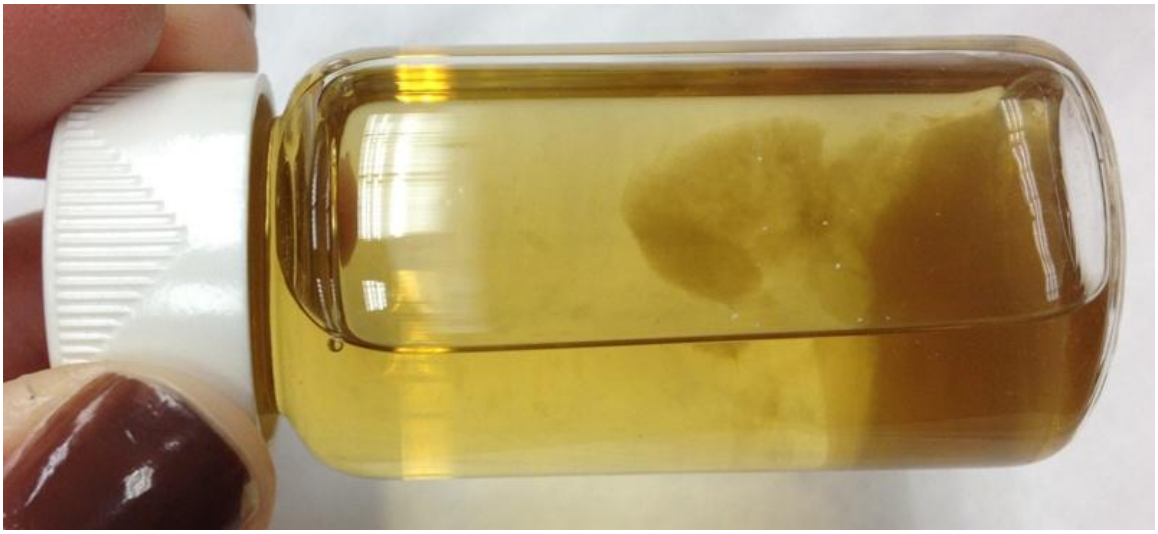

Figure 1.6: Close up of gel-like precipitate, from sample incubated at 35°C (95°F).

IDEXX COLILERT® USING THE QUANTI-TRAY/2000® METHOD

The IDEXX Colilert® Quanti-Tray/2000® method tests for the presence of both total coliform and *Escherichia coli (E. coli)* through the utilization of a powdered medium. The presence of total coliform bacteria is confirmed by a color change of the sample from clear to yellow in natural light, while the presence of *E. coli* is indicated by the fluorescence of the sample under ultraviolet lighting.[2]

This test is much more sensitive than the Hach PathoScreen™, with a much smaller range of recommended temperatures [35°C (95°F), ±0.5°C]. In fact, the Colilert® Quanti-Tray/2000® test is not *officially* valid if incubated outside of that range. However, this research aimed to gather data on whether or not this test yielded any results at temperatures outside this range. If so, it could mean the test could conceivably be used in rural area of developing countries with no access to incubators.

PROCEDURE

The water sample used for the Quanti-Tray/2000® tests was the same as that used for the PathoScreen™ tests: a diluted sample from the West Side Wastewater Treatment Center in Fayetteville, Arkansas. The sample was prepared according to the instructions given in the IDEXX manual for Colilert® using the Quanti-Tray/2000® enumeration procedure. 100 mL of the sample were measured into a sterilized vessel, and one packet of the Colilert® powder medium was added. Once the medium was added, the vessel was securely capped and shaken vigorously until all the powder had dissolved. This mixture was then poured into the Quanti-Tray/2000® enumeration tray, with care taken to ensure an appropriate amount of the sample was present in each well. The tray was then heat sealed.

The analytical procedure recommends the use of the official Quanti-Tray/2000® tray sealer to seal the enumeration trays; however, it was found during the course of this research that an iron set on the low setting (slightly above that for Nylon) works very well in the absence of an actual tray sealer from IDEXX. This makes the use of the Quanti-Tray/2000® test much more practicable for field use purposes than it would be otherwise.

