Implementation of a Multiplex Molecular Gastrointestinal Pathogen Panel: The Memorial Healthcare System Experience

Rodney C. Arcenas, PhD, D(ABMM)[1,2], M. Liliana Bedoya, MBA, BS, MT(AMT)[2], Frederick L. Kiechle, MD, PhD[1,2]

[1]Pathology Consultants of South Broward, Hollywood, FL and [2]Memorial Healthcare System, Hollywood, FL

Abstract (Revised and Updated)

Background: Infectious gastroenteritis can be a serious medical and economic burden to healthcare institutions. Many of the clinical symptoms overlap making it difficult for clinicians to make an accurate diagnosis without the use of laboratory testing. Traditional microbiological methods for stool pathogen testing have long been a mainstay in the clinical laboratory which can be time consuming, labor intensive, and involve multiple tests if bacterial, viral, and parasitic pathogens are being investigated. There is a shift towards syndromic testing involving multiplex molecular methods. This abstract describes the experience at the Memorial Healthcare System of implementing the Luminex xTAG® GastroPathogen Panel (GPP).
Methods: The GPP detects 13 (bacterial, viral, and parasitic) pathogens. This assay was performed as per package insert instructions utilizing the NucliSENS® easyMAG (bioMerieux) for stool extractions. Samples were obtained from Zeptometrix NATrol™ (11), Luminex (119), and left-over stool samples (105) from our Microbiology laboratory that were processed for culture and other non-molecular based testing. Prevalence data was also collected after test implementation (03-30-16) through 04-30-16. Workflow metrics were documented and comparisons were made to show differences between the traditional in-house testing and the newer multiplex molecular testing. GPP testing is batched and run once per day for each day of the week.
Results: The GPP offered additional stool pathogen targets compared to the Microbiology laboratory offering of culture (*Campylobacter, Salmonella, Shigella, E. coli O157,* and *Yersinia*), Shiga-toxin (EIA), and rotavirus antigen. The time to result for a sample after being received in the laboratory is approximately 6-7 hrs with the Medical Technologist manually entering the results. The Information Technology (IT) component of this test implementation was one of the bottlenecks in our process that resulted in going live with manual resulting and no interface. Our current laboratory information system (LIS) is SOFT Lab and our LIS vendor did not have any experience in setting up an interface with the Luminex Magpix instrument. We have a target turn-around-time (TAT) of 24 hrs. This 24-hr TAT is shorter when compared to our average stool culture TAT of 70.5 hrs. Additional send-out testing also takes multiple days to get results. GPP testing of the left-over stool samples showed additional detections for Norovirus that is normally not ordered or tested for routinely within our healthcare system. Prevalence data after test implementaiton showed Norovirus to be the most prevalent pathogen among pediatric and adult patients tested. Our Microbiology stool bench was discontinued allowing those FTEs to be devoted other responsibilities as well as being able to develop and implement new testing.
Conclusion: Implementation of the GPP within our healthcare system has given clinicians a wider breadth of GI pathogens than what we used to offer prior to molecular testing. The shorter TAT has allowed clinicians to act appropriately sooner based on the availability of results. The discontinuation of the stool bench in our Microbiology lab has increased our FTE resources to allocate to other responsibilities such as implementation of new testing and other bench-work that requires more hands-on time.

Materials and Methods

Samples
- Zeptometrix NATrol™ samples
- Luminex provided samples
- Clinical diarrheal stool samples
  - Raw and Cary-Blair diarrheal stool

Instruments
- easyMag Nucleic Acid Extractor (bioMerieux)
- Thermalcycler (Eppendorf)
- Luminex MAGPIX®

Reagents
- xTAG® GPP reagents. Assay performed as per manufacturer's protocol.

Data Analyses
- Data collection involved our IT Laboratory team and use of our LIS (SOFTLab)
- Data analyses were done using Microsoft Excel (Microscoft)

Results – Workflow/Methodology Comparisons

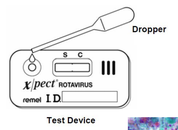
Method	Tests For	TAT	Positivity Rate (2014 volumes)
Stool Cx	5 bacterial pathogens	48-72 hrs	12.5% (5,516)
Ova & Parasite Exam	Parasite Eggs and Pathogens	24hrs	0.6% (1,664)
Modified Acid-Fast Stain (for Cryptosporidium, Cyclospora, Isospora)	3 Parasites	24 hrs	4.2% (120)
Rapid Rotavirus Antigen	Rotavirus	~30 minutes	12.2% (377)

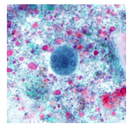
Luminex GPP		
Bacteria	*Campylobacter*	24 hr TAT (actual testing time approx 8 hrs); testing is 1x/day for 7 days/week
	E. coli O157	
	Enterotoxigenic *E. coli*	
	Salmonella	
	Shigella	
	Shiga Toxin	
	V. cholerae	
Viruses	Adenovirus 40/41	
	Norovirus I/II	
	Rotavirus	
Parasit	*Cryptosporidium*	
	E. histolytica	
	G. lamblia	

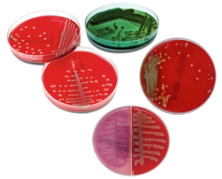
Results – Traditional In-house Testing Time Estimates

