A Novel Lysis Method for the Purification (Gram +) and (Gram –) Bacterial gDNA from Food Samples

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1. Introduction

Pathogen detection from raw and processed food samples requires specialized testing to identify and quantitate the presence of harmful bacteria. Testing is performed by a variety of organizations including contract food testing labs, food manufacturers, academic researchers, and consumer protection agency labs.

Most of the contract food testing labs test for pathogens using direct amplification solutions whereas academic researchers and government agencies preferred qPCR based methods that start with purified bacterial gDNA.

Direct amplification methods do reduce some of the pre-processing steps however, we have learned that in many cases the presence of sugars, fats, and oils, inhibit direct amplification assays.

Here we present two Maxwell RSC protocols (Method 1 and Method 2) designed for the efficient purification of Gram - and Gram + bacterial amplifiable DNA from raw and processed food samples.

The goal was to have customers evaluate and compare the two methods and provide performance and preference feedback.

Here we present both customer evaluation data and Promega testing data demonstrating a preference for Method 1.

2. Reagents and Instrument

The PureFood Pathogen kit (Catalog # AS1660) includes:

- 15ml Lysis Buffer A
- 25ml Lysis Buffer B
- 48 Maxwell® RSC Cartridges (RSCJ)
- 50 Maxwell Plungers
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer

Figure 1. Maxwell RSC and Quantus

Instrument:
Maxwell® RSC Instrument, (Cat. #AS4500)

- Purifies 1 to 16 samples of DNA or RNA in less than 40 minutes.
- Controlled by software user interface on a Windows 8 Surface Pro tablet
- Integrated quantitation with Quantus™ Fluorometer

3. PureFood Pathogen Prototype Protocols

During development collaborating customers were provided early access kits along with two protocol versions to evaluate.

Both protocols were summarily designed to:

- Disrupt the cell walls of Gram + bacteria and the cell envelopes of Gram – bacteria without the need for multiple protocols or separate reagents; *Salmonella, E. coli,* and *Listeria*
- Minimize the carry-over of potential PCR inhibitors from the food matrices, i.e. fats and oils

The two methods evaluated differed by sample input volume and the addition of a centrifugation step:

Method 1: **800 µl** of media/food sample input volume
Method 2: **1000 µl** of media/food sample input volume and a centrifugation step

Method 1 - Current PureFood Pathogen Prototype Protocol

1. Add **800ul** of Media/food sample to 1.5ml tube
2. Add **200ul** of **Lysis Buffer A**-vortex to mix
3. Incubate at **56°C, 4-5min** on Thermomixer shaking at 1000-1200 rpm
4. Add **300ul** of **Lysis Buffer B** -vortex to mix
5. Add to well-1 of Maxwell FFS cartridge
6. Elute with **50ul** of Elution Buffer

Method 2 - Alternative Method Tested

1. *Add **1ml** of Media/food sample to 1.5ml tube*
2. *Centrifuge 1ml of culture **10,000** **x g*** in microfuge**, 5min** to pellet (remove supernatants via pipet)
3. Add **200ul** of **Lysis Buffer A**-vortex/and or pipet to mix
4. Incubate at **56°C, 3-4min** on Thermomixer shaking at 1000-1200 rpm
5. Add **300ul** of **Lysis Buffer B**-vortex to mix
6. Add to well-1 of Maxwell FFS cartridge
7. Elute with **50ul** of Elution Buffer

3. Sample Enrichment

Standard Enrichment Method

Pre-enrichment:

- Place **25g** food sample into a Seward Stomacher® enrichment bag
- Add **225ml** of pre-warmed buffer (**Buffered Peptone Water** or **½ Fraser**)
- Add select amount of **bacteria CFUs** to **Stomacher** bags
- Seal the bag and homogenize using a Stomacher device
- Incubate at **37°C** for **24 hours**
- Process **1ml** of sample for comparisons

In-house Bacteria Prep:

- ➢ Bacteria samples are grown as 50ml overnight broth cultures
- ➢ Serial dilutions are performed
- ➢ Added 0.1ml of selected dilutions to Stomacher bag
- ➢ Selected dilutions are plated on media to determine approximate CFUs added to Stomacher sample

5. Analysis and Results

Comparison of Promega's Direct lysis and Pellet methods for the purification and identification of Listeria innocua spiked into ground beef samples at selected CFU (Figure 2).

