Evaluation of DNA/RNA Protect Swabs with Shigella Sonnei Spiked Stool Samples

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Abstract

Shigella sonnei causes approximately 10,000 cases of gastroenteritis in the USA yearly. It is typically linked to community outbreaks at daycare centers and schools. Diagnosis of shigellosis is often challenging due to poor viability of the organism in transportation medium. DNA/RNA Protect™ (Sierra Diagnostics Inc. Sonora, CA) is a universal sample collection system meant to protect target DNA for up to 7 days at room temperature. The aim of this study was to evaluate the applicability of DNA/RNA Protect swabs for detection and isolation of *S. sonnei* from stool samples. Stool spiked with high (10^7 CFU/g) and low (10^5 CFU/g) levels of *S. sonnei* was sampled with DNA/RNA Protect swabs which were then left for storage at room temperature. For comparison, regular swabs were prepared and stored in Cary-Blair medium at +4ºC. Two swabs from each inoculation level were tested on days 1-7, 12, 15, 20, 22, 29, 33 and 44 by PCR from direct DNA extract and by culture on MacConkey agar. To determine the sensitivity for each sampling system, stool was inoculated with final spiking levels of 10^0 to 10^5 CFU/g and tested after 5 days. At high inoculation level both swab types tested positive by direct PCR during the whole 6 week study period. At low inoculation level all DNA/RNA Protect swabs tested positive for the first 7 days, after which 80 % were positive. Regular swabs tested positive for the first 4 days, after which only 40 % were positive. At high inoculation level both swab types yielded a culture containing *S. sonnei* during the first 7 days while at low inoculation level 6 of 14 DNA/RNA Protect and 1 of 14 regular swabs were culture positive. During the following 5 weeks culture was successful with 5 of 14 high inoculation level DNA/RNA Protect and 2 of 14 regular swabs while at low inoculation level only 2 of 14 swabs of both types yielded a culture. The limit of detection by direct PCR was 10^2 and 10^5 CFU/g for DNA/RNA Protect and regular swabs, respectively, and by culture 10^5 CFU/g for both swab types. DNA/RNA Protect swabs are comparable to conventional sampling systems for obtaining a culture and offer significant benefits with improved sensitivity for PCR detection and no need for refrigerated storage.

Introduction

- *Shigella sonnei* causes approximately 10,000 cases of gastroenteritis in the USA yearly
- Infections are especially common among young children in daycare centers (1)

- Diagnosis of shigellosis is challenging due to poor viability of the organism in transportation medium
- Conventional laboratory diagnosis involves culture of specimens on moderately selective media followed by biochemical and serological assays
- Molecular detection methods based on PCR have been described

- Sampling practices and transport methods are important factors that influence the sensitivity of diagnostic tests for shigellae
- DNA/RNA Protect™ (Sierra Diagnostics Inc. Sonora, CA) is a novel universal sample collection system meant to protect target DNA for up to seven days at room temperature

The aim

- Evaluate the applicability of DNA/RNA Protect swabs for PCR-based detection and isolation of *S. sonnei* from spiked stool samples
  - Evaluate the effect of long term storage of the sample swabs
  - Determine the sensitivity of the sampling system

Materials and methods

- Long term stability study
  - Stool from a healthy donor spiked at levels 10^7 CFU/g and 10^5 CFU/g of *S. sonnei*
  - Sampling with DNA/RNA Protect swabs and polyester fiber tipped swabs (Falcon 220670, BD, Sparks, MD) stored in Cary-Blair (BD)
  - Swabs stored at:
    - DNA/RNA Protect: room temperature
    - Cary-Blair: $+4^{o}C$
  - Two swabs from each inoculation level tested on days 1-7, 12, 15, 20, 22, 29, 33 and 44

- Limit of detection (LOD) study
  - Stool from a healthy donor spiked with final spiking levels of 10^0 to 10^5 CFU/g of *S. sonnei*
  - Sampling with DNA/RNA Protect swabs and polyester swabs stored in Cary-Blair; four parallel swabs from each spiking level
  - Swabs tested after five days storage at:
    - DNA/RNA Protect: room temperature
    - Cary-Blair: +4°C
  - Definition of LOD: the lowest inoculation level in which three out of four parallel swabs (75 %) were positive for *S. sonnei*