Stool Culture/O&P/Rotavirus Time Estimates		
		Technologist Time (min)
Pre-Analytical	Receiving/processing	10 min
	Stool Culture plating and other set-ups	Culture plating: 5 min
Analytical	Stool Cx Identification	6-40 minutes (not including incubation time)
	O&P	<60 minutes
	Shiga Toxin EIA	<30 minutes
	Rotavirus Antigen	18 minutes
Post-Analytical	Manual Resulting	1-5 minutes

Total Turn-Around-Time (TAT) in Hours	
Stool Cx Identification	48-72 hrs
O&P	<8 hrs
Shiga Toxin EIA	<8 hrs
Rotavirus Antigen	<1 hr

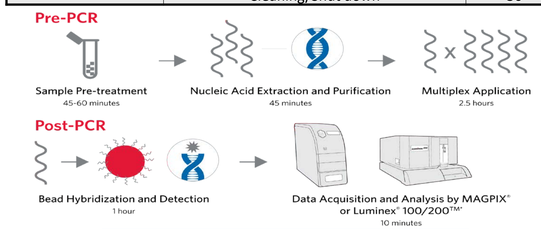






Results – GPP Workflow and Time Estimates

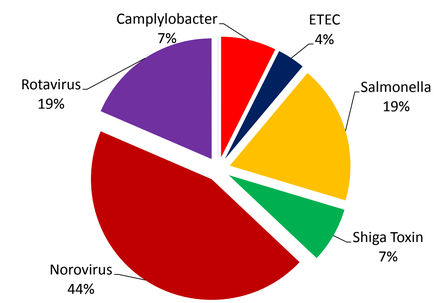
GPP Time Estimates (24 samples)	Assay Steps	Time (min)
Pre-PCR	Pre-Treatment	65
	easyMag Extraction	85
PCR	PCR Reagent Preparation	15
	Sample + PCR master mix	20
	RT-PCR	165
Post-PCR	Bead Hybridization	25
	Data Acquisition/Analysis	25
	Reporting (no instrument interface)	30
	Cleaning/Shut down	30

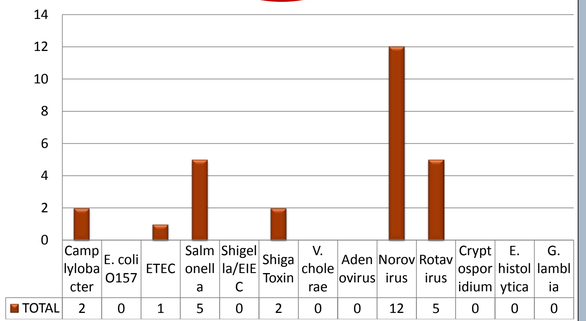


- **TOTAL testing time: 460 minutes (7.7 hrs)**
- **TOTAL TAT (Collected to Verified): 1504 min (25 hrs)**

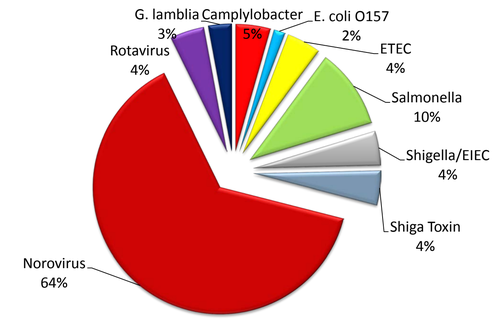
Leftover Stool Specimens from Microbiology	Total Number	GPP(+)/Micro(-) Specimens
GPP(neg)/Micro(neg)	68	▪ 8 Norovirus ▪ 4 *C. difficile* ▪ 1 *Campylobacter* ▪ 1 Adenovirus ▪ 1 *Shigella* ▪ 1 *E. coli* O157
GPP(pos)/Micro(pos)	13	
GPP(neg)/Micro(pos)	0	
GPP(pos)/Micro(neg)	16	

Results – Pediatric GPP Prevalence (March 30 – April 30)

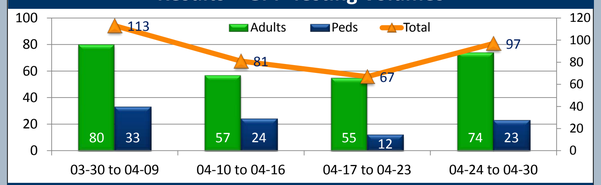




Results – Adult GPP Prevalence (March 30 – April 30)



Results – GPP Testing Volumes



Summary and Conclusions

- The GPP has resulted in time savings within the laboratory in TAT as well as Medical Technologist hands-on-time.
- The traditional Microbiology stool culture bench has been discontinued with the GPP assay as the primary stool pathogen test.
- Leftover Microbiology stool specimens were tested in the GPP and showed that 16 specimens were detected that were either not detected by culture or not tested for within our traditional routine stool pathogen work-up
  - 4/16 specimens were positive for *C. difficile* that were not detected by current methods
  - 3/16 specimens were positive for *Campylobacter*, *Shigella*, and *E. coli* O157 that were negative by culture
  - 9/16 specimens were positive for Norovirus (8) and Adenovirus (1)
- Current GPP testing is showing an under appreciation of Norovirus which is the highest incidence in the Adult and Pediatric patient populations.

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Contact information: rarcenas@mhs.net or mbedoya-gomez@mhs.net