Figure 2. qPCR Amplification of *Listeria innocua* (lin02483): 55 and 118 CFU/ground beef

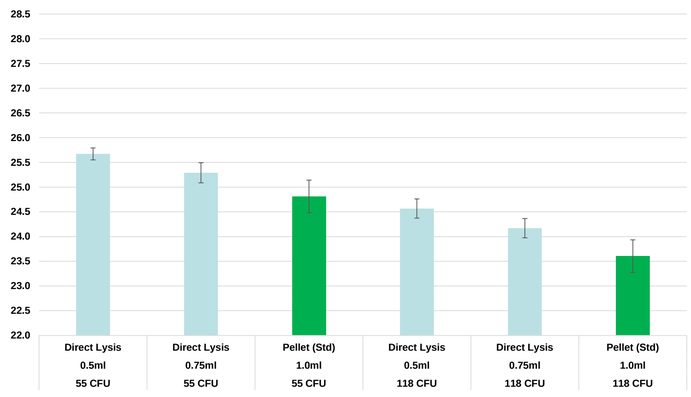


Table 1. Customer Data from Alpha Testing

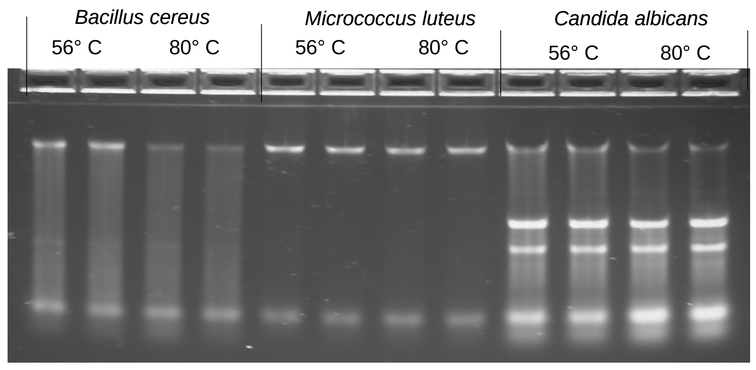
S. enteritidis				*E. coli JM101*			
Sample ID	Cq	Calculated [DNA]	DNA Copies	Sample ID	Cq	Calculated [DNA]	DNA Copies
SOP 10^-1	16.14	235.91	44971053.95	SOP 10^-1	16.51	180.17	34346129.00
SOP 10^-2	21.14	6.18	1177891.01	SOP 10^-2	20.48	9.99	1905050.08
SOP 10^-3	24.64	0.48265	92007.99	SOP 10^-3	25.70	0.22299	42508.72
SOP 10^-4	28.04	0.04055	7730.05	SOP 10^-4	28.71	0.02489	4744.80
SOP 10^-5	31.52	0.00321	612.68	SOP 10^-5	33.21*	0.00094	178.88
SOP 10^-6	35.25*	0.00021	40.47	SOP 10^-6	34.57*	0.00035	66.42
SOP 10^-7	-	-	-	SOP 10^-7	35.55*	0.00017	32.53
Method 1 10^-1	17.23	106.64	20327996.18	Method 1 10^-1	19.08	27.71	5282241.16
Method 1 10^-2	20.30	11.39	2171961.90	Method 1 10^-2	22.53	2.24	427914.43
Method 1 10^-3	24.40	0.57486	109585.55	Method 1 10^-3	26.19	0.15605	29748.07
Method 1 10^-4	28.01	0.04145	7900.84	Method 1 10^-4	30.08	0.00917	1749.03
Method 1 10^-5	32.33	0.00178	339.61	Method 1 10^-5	32.93*	0.00115	219.36
Method 1 10^-6	33.43*	0.00080	152.40	Method 1 10^-6	33.08*	0.00103	196.65
Method 1 10^-7	36.09*	-	0.00	Method 1 10^-7	36.83*	0.00007	12.80
Method 2 10^-1	17.92	64.51	12297088.88	Method 2 10^-1	20.11	13.08	2494374.54
Method 2 10^-2	20.94	7.15	1362630.87	Method 2 10^-2	23.97	0.78632	149896.15
Method 2 10^-3	25.98	0.18185	34665.35	Method 2 10^-3	27.09	0.08101	15442.93
Method 2 10^-4	28.99	0.02030	3869.32	Method 2 10^-4	30.09	0.00911	1736.33
Method 2 10^-5	33.00	0.00109	208.45	Method 2 10^-5	34.23*	0.00045	85.09
Method 2 10^-6	34.18*	0.00046	88.25	Method 2 10^-6	37.68*	0.00004	6.89
Method 2 10^-7	-	-	0.00	Method 2 10^-7	37.99*	0.00003	5.50
* under LOQ							

DNA concentration was determined using qPCR assay and DNA concentration and DNA copy numbers were calculated based on Cq values.