- Testing of the swabs
  - Each swab was:

    1. cultured on MacConkey agar → a sweep from the first streaking quadrant and typical (lac neg.) colonies tested for *ipaH* gene by PCR

    2. used for either manual (PureGene® DNA Purification Kit, Gentra Systems Inc., Minneapolis, MN) or automatic (MagnaPure, Roche Diagnostics, Mannheim, Germany) DNA extraction → extracts tested for *ipaH* gene by PCR

- PCR analysis of DNA templates
  - Hybridization probe –based real-time PCR using LightCycler™ (Roche Diagnostics)
    - Primers and probes for *ipaH* (5′→3′):

EIEC-1 gTT CCT TgA CCg CCT TTC CgA
EIEC-2 gCC ggT CAg CCA CCC TCT gA
EIEC-HP1 TgC gTT TCT ATg gCg TgT Cgg-[FAM[1]]
EIEC-HP2 [Red 640[2]]-Tg ACA gCA AAT gAC CTC CgC ACT-Ph[3]
[1] Fluorescein
[2] LightCycler Red 640
[3] Phosphate

    - 20 µl reaction mixture consisted of:

2 µl of 10x reaction mix (LightCycler – FastStart DNA Master Hybridization probes; Roche Diagnostics), 0.5 µM of each primer, 0.2 µM of each probe, 3.0 mM of $MgCl_2$ and 2 µl of DNA template

    - Amplification conditions:

40 cycles of 95ºC 10 s, 50ºC 20 s and 72ºC 30 s

Results

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Day	DNA/RNA Protect		Cary-Blair	
	10^7 CFU/g[1]	10^5 CFU/g[1]	10^7 CFU/g[1]	10^5 CFU/g[1]
0	1 / 1	1 / 1	1 / 1	1 / 1
1	2 / 2	2 / 2	2 / 2	2 / 2
2	2 / 2	2 / 2	2 / 2	2 / 2
3	2 / 2	2 / 2	2 / 2	2 / 2
4	2 / 2	2 / 2	2 / 2	2 / 2
5	2 / 2	2 / 2	2 / 2	1 / 2
6	2 / 2	2 / 2	2 / 2	2 / 2
7	2 / 2	2 / 2	2 / 2	0 / 2
12	2 / 2	1 / 2	2 / 2	1 / 2
15	2 / 2	2 / 2	2 / 2	0 / 2
20	2 / 2	2 / 2	2 / 2	0 / 2
22	2 / 2	2 / 2	2 / 2	2 / 2
29	2 / 2	1 / 2	2 / 2	2 / 2
37	2 / 2	2 / 2	2 / 2	0 / 2
44	2 / 2	1 / 2	1 / 2	0 / 2

[1] Number of swabs positive / number of swabs tested

Table 1. Detection of *S. sonnei* from stool spiked at 10^7 CFU/g and 10^5 CFU/g levels of target. DNA was extracted directly from the stool swabs and tested by PCR.

- PCR directly from stool extracts:
  - At 10^7 CFU/g, both swab types tested positive during the whole 6 week study period
  - At 10^5 CFU/g level, DNA/RNA Protect swabs tested positive for the first 7 days, after which 80 % were positive
  - At 10^5 CFU/g level, Cary-Blair swabs tested positive for the first 4 days, after which only 40 % were positive

