

Lishtot TestDrop Pro Test Protocol and Interpretation

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EcoConcern Team

ENPHO

MIT H2O-2B Team at Final D-Lab Showcase

Overview

We set up our lab at ENPHO, in Kathmandu, Nepal during Jan 7 – 10, 2019 and began testing a total of five products on Friday, Jan. 11. The test period ran two full weeks (six day work weeks) from Jan. 11 – 25. We presented our results to our partners, ENPHO and EcoConcern, as well as some potential clients and key stakeholders, on Jan 25, 2019.

Reference Test IDEXX Quanti-tray “gold standard”

Five (5) Product Options Tested

1. P/A (30 mL), P/A (8) mL plus Compact Dry (1 mL)
2. Compact Dry only (100 mL)
3. Lishtot TestDropPro (100 mL)
4. Aquatest + RUG (50 mL)
5. ECC (1 mL and 10 mL)

Volume of Field Sample Needed per Test per Day

	Total Vol sample needed (assuming duplicates on all tests)	Units of Test Supplies brought to Nepal
P/A (20 ml), P/A (8 ml)	40 ml 16 ml	400 400
Compact Dry (1mL)	12 ml	400
Compact Dry (100ml)	200 ml	400
Lishtot (100 mL per test)	200 ml	25 Devices and several hundred cups
Aquatest + RUG (50 mL)	100 ml	Make up ourselves
ECC (1 mL test, 10 mL test)	22 ml	Make up ourselves
IDEXX Colilert (100 mL)	200 ml	400
Total Volume Needed Per Test	780 ml = minimum needed for each sample	

The diversity of test product volumes was a function of our desire to test as low a volume as possible in some instances and to use the “standard” 100 mL sample size in other instances. It also depended partly on having 100s of certain sized bottles at hand for sterilization at the ENPHO lab.

There were six people on the Products R&D Testing team (5 MIT, 1 EcoConcern). Different team members had primary responsibility for different products so that we would get consistent data from the same person doing the same tests every day. Several people wore multiple hats, such as Ike collecting all the daily samples and also doing the data analysis for Lishtot. Amber doing Lishtot AND IDEXX, etc. Swooch assisting as needed.

Protocol IDEXX Quanti-Tray®¹

1. Add contents of one pack of Colilert to a 100 mL water sample in a sterile vessel.
2. Cap vessel and shake until dissolved.
3. Use one hand to hold a Quanti-Tray upright with the well side facing the palm
4. Squeeze the upper part of the Quanti-Tray so that the Quanti-Tray bends toward the palm.
5. Open the Quanti-Tray by pulling the foil tab away from the well side. Avoid touching the inside of the foil or tray
6. Pour sample/reagent mixture directly into the Quanti-Tray avoiding contact with the foil tab. Allow foam to settle.
7. Place the sample-filled Quanti-Tray onto the rubber tray carrier of the Quanti-Tray Sealer with the well side (plastic) of the Quanti-Tray facing down to fit into the carrier
8. Seal according to the Quanti-Tray Sealer instructions
9. Place the sealed tray in a 35±0.5°C incubator for 24 hours.
10. Count the number of large and small positive wells and refer to the Quanti-Tray/2000 most probable number (MPN) table to find the MPN.
11. Dispose of media in accordance with good laboratory practices

¹ Instructions adapted from <https://www.idexx.com/files/quanti-tray-2000-procedure-en.pdf>

Lishtot TestDrop Pro Set-Up: (Susan)

We had 20+ TestDrop Pro devices with us in Nepal. We numbered each device. Of that set, we randomly selected five as the ones we would test during the three weeks of testing.

Those five were #3, #13, #14, #15 & #17.

On some days, Amber ran tests alone. On other days, two sets were run concurrently by Amber and Swooch.

On Day 1 (1/11/19) and Day 2 (1/14/19), for each TestDropPro test, we first tested a blank sample using bottled water. We used these tests as a control to verify that the TestDrop Pro gives consistent results with clean water. Following that, we tested the Kathmandu environmental samples that Ike collected each day (typically 10 unique samples). Concurrently, other team members were running the other four+ products we were testing.

