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Evaluation of STEC detection system - bioMérieux VIDAS®/GENE-UP® Top7 STEC Detection System

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Abstract

Introduction: Testing of beef products for the presence of Shiga toxin-producing *E. coli* (STEC) typically relies on detecting genes that encode Shiga toxin (*stx*), intimin (*eae*) and specific O-antigens. STEC test systems that incorporate additional gene targets or utilise an STEC concentration procedure may assist in decreasing the number of potential positives (PPs) that require culture confirmation.

***Purpose*:** Evaluate the performance of bioMérieux GENE-UP® in combination with VIDAS® STEC Top7 on Australian manufacturing beef enrichment broths.

Methods: One hundred manufacturing beef enrichment broths previously identified as PP for a Top7 STEC serogroup were included in the study. Seventeen of the 100 PP manufacturing beef broths were confirmed positive for O157 (9), O26 (6), O111 (1) or O103 (1) using a DAWR approved confirmation method. Each broth was subsequently tested using the bioMérieux GENE-UP® STEC Top7 system in combination with VIDAS® which involved PCR analysis of the VIDAS® ESPT1 eluate for *stx*, *eae*, O157 and Big6. For comparison, all samples were screened for Top7 STEC using an in-house PCR approach utilising previously published FSIS primers and probes for Big6 STEC and an O157 specific PCR.

Results: A total of 28 PPs were identified using the bioMérieux screening test method (ESPT1/GENE-UP®). In comparison 77 PPs were identified using the in-house PCR approach. The cohort of 28 PPs identified using the bioMérieux screening test method included 16/17 (94%) confirmed STEC samples. One sample (BM52) was not determined to be PP using the bioMérieux screening test method due to a low concentration of *eae* (1.50 $\log_{10}$ gene copies/mL) following ESPT1 preparation. In comparison, the in-house PCR approach identified all 17 confirmed samples.

Significance: In Australia, the ratio of confirmed STEC positive samples to PPs remains low. In this study the bioMérieux GENE-UP®/ VIDAS® STEC Top7 test system utilized an STEC concentration step to reduce the number of PPs requiring culture confirmation by 49% whilst identifying 94% of confirmed STEC samples. Such reductions could provide economic benefits to Australian beef processors.

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1 Background

Australian beef exporters wishing to send raw non-intact beef products to the USA must meet FSIS regulations around specific serotypes of Shiga-toxigenic *E. coli* (STEC). STEC detection systems such as BAX® and GDS®, test for the genetic targets, *stx*, *eae* and O type using gene-based methods. Despite this advanced approach, some manufacturing beef samples may be potentially positive (PP) for STEC (from a screening test) without containing a culturable STEC organism. In this scenario, beef exporters face unwanted distribution delays and economic impacts. Novel approaches to STEC detection may incorporate additional genetic targets that enhance the specificity of the detection system and subsequently reduce the impact on beef producers. bioMérieux, in partnership with Meat & Livestock Australia Donor Company (MDC), contracted CSIRO to conduct a study to analyse the performance of the bioMérieux VIDAS®/GENE-UP® Top7 STEC detection method on enriched manufacturing beef samples from Australian beef manufacturers.

2 Project objectives

Evaluate the performance of the bioMérieux VIDAS®/GENE-UP® Top7 STEC detection method on manufacturing beef enrichment broths that have previously been identified as PP using either the BAX® or GDS® test systems.

3 Methodology

3.1 Initial STEC screening

The majority of STEC screening in Australia is presently conducted using either the BAX® System Real-Time PCR STEC Suite (Hygiena) or the Assurance GDS® MPX Top7 STEC (BioControl). Enrichment and screening of manufacturing beef enrichment broths for STEC was conducted as described at http://www.agriculture.gov.au/SiteCollectionDocuments/biosecurity/export/meat/elmer-3/approved-methods-microbiological-testing.pdf. Briefly, BAX® samples were generated by

enriching 375 g of manufacturing beef in 1.5 L of pre-warmed (45-46°C) MP enrichment broth (Hygiena) at 39-42°C for 12-24 h. GDS® samples were generated by enriching 375 g of manufacturing beef in 1.5 L of pre-warmed (42°C) mEHEC medium (BioControl) at 42°C for a minimum of 10 hours. Enrichment and screening can be conducted at on plant laboratories (abattoir) or at centralised commercial testing laboratories. All samples included in this study were initially screened and determined to be PP for Top7 STEC using either the BAX® or GDS® test systems.