Day	DNA/RNA Protect		Cary-Blair	
	10^7 CFU/g[1]	10^5 CFU/g[1]	10^7 CFU/g[1]	10^5 CFU/g[1]
0	0 / 1	0 / 1	0 / 1	0 / 1
1	2 / 2	1 / 2	2 / 2	1 / 2
2	2 / 2	1 / 2	2 / 2	0 / 2
3	2 / 2	1 / 2	2 / 2	0 / 2
4	2 / 2	1 / 2	2 / 2	0 / 2
5	2 / 2	0 / 2	2 / 2	0 / 2
6	2 / 2	0 / 2	2 / 2	0 / 2
7	2 / 2	1 / 2	1 / 2	0 / 2
12	1 / 2	0 / 2	0 / 2	0 / 2
15	0 / 2	0 / 2	0 / 2	0 / 2
20	1 / 2	0 / 2	0 / 2	0 / 2
22	1 / 2	1 / 2	1 / 2	0 / 2
29	1 / 2	1 / 2	1 / 2	2 / 0
37	1 / 2	0 / 2	0 / 2	0 / 2
44	0 / 2	0 / 2	1 / 2	0 / 2

[1] Number of swabs positive / number of swabs tested

Table 2. Detection of *S. sonnei* from stool spiked at 10^7 CFU/g and 10^5 CFU/g levels of target. Stool swabs were cultured on MacConkey agar and overnight growth tested by PCR.

- Culture and PCR from the growth during the first seven days of storage:
  - At 10^7 CFU/g, both swab types yielded a culture containing *S. sonnei*
  - At 10^5 CFU/g, 6 of 14 DNA/RNA Protect and 1 of 14 Cary-Blair swabs were culture positive

- Culture and PCR from the growth during the storage weeks 2-6
  - Culture was successful with 5 of 14 10^7 CFU/g inoculation level DNA/RNA Protect and 2 of 14 Cary-Blair swabs
  - At 10^5 CFU/g, only 2 of 14 swabs of both types yielded a culture

Inoculation level (CFU / g)	DNA/RNA Protect		Cary-Blair	
	Direct PCR[1]	MacConkey[1]	Direct PCR[1]	MacConkey[1]
10^5	4 / 4	3 / 4	4 / 4	3 / 4
10^4	4 / 4	2 / 4	2 / 4	2 / 4
10^3	3 / 4	0 / 4	0 / 4	0 / 4
10^2	3 / 4	0 / 4	0 / 4	0 / 4
10^1	1 / 4	0 / 4	0 / 4	0 / 4
10^0	0 / 4	0 / 4	0 / 4	0 / 4

[1] Number of swabs positive / number of swabs tested

Table 3. LOD of DNA/RNA Protect and Cary-Blair sampling systems for *S. sonnei* from stool by PCR directly from stool extracts and growth on MacConkey

- Direct PCR from the swab extracts:
  - DNA/RNA Protect: 10^2 CFU/g
  - Cary-Blair: 10^5 CFU/g
- PCR from the overnight growth on MacConkey:
  - DNA/RNA Protect: 10^5 CFU/g
  - Cary-Blair: 10^5 CFU/g

- The effect of the sampling system on the PCR amplification efficiency
  - DNA extracted from the DNA/RNA Protect swabs amplified more efficiently than that extracted from Cary-Blair swabs (Fig. 1 and 2)