The samples have been collected from various locations in Kathmandu as you can find in Appendix A.

TestDrop Testing procedure

The layout for the TestDropPro testing was to place a clean, never used plastic cup on a marked spot on the lab bench. First, we filled that clean cup with bottled water and tested that with #3, #13, #14, #15 & #17 and noted our results.

Then, we took a new plastic cup, poured in the environmental sample, and tested it again with #3, #13, #14, #15 & #17.

For each test, we filled the cup with 100 mL of sample (given that that is the standard sample size for the IDEXX reference product). Also, so as to be consistent in terms of the volume parameter.

The notation used was “0” for blue light, “1” for red light on the TestDropPro

On Day 1 (1/11/19) and initially on Day 2 (1/14/19) of TestDropPro testing, we were pushing the button that made the most sense for the given sample. So, if it was the bottled water sample, we pushed the bottled water button. On those first days, we did not record the app result. Beginning on 1/15/19, we stopped recording the blanks (bottled waters results) and began recording the app result (Kathmandu sample) for each for #3, #13, #14, #15 & #17

Lishtot Test Drop Pro Procedure (Amber from Lishtot Website and Literature)

1. Make sure your cup, hand, device are dry initially
2. Pour sample water into clean plastic cup just over halfway full
 - a. If using bottled water, unopened works best



3. Swirl water in cup for 10 seconds and tilt on one side (the side with the testing location) until the water reaches the top of that side.
 - a. If water droplets remain on the side of the cup, swirl again to until the sides above the waterline are as clear as possible.
 - b. Ensure swirl on the cup is high enough (at least 3 finger widths above test location)
4. Set the cup down on a hard flat surface and let it settle
5. Position your device above the water line (by a finger's width) four inches (10 cm) away
6. Hold down appropriate button (bottled water, faucet for tap water, or trees for natural sources) and slowly move device along a straight axis until it comes into contact with the plastic above the water line
 - a. Device should blink yellow as you move it along
7. When it touches the cup, wait one extra yellow blink and release
8. You will see a red light (positive, contaminated) or blue light (negative, clean)
9. Wait 3 seconds between tests and re-swirl before each test

Some additional notes from Amber Van Hemel -- Week of Jan. 7 – 11, 2019:

- We chose the volume 100 mL for the TestDrop Pro to be consistent with concurrent tests we are running (e.g. IDEXX, membrane filtration with CompactDry).
- We've seen recommendations for swirling for 5 seconds (in the video) and 10 seconds on their website FAQs -- <https://www.lishtot.com/faq.html> but we are going to go with the website recommendation which says "fill a cup to over one half full and swirl gently for about ten seconds tilting on one side just until the water reaches the top of that side. Let the water settle" We are interpreting this as a swirl then tilt and test on the tilted side
- When you connect the devices to the app, you can see the last ten tests performed. Theoretically after every 10 trials, we are able to connect it for some high resolution data
- We have discussed running blanks using either distilled water or bottled water, and have settled on doing blank, sample, blank, sample, blank, sample etc. (SM comment: We settled on bottled water initially because our host lab didn't have readily available volumes of distilled water and we were using a lot of water).

Interpretation of Results (Ike)

Attention is turned to the attached results (Appendix B). For each of the sixty samples tested, five data scores were collected, one for each unique TestDrop Pro device. For data interpretation, three out of five tests were used to determine if a sample was clean of E. coli or contained E. coli at some concentration. Using the "majority samples" approach, 90% of E. coli samples were identified, with the higher the concentration of E. coli leading to higher consistency of detection. As one may note in Appendix B, detection of "clean" water samples was low. After it became clear from IDEXX coliform testing, that all water samples in Nepal that were tested with the

Lishtot device had coliforms present, averaging all five readings for any given sample yielded 96.7% of the samples matching the coliform pattern in the E. coli-free water. Please see Appendix A that shows source of water tested as well as its natural lode of E. coli as determined by testing. The TestDrop Pro performed properly with bottled water.