Once the trays were sealed, with care taken to ensure complete sealing of the trays so that samples may not 1) leak out and contaminate the environment, or 2) be contaminated or otherwise rendered void by water leaking in from a water bath incubator, they were placed into incubators set at temperatures of: 30°C, 32.5°C, 35°C, 37.5°C, and 40°C (86°F, 90.5°F, 95°F, 99.5°F, and 104°F). The trays were left to incubate for 24 hours, with results being checked periodically beforehand, and through 28 hours. Those trays which were incubated in the water bath incubator were placed in gallon-sized slide lock sipper bags prior to being placed in the incubator.

[2] *Colilert® Quanti-Tray/2000® Enumeration Procedure.* IDEXX Laboratories, Inc. Westbrook, ME.

RESULTS

This test yielded hit-and-miss results. The tray incubated at 30°C (86°F) yielded positive results beginning shortly before 21 hours. The tray incubated at 40°C (104°F), yielded no results, even after 28 hours of incubation. These results suggest that the test may be able to be used in cooler climates when there is no incubator available, but that in warm climates, the test must be stored in a shady area in order to be usable.

However, further testing is needed on this particular question. In initial tests performed at 32.5°C, 35°C, and 37.5°C (90.5°F, 95°F, and 99.5°F), the tests all failed for reasons unknown. A new water sample was obtained and the tests performed again at all temperatures. However, during this test, the temperatures in two of the incubators fluctuated wildly throughout the incubation period, and thus those tests were rendered inconclusive due to temperature variation. Nevertheless, one conclusion was able to be made from these tests: the Quanti-Tray/2000® appears to be quite sensitive to temperature change (making it impractical for use in field conditions without a reliable source of temperature control,) and response times vary based on the value of the most probable number (MPN) of total coliform per 100 mL sample yielded by the test. Regardless of the accuracy of the actual value of the MPN indicated by the test, the wells showed a relatively steady rate of development once color change began to occur. Below is pictured the development of the tray incubated at 30°C (86°F), beginning at 21 hours of incubation through 27 hours of incubation.

Figure 2.1: Tray incubated at 30°C (86°F) after 21 hours of incubation. At this time, 10 large wells and five small wells indicate a positive hit.

