The utility of the NanoCHIP® Gastrointestinal Panel (GIP), a molecular-based test for simultaneous detection of gastrointestinal protozoan parasites and bacterial infections, in a community laboratory

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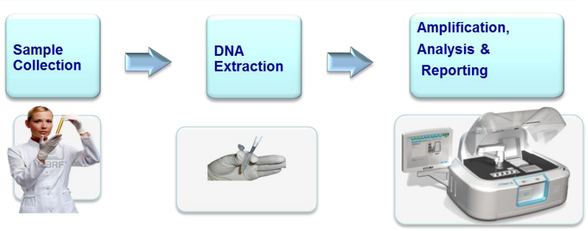
Background

Infectious Gastroenteritis is a global health problem associated with extremely high morbidity and mortality rates. Accurate diagnosis is crucial to allow appropriate and timely treatment. Stool testing at the microbiology laboratory is currently a complex, time consuming and cumbersome process, demanding highly qualified personnel and application of a wide range of techniques. Thus, workload, lab space and turnaround time are high and costly. Savyon Diagnostics has recently finalized the development of a novel molecular-based diagnostic screening test for simultaneous detection of nine bacterial and protozoan parasitic pathogens tailored specifically according to the demands of a typical laboratory at community setting. The test was developed on Savyon proprietary NanoCHIP® molecular electronic microarray system. The bacterial panel includes *Salmonella*, *Shigella* and *Campylobacter spp.* The parasitic panel is composed of *Entamoeba histolytica*, *Entamoeba dispar*, *Giardia lamblia*, *Dientamoeba fragilis*, *Cryptosporidium spp.* and *Blastocystis spp.* The simultaneous detection of all these pathogens in both panels is enabled due to the high multiplex capabilities that characterize the NanoCHIP® platform, that together with testing multiple patient samples in the same run offers a powerful and unique medium-high throughput screening tool. The system demonstrates high performance, versatility in the panel composition according to lab specific needs, minimal hands-on time, vast reduction in the workload of the lab (detection of parasites and bacteria in the same runtime) and cost-effectiveness. The system is compatible with a variety of automatic DNA extraction systems and thus is capable to fully integrate in the routine workflow of clinical laboratories of various sizes.

The NanoCHIP® Workflow



Materials & Methods

- *A clinical study composed of prospective and retrospective samples was conducted at Meuhedet Health Services, Rosh Haayin Laboratory, Israel, during February – April 2014 in symptomatic patients:*
  - *Retrospective samples: 46 positives*
  - *Prospective samples: 150 (31 positives; 119 negatives)*
- *The DNA was extracted by the Bullet PRO® system (Diasorin), amplified by PCR and processed by the NanoCHIP® NC400 instrument (Savyon)*
- *Results were compared to standard methods used routinely by the lab, i.e. culture and microscopy*
- *Discrepant analysis was carried out by Diagenode® RT-PCR assays (Mikrogen) and/or by sequencing*

The performance parameters following the prospective study demonstrate the utility of The NanoCHIP GIP® test to be used routinely for screening purposes in the microbiology lab in the community

Objective

The aim of this work was to demonstrate the utility of the NanoCHIP® GIP test for screening of gastro-enteric bacterial and parasitic pathogens in stool specimens in a typical laboratory at community setting

The NanoCHIP® GIP Combi II Panel

Parasites	LOD (CFU/mL)	Bacteria	LOD (CFU/mL)
Giardia lamblia	5.00E+03	*Salmonella*	1.00E+05
Cryptosporidium	ND	*Shigella*	1.00E+04
E. histolytica	5.00E+03	*Campylobacter*	1.40E+04
E. dispar	5.00E+03		
D. fragilis	5.00E+03		
Blastocystis spp.	ND		