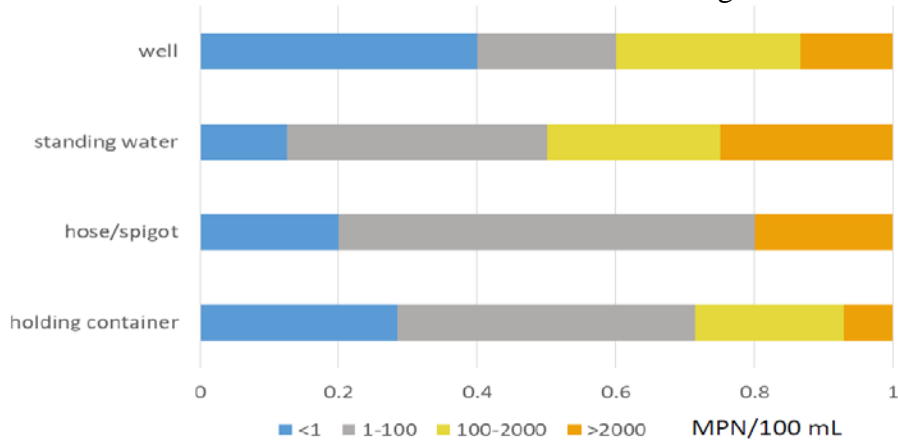
Analysis and Conclusions

The project goal is to find a simple, non-expensive E.coli test for developing countries, it is clear that the TestDrop Pro is reusable and the cost per \$0.01 per test though the upfront costs are higher than for other tests (Appendix C). Lishtot TestDrop was the only system tested that did not require adding something to the water or require an incubation time. It was also the fastest system to results of those we tested. The ability of the TestDrop to identify the coliforms present in all sixty field samples has two implications. The first is that the TestDrop, in its current configuration, may require additional testing and fine-tuning for routine use in untreated water in developing countries, as its sensitivity to many (non-target) forms of contamination makes it too sensitive for testing in such challenging environments. On the other hand, application of the TestDrop Pro to public water systems, filtered and reverse osmosis water, and the like might be ideal, as the device can identify contaminations quickly and with high apparent reproducibility. Safe water should not have coliforms; if coliforms or other contaminants are present, the Lishtot technology should be able to detect them quickly and at a low level of false readings. This paradigm-shifting product can be very useful in various applications and it is worth further development. We plan to further test the TestDrop Pro with one of our MIT students working in Israel with Lishtot this summer 2019.