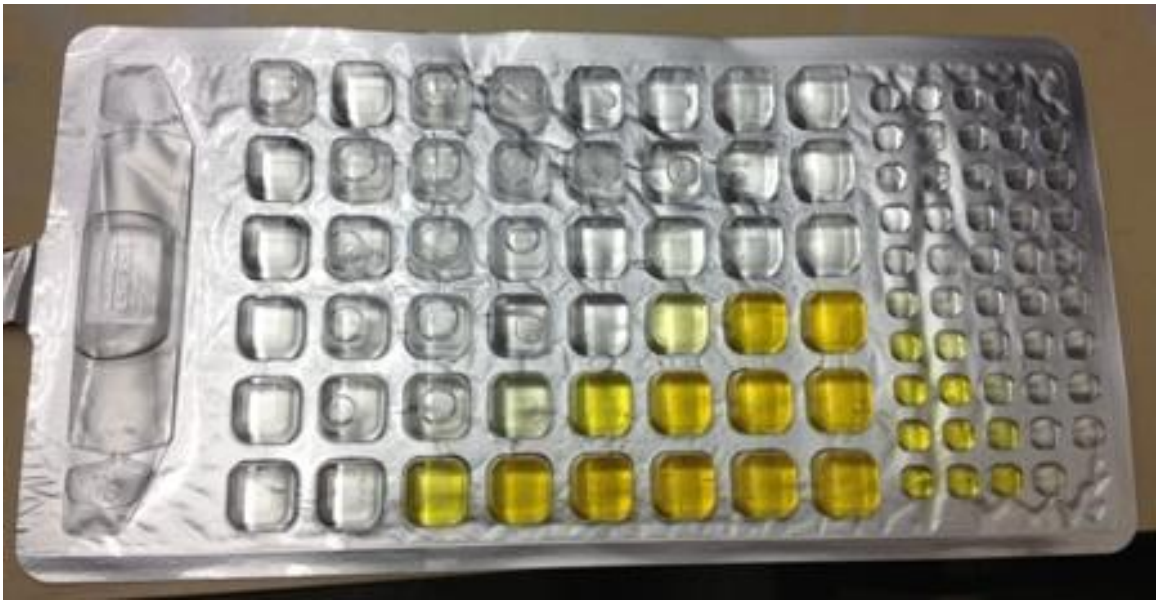

Figure 2.2: Tray incubated at 30°C (86°F) after 24 hours of incubation. At this time, 14 large wells and 10 small wells indicate positive hits.

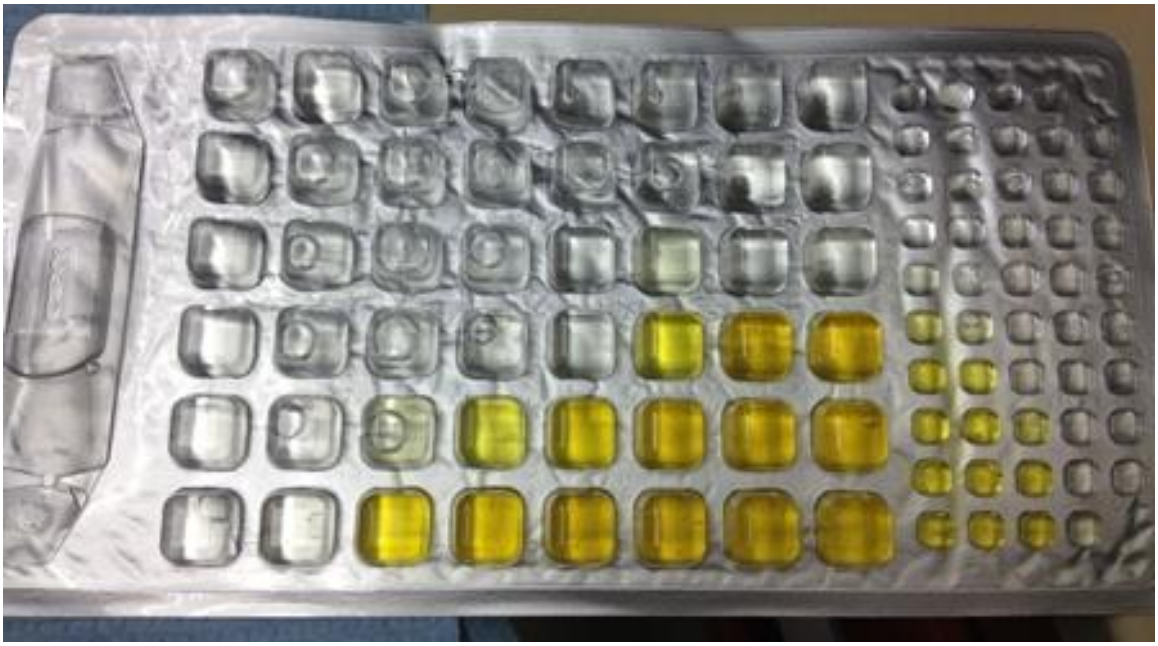

Figure 2.3: Tray incubated at 30°C (86°F) after 25 hours of incubation. At this time, 16 large wells and 12 small wells indicate positive hits.