Based on the data presented (*Table 1)* the customers preference was for Method 1. Due to DNA concentration and DNA copy numbers as well as the simplification by removing that centrifugation step as compared to Method 2.

Method 1 was also tested for the ability to purify gDNA from more challenging species of bacteria and fungal species (*Figure 3*).

Figure 3. Lysis Temp Comparisons-Direct Lysis Method



Method will lyse samples at 56°C in the purification protocol

7. Competitive Benchmarking

Benchmarking was conducted comparing the Promega PureFood Pathogen kit to CONGEN SureFast Prep and Qiagen *mericon* kits. The PureFood Pathogen kit samples were processed using Method 1 and all other samples were processed using the manufactures recommended protocols.

Enrichment Method

- Select inoculums of Listeria innocua placed in Stomacher bag + 225ml of select media
- Sample incubated at 37°C, 22hrs
- 1ml aliquots processed as described per method

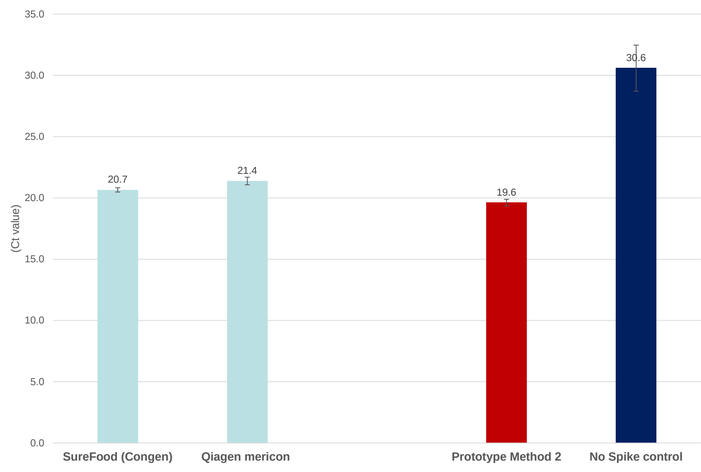
Competitor Methods

- Qiagen mericon DNA Bacteria Kit
- CONGEN SureFast Prep Bacteria Kit

No spike controls
PCR reactions were run under the same conditions except that Listeria was not added. N= 4 for all runs. Prototype is the Promega alpha test kit.

Prototype Method Comparison on Whole Milk

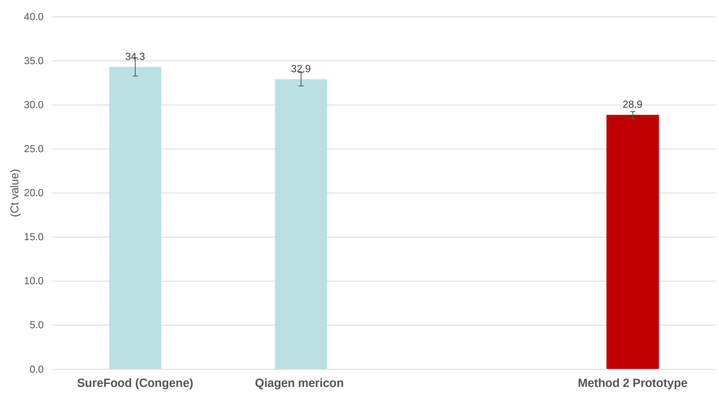
Figure 4. qPCR amplification of Listeria innocua (lin02483 target): 34 CFU/Whole Milk



Samples spiked with Listeria innocua at 34 CFU per inoculum

Prototype Method comparisons using ground beef

Figure 5. qPCR amplification of Listeria innocua (lin02483): 19 CFU CFU/Ground Beef



Listeria innocua samples at 19 CFU inoculums

9. Conclusion

We have developed a magnetic particle-based method for the efficient purification of Gram - and Gram + bacterial amplifiable DNA from raw and processed food samples.

- Internal testing and customer alpha testing demonstrated a preference for Method 1 which is now the *Current PureFood Pathogen Prototype Protocol*
- The system can process 1 to 16 on the Maxwell® RSC.
- The chemistry gives equivalent to superior yields to silica-based system methods as determined by qPCR.
- The chemistry is also robust to handle fungal samples and challenging Gram + such as *Micrococcus luteus.*
- This chemistry will continue through development process with a target release of September 2017.