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Application Note

eMcollect Sponge on PCR Detection

Detection of *Cronobacter*, *E. coli* O157, *Listeria* and *Salmonella* using eMcollect sponges, with and without neutralizers, in combination with Assurance® GDS PCR assays

ISO 18593 describes different methods for surface sampling in the food chain environment "...in order to detect and enumerate culturable microorganisms such as pathogenic or non-pathogenic bacteria or yeasts and moulds...", adding "In cases where residues of disinfectants are expected, appropriate neutralizers should be added to the diluent or media before sampling, to prevent any inhibitory effect of the disinfectants on the growth of microorganisms..." [1]

The eMcollect sponges were developed to collect environmental monitoring samples in the food and beverage industries in accordance with the ISO 17604 and ISO 18593 standards. The eMcollect product portfolio includes items with and without neutralization agents (sodium thiosulfate, lecithin, L-histidine and Tween®) in either Buffered Peptone Water (BPW) or distilled water. These neutralizers allow the subsequent detection of microorganisms even in the presence of disinfectant residues from sanitization routines on the sampling surface.[2]

The Assurance GDS system includes qPCR assays that detect specific food-borne pathogens. Assurance® GDS are PCR assays that detect specific food-borne pathogens. They use Immunomagnetic Separation (IMS) technology before the PCR step, whereby beads bind the contained target pathogens to concentrate and separate them from inhibitory background materials, a procedure that significantly improves the sensitivity of the assay.

The aim of this application note was to evaluate the impact of using eMcollect sponge sticks containing neutralization agents in their liquids on *Cronobacter*, *Escherichia coli* O157, Listeria and *Salmonella* detection by immunomagnetic separation and qPCR.

Materials

Product Name	Cat. No.
EcoCult® Buffered Peptone Water*	**1401410500**
ReadyStream® (Media bag), GranuCult® prime Buffered Peptone Water	**5.74846**
mEHEC® Broth	**103686BC**
Half FRASER broth (base) with antibiotics, GranuCult® prime	**1.00025**
FRASER *Listeria* Ammonium iron (III) Supplement	**1.00092**
Tryptic Soy Agar - Ready-to-use Settle Plates	**1.46004**
eMcollect sponge stick with BPW at 10% neutralizing agent	**A04550**
eMcollect sponge stick with BPW	**A04551**
eMcollect sponge stick with water + 10% neutralizing agents	**A04552**
Assurance® GDS Rotor-Gene® Q Thermocycler	**73070BC**
Assurance® GDS *Salmonella* Tq Kit	**71008BC**
Assurance® GDS *Cronobacter* Tq II Kit	**71038BC**
Assurance® GDS *Listeria* spp. Tq Kit	**71009BC**
Assurance® GDS *Listeria monocytogenes* Tq Kit	**71010BC**
Assurance® GDS EHEC ID for *E. coli* O157:H7 Kit	**71037BC**

Table 1: Culture media, Assurance® GDS kits [3] and thermocycler used in this application study. The validation procedures are listed in the literature paragraph. *In combination with *Salmonella* incubation.

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Microorganism	WDCM	Cat. No. Vitroids™, LENTICULE®
Salmonella enterica subsp. *enterica* NCTC 6017	WDCM 00030	**VT000306-10EA**
Listeria monocytogenes serovar 4b CECT® 935	WDCM 00021	**VT000216-10EA**
Escherichia coli O157 (NT) NCTC 12900	WDCM 00014	**CRM12900L-10EA**
Cronobacter sakazakii (Farmer et al.) Iversen et al. ATCC® 29004*	-	-

Table 2: Microorganism strains used in this study (**ATCC**© = American Type Culture Collection **www.atcc.org**; **NCTC** = National Collection of Type Cultures **www.culturecollections.org.uk**; **WDCM** = World Data Centre for Microorganisms a global microbial resources data center, to improve access to microbial data). Vitroids™ and LENTICULE® discs contain specific viable microorganisms in a certified quantity. **Cronobacter sakazakii* were used from our in-house strain collection.

Test protocol

The preparation of the test method was performed as follows: Generally, two samples per eMcollect product were processed for each test protocol. The eMcollect sticks were given into the original sampling bags.