Results

Table 1. Results before and after analysis of discrepancies

	Results vs. Conventional Methods						Discrepant Analysis Results					
Pathogen	TP	FP	TN	FN	Sensitivity	Specificity	TP	FP	TN	FN	Sensitivity	Specificity
Giardia	34	2	157	3	92	99	35	1	159	1	97	99
Campylobacter	9	8	178	1	90	96	17	0	179	0	100	100
Shigella	15	16	164	1	94	91	31	0	165	0	100	100
Salmonella	3	1	190	2	60	99	4	0	192	0	100	100
Cryptosporidium	1	1	194	0	100	99	2	0	194	0	100	100
E. histolytica	3*	0	193	0	N/A	100	3	0	193	0	100	100
D. fragilis	2	33	161	0	100	83	35	0	161	0	100	100
B. hominis	3	25	168	0	100	87	28	0	168	0	100	100

* Defined by the lab as *Entamoeba coli*

According to the discrepancy analysis, the NanoCHIP® GIP detected 87 positive samples that were defined originally as negatives by the conventional methods

7 samples that were detected as negatives by the NanoCHIP® GIP Combi II were confirmed as false positive detection by the conventional methods

Table 2. Prospective study results

Pathogens	TP	FN	TN	FP	Sens	Spec	PPV	NPV
B. hominis	21	0	129	0	100%	100%	100%	100%
D. fragilis	27	0	123	0	100%	100%	100%	100%
*Salmonella**	1	0	149	0	100%	100%	100%	100%
Shigella	19	0	131	0	100%	100%	100%	100%
Campylobacter	15	0	135	0	100%	100%	100%	100%
Giardia *	5	0	145	1	100%	99%	83%	100%
*Cryptosporidium**	2	0	148	0	100%	100%	100%	100%
*E. histolytica**	2	0	148	0	100%	100%	100%	100%
E. dispar	0	0	150	0	N/A	100%	N/A	100%

	Prospective: total assay							
	TP	FN	TN	FP	Sens	Spec	PPV	NPV
Total GIP II assay	92	0	57	1	100%	98%	99%	100%

* The amount of positives is not statistically significant

Results (cont.)

Table 3 . Overall** Study Results

Pathogens	TP	FN	TN	FP	Sens	Spec	PPV	NPV
B. hominis	28	0	168	0	100%	100%	100%	100%
D. fragilis	35	0	161	0	100%	100%	100%	100%
*Salmonella**	4	0	192	0	100%	100%	100%	100%
Shigella	31	0	165	0	100%	100%	100%	100%
Campy	17	0	179	0	100%	100%	100%	100%
Giardia	35	1	159	1	97%	99%	97%	99%
*Cryptosporidium**	2	0	194	0	100%	100%	100%	100%
*E. histolytica**	3	0	193	0	100%	100%	100%	100%
E. dispar	0	0	150	0	N/A	100%	N/A	100%

	Overall assay							
	TP	FN	TN	FP	Sens	Spec	PPV	NPV
Total GIP II assay*	76	1	118	1	99%	99%	99%	99%

*The amount of positives is not statistically significant
** Overall results in retrospective and prospective samples
The rate of mixed infections: 36 out of 196 (18%) samples

Discussion & Summary

- The NanoCHIP® GIP Combi II has a higher detection yield compared to the conventional methods and the overall performance is better
- Improved detection is observed in all the pathogens composing the panel
- Proven differentiation between *histolytica* and *dispar Entamoeba* sub-types
- Reliable differentiation between *Blastocystis* / *D. fragilis* / *E. histolytica* and a higher positive rate in regard to these pathogens
- A procedure that is compatible with the laboratory needs in terms of time-to-result
- Efficient detection of mixed infections in one assay
- User-friendly, objective and clear interpretation of results without need for special skills
- Reduction in lab workload by avoiding separate procedures for bacterial and parasitic detection
- Overall, the NanoCHIP® GIP Combi II has demonstrated its utility in the community setting laboratory for reliable detection of bacterial and parasitic gastrointestinal infections and screening purposes
- The test presents significant advantages compared to currently used methods, mainly in terms of performance, minimal hands-on time, improved laboratory workflow, and potential assimilation as part of a fully automated process