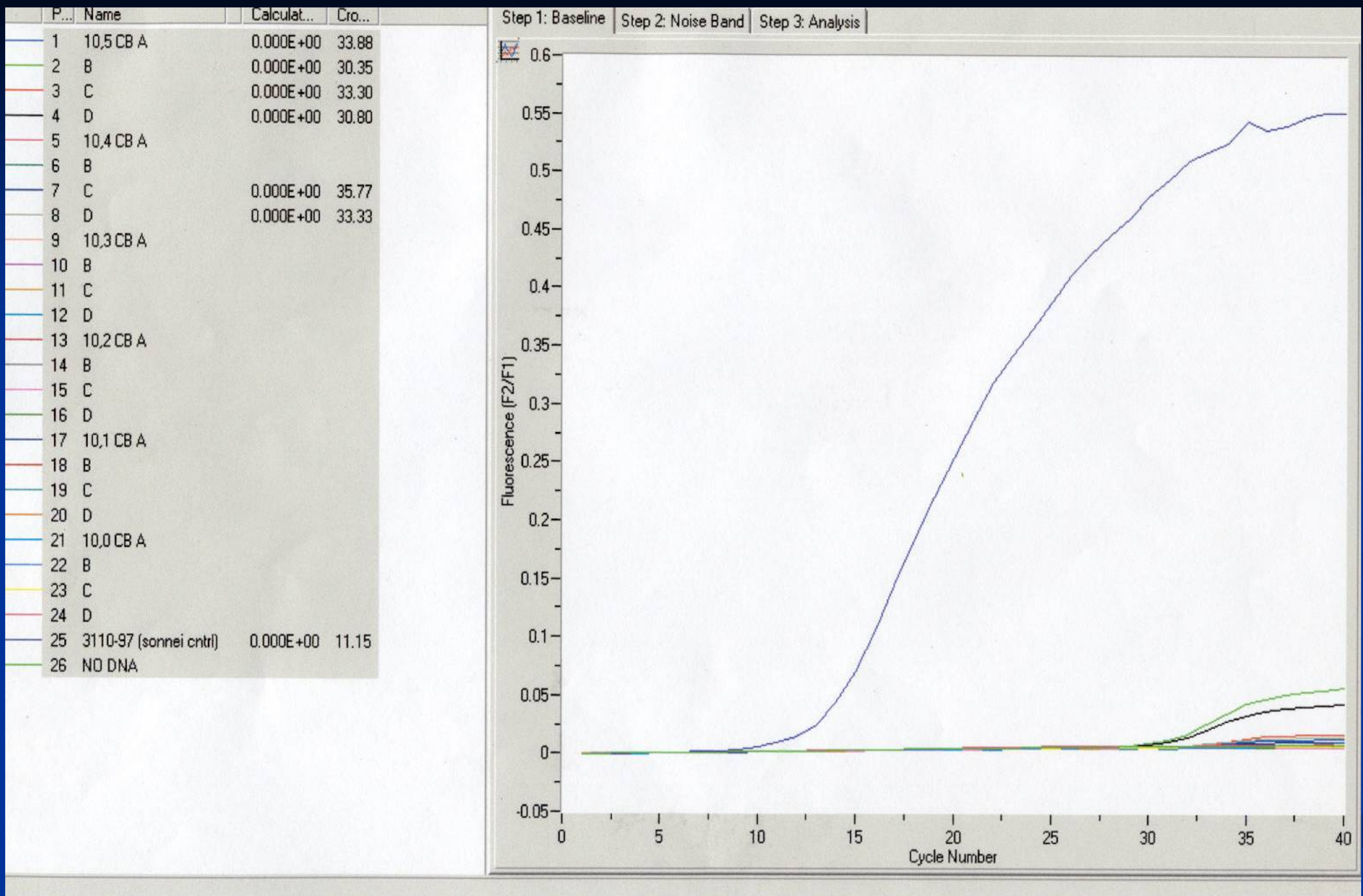

Figure 1. Fluorescence accumulation in the LightCycler *ipaH* assay when DNA extracted directly from the Cary-Blair swabs was used as a template for PCR. LOD 10^5 CFU/g for *S. sonnei* was observed.