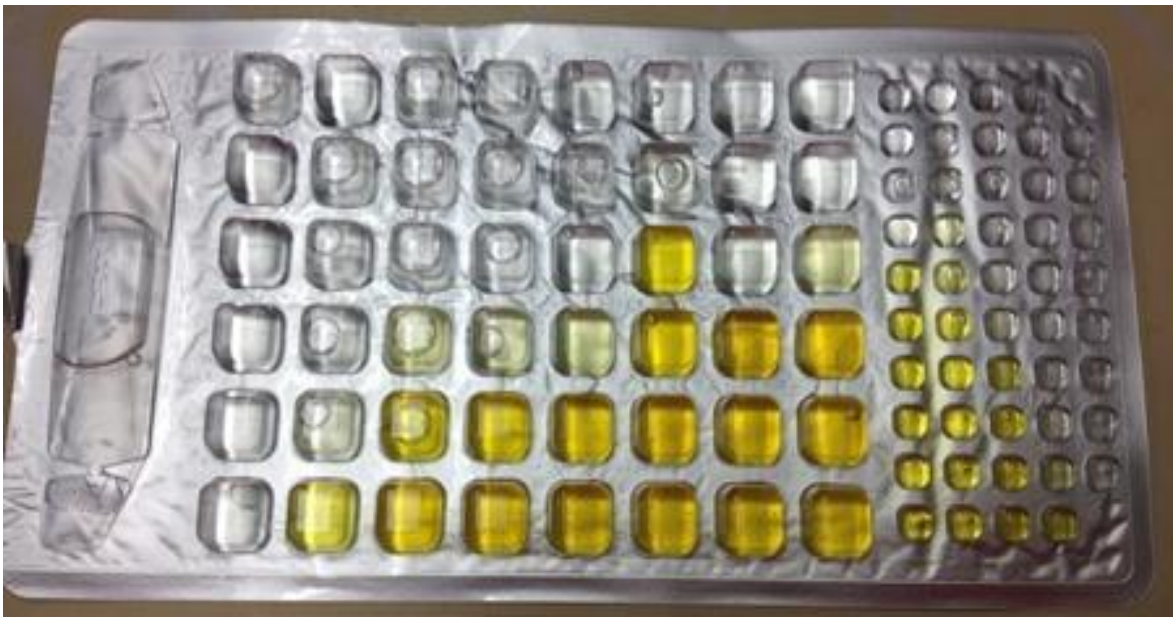

Figure 2.4: Tray incubated at 30°C (86°F) after 27 hours of incubation. At this time, 21 large wells and 19 small wells indicate positive hits.

Below is a graph indicating the rate of development for this test visually:

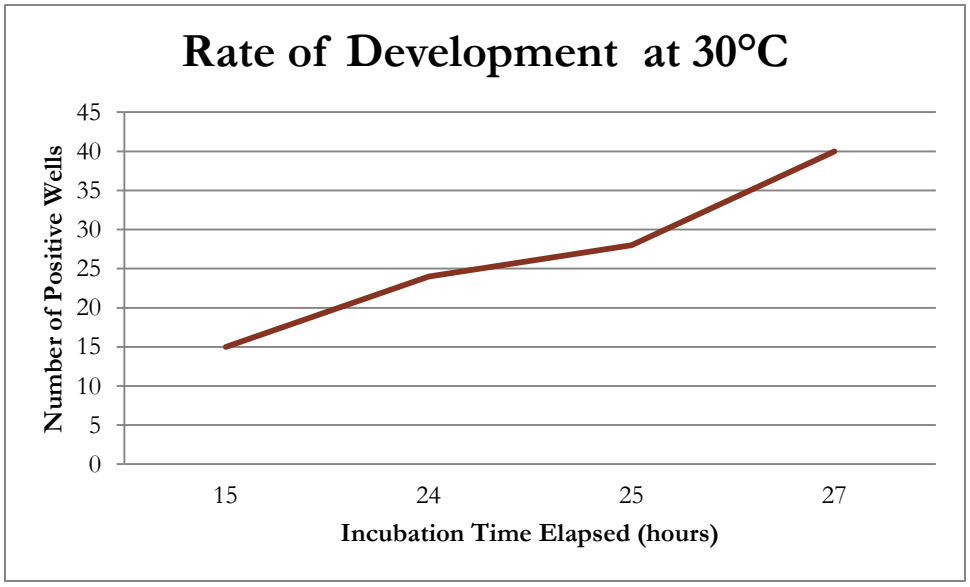

Figure 2.5: Visual representation of the rate of positive result development at 30°C (86°F).