Appendix A: Source Water

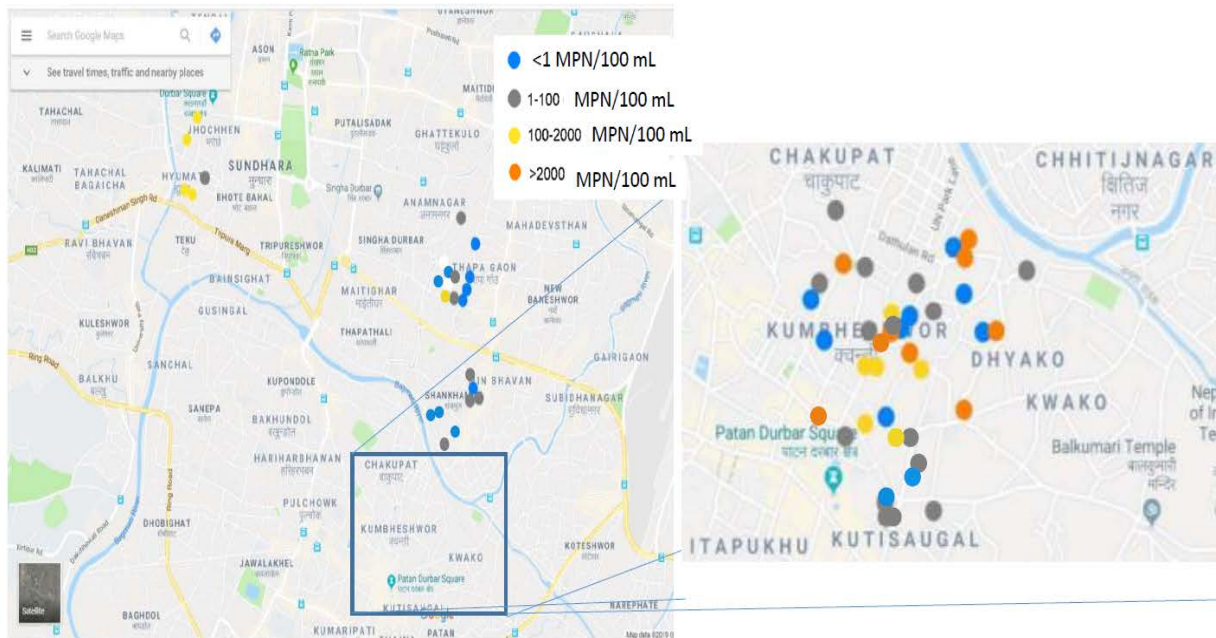
Source Sample Log					
Duplicates a & b were performed on each source water					
Date	#	Source	Description or Comment	Date sampled	< 24 hr holding in frig
1/11/2019	1	Bagmati River	10 ⁻⁵ Dilution (w/ tap water)		
	2	ENPHO Tap Water			
	3	Bottled Water	AQUA Ruis		
1/14/2019	4	Bagmati River	10 ⁻⁴ Dilution (w/ tap water)		
	5	Lily Pond at sunset Hotel	slightly discolored		
	6	Running water outdoors at Sunset Hotel	more discolored		
	7	Nepali Police tap water			
	8	Cleaning water	from a small restaurant		
	9	Surface runoff	scooped from a puddle		
	10	Bagmati River	Different point (true Bagmati)		
1/15/2019	11	Bore well water - Jhankhel	post-filter water / iron	15-Jan	same day analysis
	12	Bore well after filter - Jhankhel		15-Jan	same day analysis
	13	Tap water -Jhankhel		15-Jan	same day analysis
	14	Stone tap -Jhankhel		15-Jan	same day analysis
	15	Bangalamukhi Temple - drinking water	from roof tank	15-Jan	same day analysis
	16	Bhuichi Stone Tap		15-Jan	same day analysis
	17	omitted in order to start with #21			
	18	omitted in order to start with #22			
	19	omitted in order to start with #23			
	20	omitted in order to start with #24			
1/16/2019	21	Imukhel	outdoor spigot	15-Jan	< 24 hr holding in frig
	22	Imukhel	standing pool	15-Jan	< 24 hr holding in frig
	23	Imukhel	well	15-Jan	< 24 hr holding in frig
	24	Imukhel	bucket for laundry	15-Jan	< 24 hr holding in frig
	25	Thapagaul (spelling?)	hose	15-Jan	< 24 hr holding in frig
	26	Anamnagar	bucket for dishes	15-Jan	< 24 hr holding in frig
	27	Dhyako	hose	16-Jan	same day analysis
	28	Dhyako	well	16-Jan	same day analysis
	29	Mangal Bazaar	hose	16-Jan	same day analysis
