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Validation of Luminex xTAG Gastrointestinal Pathogen Panel with Stool Specimens in S.T.A.R. Buffer

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Introduction

Gastrointestinal infections in both pediatric and adult patients account for significant morbidity and mortality worldwide. Diarrheal disease can be caused by a number of pathogens including viruses, bacteria, and parasites. For detection of enteric pathogen set, conventional diagnostic procedures involve culture, microscopy, and/or stool antigen tests. A newly FDA-approved test procedure, xTAG® Gastrointestinal Pathogen Panel (GPP) which includes 15 targets (4 pending approvals), overcomes most of those shortcomings and offers an economical and efficient management for clinical patients. This study evaluates and validates the analytical and clinical performance of the GPP assay.

Method

Two hundred and one (201) stool specimens were collected from 140 known (+) banked samples of Luminex®, 51 clinical patients, and 10 normal volunteers. For sample preparation, 1ml of S.T.A.R. (Stool Transport and Recovery) buffer with the internal control (MS2) was added to each tube (Abbott Master Mix Tube) containing stool-collected on the swab. After mixing thoroughly, incubate the tubes at 37^{0}C water bath for 5 minutes. Store the tubes at –20^{0}C until use. Thaw the tubes at 37^{0}C water bath at least 5 minutes before centrifugation at 2000 rpm for 5 minutes. Remove any bubbles on the surface of the solution before testing procedure. Then, the samples were isolated with an open-mode protocol on Abbott's m2000 sp automatic instrument. The GPP kits (Luminex) were used following the manufacturer's instructions. The nucleic acid was amplified in a single multiplex RT-PCR reaction in a ProFlex thermocycler (Applied Biosystems), and the amplicon was hybridized with xMAP bead. Both the bead ID and the target reaction were detected by the Luminex^{200}. The target-specific cut-off value was used with TDAS LSM software (xTAG® Data analysis Software LSM) to determine the presence/absence of the pathogens in the testing samples.

In addition, stored (-20^{0}C) positive patient specimens were frozen and thawed for one to four times during a period between 2 weeks to five months to evaluate the effects of freeze/thaw cycles on the sample stability in STAR buffer.

Results

Each target was tested at least 6 times and all of them showed expected (+) results (Table 1 & 2). All normal stool samples were tested negative (data not shown). The external testing results were available for those 51 patients. Thirty-one out of fifty-one patients were tested negative both with GPP and outside lab (data not shown). The other 20 patient results were identified (+) targets either by GPP or outside lab (Table 3). Results of eight patients were found completely matched between two labs. There were 4 (+) results from GPP but were negative by outside lab. Another 6 (+) results picked up by GPP but they were not ordered by physicians. Two *E. histolytica dispar* (+) were not detected by GPP. A total of 17 positive samples were included in the freeze/thaw study (Table 4). All specimens were stable for at least two weeks with various freeze/thaw cycles, and some could be stable up to five months. However, targets with low signals (close to cut-off value, shown in **bold**) did not show reproducible results after two freeze/thaw cycles. No single reaction showed the inhibitory substance.

Table 1: Accuracy and Reproducibility of Banked Specimens from Lunimex

Banked Samples	Signals (MFI)*	Banked Samples	Signals (MFI)*
Cryptosporidium	601	Rotavirus A	2532
Cryptosporidium	1286	Rotavirus A	2914
Cryptosporidium	591	Rotavirus A	2763
Cryptosporidium	886	Rotavirus A	4333
Cryptosporidium	1503	Rotovirus A	4736
Cryptosporidium	1575	Rotovirus A	3141
E.Coli. 0157	1819	*C. difficile* A/B	1960/2480
E.Coli. 0157	2005	*C. difficile* A/B	1492/1814
E.Coli. 0157	374	*C. difficile* A/B	1504/1548
E.Coli. 0157	2298	*C. difficile* A/B	1371/1831
E.Coli. 0157	1905	*C. difficile* A/B	1537/2102
E.Coli. 0157	2830	*C. difficile* A/B	2050/2008
STEC	1327/1301	*Shigella*	1721.5
STEC	1498/1339	*Shigella*	1803
STEC	653/252	*Shigella*	1771
STEC	1323/1462	*Shigella*	1770.5
STEC	1498/1339	*Shigella*	2185
STEC	1960/1407	*Shigella*	2028
ETEC LT/ST	1587.5/1741	*Campylobacter*	4787.5
ETEC LT/ST	2147/2351	*Campylobacter*	4000
ETEC LT/ST	3277/3423	*Campylobacter*	3392
ETEC LT/ST	3155.5/3472	*Campylobacter*	3546
ETEC LT/ST	3717/3939	*Campylobacter*	3801
ETEC LT/ST	3173/3389	*Campylobacter*	3985
Norovirus GI/GII	4528	*Salmonella*	1559/1420
Norovirus GI/GII	1494	*Salmonella*	2553/2322
Norovirus GI/GII	4426	*Salmonella*	4140.5/4032
Norovirus GI/GII	2136	*Salmonella*	4731/3967
Norovirus GI/GII	5109	*Salmonella*	4091/3776
Norovirus GI/GII	4780	*Salmonella*	4026/3598
Norovirus GI/GII	764	*Salmonella*	3615.5/3577
Giardia	925	*Giardia*	2288.5
Giardia	2282	*Giardia*	2169
Giardia	2304	*Giardia*	961