COMPARISON OF PERFORMANCE AND RECOMMENDATION

From the data and results yielded by this investigation, it is clear that the Hach® PathoScreen™ tests performed better at a wider range on temperatures than did the Colilert® medium using the Quanti-Tray/2000® enumeration method. However, as only three trials were conducted using the Colilert® with Quanti-Tray/2000® method, there are

questions remaining which were outside the scope of this investigation. For example, could the temperature of the equipment used to seal the Quanti-Tray/2000® enumeration tray possibly affect the outcome of the test results? In this investigation, an inexpensive iron was used, in order to replicate a situation which may occur in the sorts of environments that are of interest for the purposes of this study (tray sealers from IDEXX are both cumbersome and costly, two virtually prohibitive features working against their use in developing countries, particularly in rural areas.) Could the hotter temperature of the iron have affected the powder medium in some way? The Colilert® Quanti-Tray/2000® method (in addition to being more sensitive to changing conditions) is more expensive and complicated to carry out. One must purchase (and then transport) the tray sealer for the tests. A used sealer can cost more than $200 USD. Along with the fact that the test kits themselves are more expensive than the PathoScreen™ field test kits, the price point of this test alone is a virtually prohibitive quality in many regions where drinking water quality testing is needed.

The Hach® PathoScreen™ field test kit is relatively affordable. The cost of one field kit is $47.05 USD; one kit contains five reusable glass scintillation vials, sterilizing liquid, and enough powdered growth medium (contained in pre-measured foiled pillows to increase shelf life) for 100 presence/absence tests using 20 mL samples or 20 Most Probable Number (MPN) tests.[3] Supplementing the assertion that the PathoScreen™ test is the most reliable and practical for use in the field (particularly in rural areas of developing countries) is the fact that the PathoScreen™ tests do not have to be placed in the glass scintillation vials for incubation. Indeed, they can be successfully carried out in any sort of sealed, sterilized container. For example, regular water bottles have been used successfully in field tests using the PathoScreen™ growth medium.

CONCLUSION

For many regions where the monitoring of drinking water quality is necessary and essential to the health and well being of the population which relies upon it, simple, easy to interpret indicator species tests are of vital importance. Often, reliable sources of electricity are a rarity, with rolling blackouts common in urban areas of developing countries, while no electricity may be the norm in rural areas. Technical expertise is also a rarity in these situations. This study found that, overall, the Hach® PathoScreen™ test is more reliable, easier to perform, and easier to interpret than the Colilert® test using the Quanti-Tray/2000® enumeration method, a common form of MPN test. The Quanti-Tray/2000® tests yielded shaky and bizarre results, with positive results being indicated at temperatures five degrees below the recommended incubation temperature and no results at all being indicated at the actual recommended incubation temperature of 35°C (95°F). The global water sanitation community would benefit greatly from further investigation of this subject in general and the sensitivity and accuracy of the Colilert® test using the Quanti-Tray/2000® method in particular.

[3] From the PathoScreen Field Test Kit product page on the Hach® website: http://www.hach.com/pathoscreen-field-test-kit/product?id=7640249603

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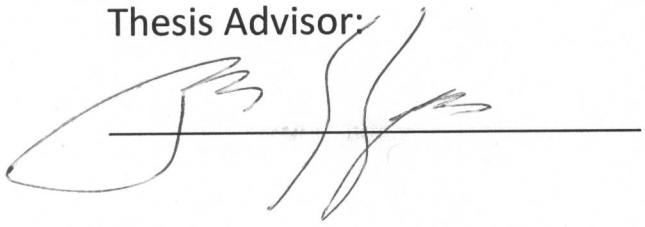
This thesis is approved.

Thesis Advisor:



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