	30	Dhyako	bucket for dishes	16-Jan	same day analysis
1/17/2018	31	Thapa Gaon	bucket for dishes	16-Jan	< 24 hr holding in frig
	32	Anamnagar	hose	16-Jan	<24 hr holding in frig
	33	Kumbheshwor	well	16-Jan	<24 hr holding in frig
	34	Kumbheshwor	well	16-Jan	<24 hr holding in frig
	35	Mangal Bazaar	well	16-Jan	<24 hr holding in frig
	36	Kumbheshwor	well	16-Jan	<24 hr holding in frig
	37	Imukhel	standing water	16-Jan	<24 hr holding in frig
	38	Imukhel	hose	16-Jan	<24 hr holding in frig
	39	Imukhel	bucket	16-Jan	<24 hr holding in frig
	40	Chakupat	bucket for laundry	16-Jan	<24 hr holding in frig
1/18/2019	41	Mangal Bazaar	well	17-Jan	<24 hr holding in frig
	42	Mangal Bazaar	hose	17-Jan	<24 hr holding in frig
	43	Ekhalkhu	standing water	17-Jan	<24 hr holding in frig
	44	Ekhalkhu	well	17-Jan	<24 hr holding in frig
	45	Mangal Bazaar	pipe, run-off	17-Jan	<24 hr holding in frig
	46	Hyumat	bucket	17-Jan	<24 hr holding in frig
	47	Hyumat	well	17-Jan	<24 hr holding in frig
	48	Hyumat	bucket	17-Jan	<24 hr holding in frig
	49	Chikanmugal	standing water	17-Jan	<24 hr holding in frig
	50	Chikanmugal	large water storage tank	17-Jan	<24 hr holding in frig
1/21/2019	51	Shreenagar	tap	21-Jan	same day analysis
	52	Shreenagar	jug	21-Jan	same day analysis
	53	Shreenagar	running water	21-Jan	same day analysis
	54	Shreenagar	outdoor spigot	21-Jan	same day analysis
	55	Shreenagar	standing water	21-Jan	same day analysis
	56	Imukhel	outdoor spigot	21-Jan	same day analysis
	57	Kumbheshwor	well	21-Jan	same day analysis
	58	Kumbheshwor	bucket	21-Jan	same day analysis
	59	Kumbheshwor	well	21-Jan	same day analysis
	60	Mangal Bazaar	bucket for laundry	21-Jan	same day analysis