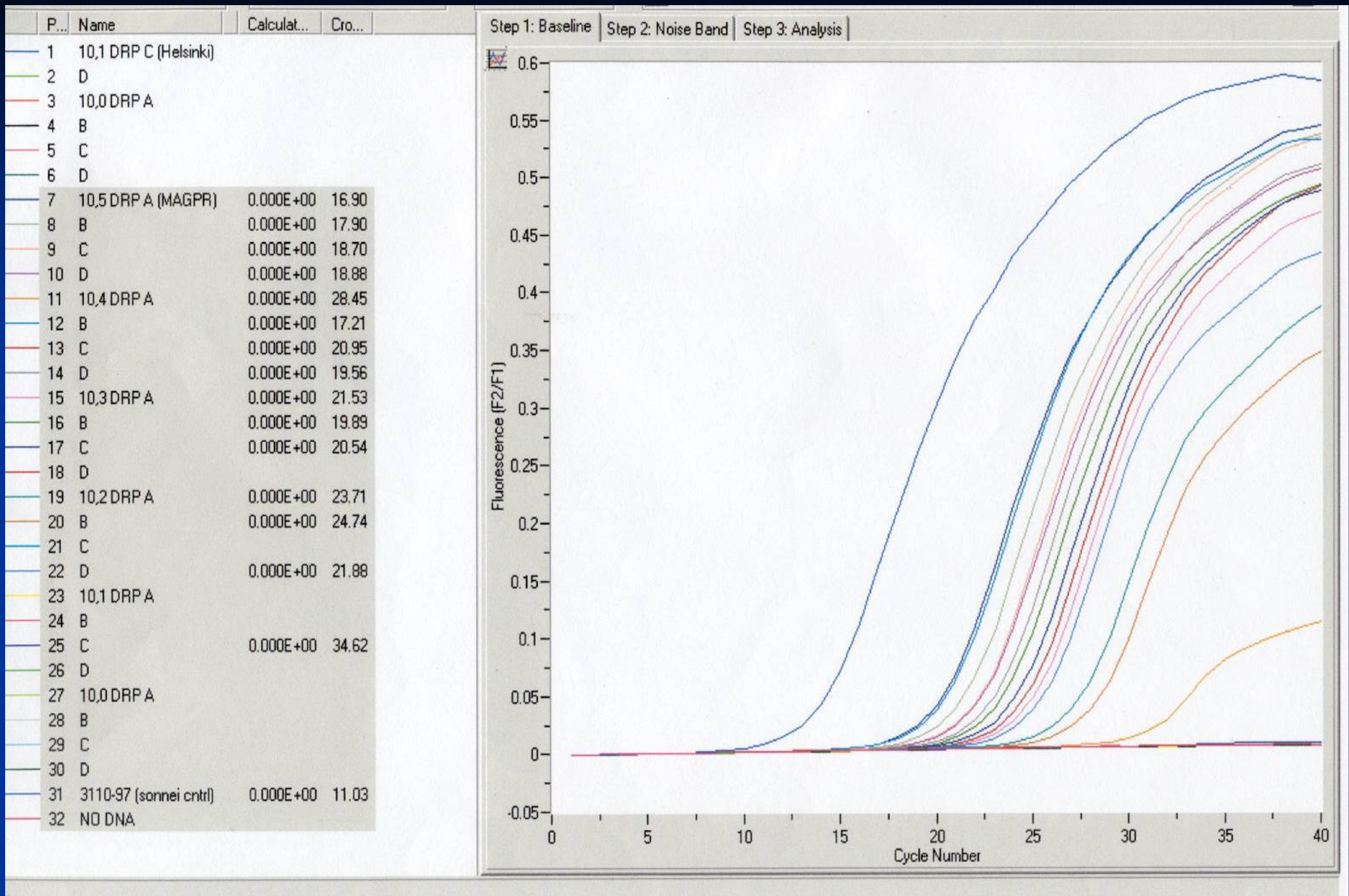

Figure 2. Fluorescence accumulation in the LightCycler *ipaH* assay when DNA extracted directly from the DNA/RNA Protect swabs was used as a template for PCR. LOD 10^2 CFU/g for *S. sonnei* was observed.

Conclusions

- DNA/RNA Protect offers significant benefits with improved sensitivity for PCR detection and no need for refrigerated storage
- The improved sensitivity of the *ipaH* PCR assay for detecting *S. sonnei* using DNA/RNA Protect swabs compared to Cary-Blair swabs may be due to their better protection and reduced dilution of target DNA

- For direct detection by PCR, DNA/RNA Protect swabs were effective at least up to 44 days

- DNA/RNA Protect is comparable to conventional sampling systems for obtaining a culture of *S. sonnei* from stool

References

1. Shane, A. L., N. A. Tucker, J. A. Crump, E. D. Mintz, and J. A. Painter. 2003. Sharing Shigella: risk factors for a multicommunity outbreak of shigellosis. Arch. Pediatr. Adolesc. Med. 157, 601-603.