- For each sample, the sponge of a sponge stick (with or without neutralizer) was given into an eMcollect sampling bag along with the media, then inoculated and incubated (see details below).

Before incubation, homogenization was performed. The homogenizing steps was done in a stomacher at speed level 3 for 30 seconds. The samples were incubated according to the specific strain's test method.

Incubation temperature and duration:

- *Cronobacter sakazakii* ATCC® 29004: 100 mL of prewarmed BPW were added and the sample bags incubated for 24 hours at 37 °C.
- *Escherichia coli* O157 (NT) WDCM 00014: Along with 100 mL of prewarmed mEHEC® broth, the sample bags were incubated for 20 hours at 41.5 °C.
- *Listeria monocytogenes* serovar 4b WDCM 00021: 100 mL of prewarmed half FRASER broth (with FRASER supplement) was added, and the sample bags incubated for 24 hours at 30 °C.
- *Salmonella enterica* subsp. *enterica* WDCM 00030: Along with 100 mL of prewarmed BPW the sample bags were incubated for 18 hours at 37 °C.

After the required incubation period, the sample bags were taken out of the incubator and homogenized by hand. Afterwards, sample preparation for PCR was performed according to the Assurance® GDS Kit user guide's procedure (see **Table 1** for the different Assurance® GDS Kits used). For each PCR test trial 1 mL of sample was used (except Assurance® *Cronobacter* PCR Kit: 300 µL of sample plus 700 µL of wash solution).

- For the positive controls two 100 mL samples of each medium were spiked with their corresponding microorganism suspension and incubated with the other samples in parallel. The positive controls contained no eMcollect product.
- Negative controls containing media but neither an eMcollect product nor any microbial strain suspension were also incubated.

Results

The objective of this application note study was to assess any potential inhibitory effects on the PCR reaction of using eMcollect sponge sticks (with or without neutralizer, in BPW or water).

- The negative control samples, containing neither a microorganism suspension nor an eMcollect product, yielded negative PCR detection results (not shown in tables).
- All internal controls to verify the reliability of the amplification process were positive.
- **All sample replicates of all sponge stick product variants (with or without neutralizer in BPW or water) yielded positive detection results with their corresponding Assurance® GDS kit, indicating there was no inhibition of PCR amplification in any of the tests.**

In **Tables 4** to **8** below, the amplification results are listed in more detail, also showing the inoculation levels.

Cronobacter Test Results – inoculation level average: 6 CFUs of *Cronobacter* sakazakii

Number of samples	Samples inoculated with *Cronobacter* (2 replicates per sample)	PCR result*
1	Positive sample BPW	positive
2	Sponge stick BPW without neutralizer	positive
2	Sponge stick BPW with neutralizer	positive
2	Sponge stick water with neutralizer	positive

Table 4: Assurance® GDS *Cronobacter* PCR detection results, with the two replicates per sample yielding the same results in all cases. *as reported by the software

***Escherichia coli* O157 Test Results** - inoculation level average: 1 CFU of *Escherichia coli* O157

Number of samples	Samples inoculated with *E. coli* O157 (1 replicate per sample)	PCR result*
2	Sponge stick BPW without neutralizer	positive
2	Sponge stick BPW with neutralizer	positive
1	Sponge stick water with neutralizer	positive

Table 5: Assurance® GDS EHEC ID PCR detection results, with the one replicate per sample yielding the same results in all cases. *as reported by the software

The number of PCR replicates per sample bag had to be reduced to one to account for product availability. Because of a technical issue with the stomacher bag, a positive control was not a part of the *E. coli* O157 detection test trial.