Table 2. Accuracy and Reproducibility of 4 Pending FDA Approvals

Banked Samples	Signals (MFI)	Banked Samples	Signals (MFI)
Adenovirus 40-41	1609	*Vibrio Cholerae*	1662
Adenovirus 40-41	1579	*Vibrio Cholerae*	1622
Adenovirus 40-41	1677.5	*Vibrio Cholerae*	1377
Adenovirus 40-41	1603	*Vibrio Cholerae*	1333.5
Adenovirus 40-41	1540	*Vibrio Cholerae*	1378
Adenovirus 40-41	1700	*Vibrio Cholerae*	1370.5
Adenovirus 40-41	1578	*Vibrio Cholerae*	1318
Adenovirus 40-41	1629	*Vibrio Cholerae*	2062
Entamoeba Histolytica	1185	*Yersinia Enterocolitica*	2032.5
Entamoeba Histolytica	867	*Yersinia Enterocolitica*	1031
Entamoeba Histolytica	1137.5	*Yersinia Enterocolitica*	984
Entamoeba Histolytica	637.5	*Yersinia Enterocolitica*	1151
Entamoeba Histolytica	852.5	*Yersinia Enterocolitica*	1030
Entamoeba Histolytica	705.5	*Yersinia Enterocolitica*	836
Entamoeba Histolytica	701	*Yersinia Enterocolitica*	839
Entamoeba Histolytica	1046	*Yersinia Enterocolitica*	1203

Table 3. Comparison of Results between Luminex GPP and External Lab

Sample ID	GPP Results	External Reference Lab	Comments
140110705	*Campylobacter*	*Campylobacter (+)*	
140210498	*Cryptosporidium*	*Cryptosporidium (+)*	
140210152	*Giardia*	*Giardia* (+)	
140410383	*Giardia*	*Giardia* (+)	
140410830	*Giardia*	*Giardia* (+)	
140510710	*Giardia*	*Giardia* (+)	
140310952	*Shigella*	*Shigella* (+)	
140510685	*Shigella*	*Shigella* (+)	
140310273	*Giardia*		*Giardia* not ordered
140310427	ETEC/LTST		ETEC/LTST not ordered
140210628	Norovirus GI/GII		Norovirus not ordered
140410416	Norovirus GI/GII		Norovirus not ordered
140310531	Norovirus GI/GII		Norovirus not ordered
140310827	*C. difficile* A/B		*C.difficile* not ordered
140310952	*Cryptosporidium*		*Cryptosporidium* not ordered
140110705	*Giardia*	*Giardia* (-)	
140310531	*Giardia*	*Giardia* (-)	
140210718	*C. difficile* A/B	*C. difficile* A/ B (-)	
140410693	ETEC/LTST	*E. coli* toxin (-)	
140410298	None	*E. Histolytica-dispar* Cysts (+)	
140410830	None	*E. Histolytica-dispar* Trophozoites (+)	

Table 4. Effect of Freeze/Thaw Cycles on the Stability of Samples in STAR Buffer

Sample ID	Results	F/T times	Storage Period
140110705	*Campylobacter*	4	five months
140210152	*Giardia*	1	one month
140210498	*Cryptosporidium*	3	four months
140210628	Norovirus GI/ GII	3	four months
140210718	*C. difficile* A/B	3	four months
140310273	*Giardia*	2	three months
140310427	ETEC/LTST	1	two weeks
140310827	*C. difficile* A/B	1	two weeks
N4	*Giardia*	1	two weeks
140410383	*Giardia*	1	one month
140410830	*Giardia*	1	three months
140510685	*Shigella*	2	one month
140510710	*Giardia*	1	one month
140610146	Norovirus GI/ GII	1	one month
140110705	*Campylobacter*, ***Giardia****	2	2 weeks
140310531	*Giardia*, **Norovirus GI/GII***	2	2 weeks
140310952	*Shigella*, ***Cryptosporidium****	2	2 weeks

Conclusion

Our study results demonstrate that GPP is superior to the conventional methods in accuracy, sensitivity, and specificity. In addition, using S.T.A.R. buffer for the isolation of stool specimens instead of pre-treating stool with the bead as recommend by Luminex streamlines the testing procedure.

References

1. xTAG GPP Package Insert for use with Luminex 100/200, Rev A MLD-032-KPI-001 Rev. A; effective Date: January 2013
2. xPONENT® 3.1 Rev. 2 Software User Manual
3. TDAS GPP (US) User Manual
4. TDASLSM (US) User Manual