

Copan new FecalSwab stabilize antigens, toxins and nucleic acids in stool specimens for the detection of Adenovirus, Rotavirus and *C. difficile*

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INTRODUCTION:

C. difficile, Rotavirus and Adenovirus are the causing agents of gastroenteritis in children and adults. Appropriate specimen collection and transport devices are essential to improve diagnosis. The FecalSwab kit (a flocked swab and a tube with 2 ml semi-liquid modified Cary Blair medium), original produced in 2008, has now been improved and is in compliance with the new CLSI M40-A guidelines for support of enteric pathogens viability. Stool samples transported in FecalSwab can be processed on the Walk Away Specimens Processor (WASP™) for bacteria culture, and can also be tested for antigens, toxins and nucleic acids detection of bacteria and viruses causing gastrointestinal infections.

OBJECTIVE:

The objective of this study was to validate the performance of new FecalSwab device for the transport of stool samples or rectal swabs for the detection of Rotavirus, Adenovirus and *C. difficile* with rapid antigens, toxins and nucleic acid with real time PCR assays.

METHODS:

Clinical stools (n=130) samples submitted to Synlab laboratory (Brescia, Italy) for the detection of Rotavirus, Adenovirus and *C. difficile* were used for this validation. The flocked swab from the FecalSwab was used to transfer the stool in the tube medium; duplicate samples were prepared from each sample.

For rapid kit antigen and toxins detection, FecalSwab samples were vortexed and 200 uL or the flocked swab were used as per kit package insert's procedure. These kits were tested:

- R-Biopharm RidaQuick Rota/Adeno comby kit.
- R-Biopharm RidaQuick *C. difficile* toxin A/B kit
- Meridian Rapid Strip Rota-Adeno kit.
- Meridian ImmunoCard Stat Rotavirus kit
- Meridian ImmunoCard Stat Adenovirus kit
- Coris Rota Adeno combistrip rapid kit

For nucleic acids detection, FecalSwab samples were vortexed and 200 uL of medium from each sample were used for nucleic acid extraction as manufacturer's procedure. Nucleic acids were extracted with:

- Qiagen QIAamp DNA Stool Mini Kit for DNA extraction from *C. difficile*
- Norgen Biotek Stool Nucleic Acid for RNA extraction from Rotavirus
- Applied Biosystem PrepSEQ Express on AutoMateExpress DNA extractor for DNA extraction from Adenovirus.

Five uL of extracted nucleic acids were tested by real time PCR as manufacturer's procedure on the ABI 7500 Real Time PCR System with the R-Biopharm RIDA®GENE Viral Stool panel II and RIDA®GENE Clostridium difficile & Toxin A/B.

RESULTS

Rapid Assays Results for viruses

Rapid Assays	Rotavirus	Adenovirus	Negative	Total
RIDA® QUICK Rotavirus/Adenovirus Combi	43	34	53	130
Coris Rotavirus and Adenovirus Combi-Strip	43	34	53	130
Meridian Rapid Strip Rota-Adeno	43	34	53	130
Meridian Immuno Card STAT!® Rotavirus	43	N/A	87	130
Meridian Immuno Card STAT!® Adenovirus	N/A	34	96	130

Rapid and Molecular Amplification Assays Results for *C. diff*

Assays	Positive	Negative	Total
RIDA® QUICK Clostridium difficile & Toxin A/B Rapid assay	30	100	130
RIDA® GENE Clostridium difficile & Toxin A/B. Amplification assay	30	100	130

Viruses Molecular Amplification Assays Results

Assays	Rotavirus		Adenovirus		Astrovirus		Total
	Pos	Neg	Pos	Neg	Pos	Neg	
RIDA® GENE Stool Viral PCR Assa	43	87	34	96	0	130	130

Sample of PCR CTs values of target and internal controls for each assay

Samples	RIDA®GENE Adenovirus 0 Time		Samples	RIDA®GENE Rotavirus 0 Time		Samples	RIDA®GENE C. Difficile & Toxin A/B.0 Time	
	Positive (CT)	Internal control (CT)		Positive (CT)	Internal control (CT)		Positive (CT)	Internal control (CT)
1	14.22	33.10	1	28.77	27.10	1	23.04	26.64
2	15.78	33.15	2	25.63	27.47	2	26.43	26.74
3	14.95	33.27	3	25.88	29.01	3	29.41	26.06
4	13.36	33.03	4	31.48	32.46	4	33.16	26.45
5	14.58	31.85	5	30.23	31.33	5	31.75	26.59
6	20.34	32.25	6	29.34	30.57	6	31.32	26.88
7	14.36	32.64	7	30.96	32.23	7	33.59	26.56
8	14.72	32.65	8	29.91	31.45	8	35.19	26.54
9	21.17	33.71	9	27.86	29.32	9	31.58	25.93
10	13.55	32.35	10	29.60	30.33	10	24.31	25.75

RESULTS:

1. Good correlation found between the original results tested by Synlab laboratory and by Copan. In the 130 stool samples tested were found 34 Adenovirus, 43 Rotaviruses, 30 *C. difficile* toxin A/B positive and 23 negative.
2. Stool samples stored in Copan FecalSwab were detected positive or negative with the following rapid assays: R-Biopharm RidaQuick Rota/Adeno comby kit, Meridian Rapid Strip Rota-Adeno kit, Meridian ImmunoCard Stat Rotavirus, Meridian ImmunoCard Stat Adenovirus kit, Coris Rota Adeno combistrip rapid kit and the RidaQuick *C. difficile* toxin A/B kit.
3. The same stool sample stored in FecalSwab, tested by real Time PCR with R-Biopharm RIDA®GENE Viral Stool panel II and RIDA®GENE Clostridium difficile & Toxin A/B confirmed the results: 34 Adenovirus positive, 43 Rotaviruses positive, 30 *C. difficile* positive and 23 negative.
4. It is important to observe that:
 - No inhibition of the amplification occurred: the pre-lysis step was essential for stool samples stored in FecalSwab for successful nucleic acids extractions
 - No interference was detected with the performance of stool samples in FecalSwab with all the rapid antigen and toxins assays.

CONCLUSIONS:

Stools samples stored and transported in Copan FecalSwab device are compatible with the R-Biopharm, Meridian, and Coris rapid antigen and toxin tests.

Equivalent results were obtained with the R-Biopharm RIDA®GENE Viral Stool panel II and Clostridium difficile & Toxin A/B Real-Time PCR assays.

The FecalSwab can be used for the collection of rectal swabs samples for the detection of Adenovirus Rotavirus antigens, *C. difficile* toxins and nucleic acids.

Stool samples can be easily transferred in the FecalSwab medium with the flocked swabs, are transported in a leak proof tube, occupy less storage space in the refrigerator and are compatible with WASP automation.