Simple and rapid method for detection of enteric pathogens in stool samples for low-resource settings

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Introduction

Infectious diarrhea is the second leading cause of morbidity and mortality in the world with 3.1 million deaths annually, mostly among children and the elderly. The optimal mode of treatment depends on identification of the pathogen. Current methods are labor- and resource-intensive and can take days to complete.

PATH is a nonprofit organization whose mission is to improve the health of people around the world by advancing technologies, strengthening systems, and encouraging healthy behaviors, especially in the developing world.

PATH Diagnostics Teams

- Develop/modify cost-effective, novel, rapid, easy-to-use, and appropriate tests.
- Collaborate with industry and other nonprofits to develop high-quality test kits and reagents.
- Increase test availability by technology transfer.
- Evaluate/recommend appropriate use of commercial tests.

Objective

To identify a simple, rapid, and cost-effective method for detecting pathogenic bacteria in stool samples in low-resource settings.

Current Pathogen Identification in Stool Culture

Time to positive identification (days)

	Median	Range
C. jejuni	1.5	1-2
E. coli O157:H	1.5	1-2
Salmonella	2.2	1-4
Shigella	2.5	2-3

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Methods

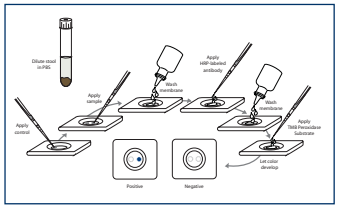
1. Dilute stool sample 1:10 in PBS.
2. Apply sample and control to cassette membrane.
3. Wash membrane with appropriate buffer.
4. Add HRP-labeled antibody.
5. Wash membrane with appropriate buffer.
6. Add TMB Peroxidase substrate.
7. Read cassette:
   - Blue spot = positive.
   - No blue spot = negative.

Positive culture sample

Positive feces sample

(negative stool on left, postive stool on right)

PATH enteric flow-through assay



Results

Shigella sp. from culture

Strain 1		Strain 2	
organisms on membrane	relative color intensity	organisms on membrane	relative color intensity
4, 970	++	2,200	++++
497	+	220	+++
50	-	22	++
5	-	2	weak +

Campylobacter from culture

Dilution	relative color intensity, beads	relative color intensity, cassette
Neat	+++	+
1:10	+++	-
1:100	++	-
1:1000	+	-

Salmonella from culture

Dilution	relative color intensity, beads	relative color intensity, cassette
1:10	+++	+++

E. coli O157:H7 from feces: results on cassette

	Dilution weight/volume			CFU/2µL loaded on membrane			%O157: H7 by SMAC culture	Real time PCR result
Sample #	1:100	1:1000	1:10000	1:100	1:1000	1:10000		
1	+	+	-	5200	520	52	97	+
2	+	+	-	14000	1400	140	64.5	+
3	+	+	-	368	837	4	99.8	+
4	-	-	-	16100	1610	161	negative	-

Conclusions

- A rapid test for enteric pathogen detection has been proposed and tested. Total time for the assay to be performed is about 10 minutes.
- The test uses one cassette and three reagents which could be in dropper-bottle format.
- The cost of all components is less than US$2 per test.
- This test could be readily adapted to a variety of enteric pathogens allowing timely treatment decisions in cases of infectious diarrhea.

References

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PATH is also currently developing other diagnostic assays based on immunochromatography and lab-on-a-card platforms, including assays for febrile illnesses, sexually transmitted diseases, and enteric diseases for use in low-resource settings.

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