***Listeria* spp. Tq Test Results** - inoculation level average: 7 CFUs of *Listeria monocytogenes*

Number of samples	Samples inoculated with L. *monocytogenes* (2 replicates per sample)	PCR result*
1	Positive sample BPW	positive
2	Sponge stick BPW without neutralizer	positive
2	Sponge stick BPW with neutralizer	positive
2	Sponge stick water with neutralizer	positive

Table 6: Assurance® GDS *Listeria* spp. PCR detection results, with the two replicates per sample yielding the same results in all cases.
*as reported by the software

***Listeria monocytogenes* Tq Test Results** - inoculation level average: 9 CFUs of *Listeria monocytogenes*

Number of samples	Samples inoculated with *L. monocytogenes* (2 replicates per sample)	PCR result*
1	Positive sample BPW	positive
2	Sponge stick BPW without neutralizer	positive
2	Sponge stick BPW with neutralizer	positive
2	Sponge stick water with neutralizer	positive

Table 7: Assurance® GDS *Listeria monocytogenes* PCR detection results, with the two replicates per sample yielding the same results in all cases.
*as reported by the software

***Salmonella* Test Results** - inoculation level average: 35 CFUs of *Salmonella enterica* subsp. *enterica*

Number of samples	Samples inoculated with *Salmonella* enterica (2 replicates per sample)	PCR result*
2	Positive sample BPW	positive
2	Sponge stick BPW with neutralizer	positive
3	Sponge stick BPW without neutralizer	positive

Table 8: Assurance® GDS *Salmonella* Tq PCR detection results, with the two replicates per sample yielding the same results in all cases.
*as reported by the software

Conclusion

To evaluate any possible negative impact of the neutralizer ingredients contained in the liquids of eMcollect sponge sticks on PCR amplification, we ran the Assurance® GDS *Cronobacter* Tq II Kit, the Assurance® GDS EHEC ID for *E. coli* O157:H7 Kit, the Assurance® GDS *Listeria* spp. Tq Kit, the Assurance® GDS *Listeria monocytogenes* Tq Kit and the Assurance® GDS *Salmonella* Tq Kit for environmental sampling on samples that had been spiked with the organisms these kits are designed to detect, and containing eMcollect sticks with and without neutralizer in either BPW or distilled water.

The internal controls of all samples including positive and negative samples were amplified. They verified the reliability of the PCR reaction.

All inoculated samples containing an eMcollect sponge stick yielded positive PCR results, irrespective of whether their liquid contained neutralizer or not.

The results demonstrate that all the eMcollect sponge stick sampling products used are compatible with all the above-mentioned Assurance® GDS kits, and that the eMcollect sponge sticks (with and without neutralizers) can thus be used in combination with PCR GDS detection for sampling and detection in food production and processing facilities.

Literature

1. ISO 18593 Microbiology of the food chain — Horizontal methods for surface sampling
2. eMcollect Swab cloths and sponges for collecting environmental monitoring samples in accordance with ISO 17604 and ISO 18593 standards-MK_FL14024EN 58769 09/2024
   - eMcollect Product Data sheet Reference A04550-DS-WW (sponge stick, BPW at 10% neutralizing agent in bag with closure label)
   - eMcollect Product Data sheet Reference A04551-DS-WW (sponge stick, BPW in bag with closure label)
   - eMcollect Product Data sheet Reference A04533-DS-WW (cloth swab, water at 10% neutralizing agent in bag with closure label)
   - eMcollect Product Data sheet Reference A04531-DS-WW (cloth swab, BPW in bag with closure label)
3. Assurance® GDS validation procedure:
   - Assurance® GDS *Cronobacter* Tq II Kit: Qualitative methods–Assurance GDS for *Cronobacter* TqII extension study Ref 2017 LR77 MCS report v1 02/03/23
   - Assurance® GDS EHEC ID for *E. coli* O157:H7 Kit: MicroVal Study 2015LR49 with the title "Qualitative Method, Renewal Validation of the Assurance® GDS for *E. coli* O157:H7 Tq".
   - Assurance® GDS *Listeria* Tq: MicroVal Study 2010LR31: Qualitative Method Matrix Extension and Renewal Study Report for the ISO 16140–2:2016 Validation of the Assurance® GDS Listeria species Tq for the Detection of *Listeria* species in a Broad Range of Foods and Environmental Surfaces
   - Assurance® GDS *Listeria monocytogenes* Tq Kit: following the MicroVal Study 2014LR32.
   - Assurance® GDS *Salmonella* Tq Kit: NF validation certificate TRA 02/12–01/09

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