Water sources used in out testing



E. coli MPN/100 mL based on IDEXX QuantiTray as Reference Test

Map of *E. coli* in Source Water Samples, by Location



Appendix B: Field Test Results with Lishtot TestDrop Pro Devices

E. COLI Level						success rate	
Correct	Wrong	Total					
0	7	12	19				36.8
<100	22	3	25				88.0
100-2000	9	1	10				90.0
2000+	6	0	6				100.0
Total E.coli	37	4	41				90.2
TOTAL:	44	16	60				73.3

Three Days of TestDrop Pro Data (1/16/19, 1/17/19 and 1/18/19)										
	Devise #3	Devise #13	Devise #14	Devise #15	Devise #17	IDEXX*		IDEXX		
	app resu	app resu	app resu	app resu	app resu	E.coli (raw data)	3 OUT OF 5 27 CUTOFF	Total coliform	Avg. Result 5 Tests	Success Rate: 96.7%
#####	21a	0	29	14	32	32	0	CORRECT	1.0	21.4
	21b	32	2	4	3	4	0	WRONG	1.0	9
	22a	32	0	0	0	23	9999	CORRECT	2.0	11
	22b	32	2	0	27	7	9999	CORRECT	2.0	13.6
	23a	32	32	26	0	32	0	CORRECT	2.0	24.4
	23b	32	32	32	0	0	0	CORRECT	2.0	19.2
	24a	18	32	0	20	27	13.5	CORRECT	2.0	19.4
	24b	32	17	0	0	0	8.6	CORRECT	2.0	9.8
	25a	16	19	32	0	32	53.5	CORRECT	2.0	19.8
	25b	32	0	18	2	32	43.5	CORRECT	2.0	16.8
	26a	32	18	32	31	8	4.1	WRONG	2.0	24.2
	26b	32	0	0	6	25	3	CORRECT	2.0	12.6
	27a	13	32	16	7	26	0	WRONG	1.0	18.8
	27b	32	32	32	4	0	0	CORRECT	1.0	20
	28a	32	32	32	0	3	0	CORRECT	2.0	19.8
	28b	0	32	9	0	0	0	WRONG	2.0	8.2
	29a	0	0	3	0	0	9999	CORRECT	2.0	0.6
	29b	32	0	22	0	0	9999	CORRECT	2.0	10.8
	30a	5	32	0	0	6	0	WRONG	2.0	8.6
	30b	32	32	0	7	32	4.1	WRONG	2.0	20.6
#####	31a	32	32	32	32	32	0	CORRECT	1.0	32
	31b	32	32	32	32	32	0	CORRECT	1.0	32
	32a	14	15	16	6	8	3	CORRECT	2.0	11.8
	32b	24	0	32	7	7	2	CORRECT	2.0	14
	33a	0	0	31	0	3	3	CORRECT	1.0	6.8
	33b	4	0	2	0	0	2	CORRECT	1.0	1.2
	34a	0	2	0	17	27	167	CORRECT	2.0	9.2
	34b	4	0	0	0	1	172.2	CORRECT	2.0	1
	35a	32	2	0	0	32	0	WRONG	2.0	13.2
	35b	15	0	29	0	32	0	WRONG	2.0	15.2
	36a	0	0	0	10	0	0	WRONG	1.0	2
	36b	4	10	16	0	2	0	WRONG	1.0	6.4
	37a	2	3	0	8	11	9999	CORRECT	2.0	4.8
	37b	1	2	0	0	0	9999	CORRECT	2.0	0.6
	38a	0	0	0	18	0	1	CORRECT	1.0	3.6
	38b	0	2	2	19	32	0	WRONG	1.0	11
	39a	2	0	0	2	0	0	WRONG	2.0	0.8
	39b	0	7	18	13	15	0	WRONG	2.0	10.6
	40a	6	26	32	1	0	0	WRONG	1.0	13
	40b	32	2	0	2	0	2	CORRECT	1.0	7.2
#####	41a	13	4	32	5	22	1299.7	CORRECT	1.0	15.2
	41b	13	0	0	2	2	816.4	CORRECT	1.0	3.4
	42a	6	32	2	32	4	66.3	CORRECT	2.0	15.2
	42b	32	28	5	0	0	78.5	CORRECT	2.0	13
	43a	32	32	9	0	11	2	CORRECT	1.0	16.8
	43b	32	0	0	5	2	74.4	CORRECT	1.0	7.8
	44a	12	7	1	32	32	9.7	CORRECT	2.0	16.8
	44b	1	0	0	2	3	4.1	CORRECT	1.0	1.2
	45a	18	1	32	24	0	9.8	CORRECT	2.0	15
	45b	13	1	32	32	6	6.3	CORRECT	2.0	16.8
	46a	10	20	7	32	0	21.6	CORRECT	2.0	13.8
	46b	3	32	32	32	18	27.2	WRONG	2.0	23.4
	47a	10	7	32	0	0	686.7	CORRECT	2.0	9.8
	47b	4	0	6	2	32	344.8	CORRECT	2.0	8.8
	48a	18	5	0	11	0	1732.9	CORRECT	2.0	6.8
	48b	12	0	10	10	26	1413.6	CORRECT	2.0	11.6
	49a	17	2	3	1	17	1299.7	CORRECT	2.0	8
	49b	15	1	1	22	32	1299.7	CORRECT	2.0	14.2
	50a	13	29	31	18	30	387.9	WRONG	2.0	24.2
	50b	21	0	32	1	16	90.9	CORRECT	2.0	14

* IDEXX result of < 1 replaced in raw data with zero (0).
 * IDEXX <1 =0.0, any positive number = 1.0; Too Numerous to Count (9999.0) = 2.0

Appendix C

EC-Kit 2.0 – Cost Comparison of Options

	1	2	3	4	5
Attributes	P/A Vial + 1 mL CompactDry ECO	Compact Dry ECO - 100 mL	REG	ECC	TestDrop Pro
Cost per test	\$1.65 / test	\$1.00 / test	≈ US\$ 0.50 / test	≈ US\$ 0.50 / test	\$0.01 / test \$50/unit; Good for 10,000 tests
Portable, Easy to Use	Yes	Yes	TBD	Yes	Yes
Interpretation	Semi-quantitative	Quantitative	Presence/Absence or Semi-quantitative	Presence/Absence or Semi-quantitative	Presence/absence
Test Duration	24 - 48 hrs	24 hrs	24 hrs	12 hours for high concentration 24 hours for lower concentration	< 1 minute