3.2 Sample collection

A total of 100 manufacturing beef enrichment broths collected between July and October 2018 were included in the study. Samples were included in the study if they were deemed potentially positive (PP) for any of the Top7 STEC of serogroups O26, O45, O103, O111, O121, O145 or O157 and had been tested using a Department of Agriculture and Water Resources (DAWR) approved STEC confirmation process. De-identified aliquots (~25 mL) of manufacturing beef enrichment broths were provided to CSIRO on a weekly basis for the purposes of conducting the bioMérieux VIDAS®/GENE-UP® Top7 STEC detection method. Details of which samples had confirmed (i.e. an STEC had been isolated) and the serogroup to which the isolate belonged were provided to CSIRO.

3.3 Additional screening

3.3.1 In-house PCR screening

DNA preparations of all 100 PP manufacturing enrichment broths were prepared using the DNeasy Blood and Tissue Kit (Qiagen). All 100 DNA preparations were screened by PCR for Big6 or O157 STEC using the primers and probes outlined in the FSIS Microbiology Laboratory Guidebook (Method Number 5B.05, Appendix 1.01; https://www.fsis.usda.gov/wps/wcm/connect/0330211c-81ab-4e97-a9f3-d425f5759ee1/MLG_5B_Appendix_1_01.pdf?MOD=AJPERES) or Perelle *et al*. (2004) [1], respectively.

3.3.2 bioMérieux VIDAS® / GENE-UP® Top7 STEC Suite

Testing of the 100 manufacturing beef enrichment broths was conducted at CSIRO using preparations prepared by VIDAS® according to the product insert (PI) (VIDAS UP E. coli Serogroups (ESPT) REF 302299308212 C - en - 2015/01) and run on a GENE-UP® thermal cycler with testing performed as per the product insert for use of the EH1, EH2 and ECO PCR kit (material no: 414153 (PI: GENE-UP STEC – *stx* & *eae*; 43-04327 A – en – 2016-03), 414154 (PI: GENE-UP STEC – Top 6; 43-04329 A – en – 2016-03) and 414152 (PI: GENE-UP E. coli O157:H7; 43-04321 D – en – 2017-05) respectively). Briefly, 800 µl of each manufacturing beef enrichment broth was immuno-concentrated on VIDAS® using the ESPT1 protocol (material no.: 30229). The resulting DNA was screened by PCR for *stx* and *eae* using the EH1 PCR kit, O157 using the ECO PCR Kit and Big6 using the EH2 PCR Kit. If the result was positive for *stx*/*eae* and O157 or *stx*/*eae* and Big6 the sample would be considered potentially positive. Samples that had previously been confirmed as positive for a Top7 STEC by the DAWR confirmation process were subsequently tested using the ESPT2 protocol (material no.:30299). If PP for Big6 then 30µl of the resulting concentrate was plated onto supplemented CHROMID® EHEC agar (material no.: 413697) and CHROMID® coli Agar (material no.: 42017). Alternatively, if PP for O157 then 30µl was plated onto both CHROMID® Agar and SMAC-CT. All plates were incubated at 37°C for 18-24 h. Following incubation, part of each typical colony was re-suspended in 200µl of water, lysed for 4-6min at 90-100°C and further diluted 1/100 in water. The lysate was subsequently analysed using the EH1, ECO and EH2 PCR assay kits on the GENE-UP® thermocycler.

3.3.3 Quantitative PCR

All PCR-based test systems will have a limit of detection below which a negative result will be obtained. In general, PCR has a theoretical limit of detection of 4.00 $\log_{10}$ copies/mL and consequently enrichment broths containing target genes at or below this concentration are a challenge for test systems. Test systems may vary in their PCR efficiency and specificity which may affect the ability to identify some gene targets which may ultimately influence the outcome of testing. The concentration of *eae* and O-antigen genes was determined using the primer and probes used for in-house PCR screening (see above). Standard curves were prepared from CSIRO

culture collection isolates and obtained by serially diluting boiled cell lysates (10 min at 100°C) of each isolate. Standard curves were generated using duplicate CT measurements for all dilutions.

4 Results and Discussion

4.1 Sample summary

Of the 100 PP samples included in the study, 48 were tested at beef processing plant laboratories and 52 were tested at a commercial laboratory. Of the commercially tested samples, 25 PPs were generated using BAX® and 27 by GDS®. An equivalent breakdown of test system usage was not available for samples screened at beef processing plants. A total of 17 manufacturing beef enrichment broths were confirmed for STEC at a commercial laboratory using a DAWR approved confirmation method with nine samples confirming for O157, six samples for O26, one sample for O103 and one sample for O111.

4.2 Detection of potential positives

A total of 28 (28%) of 100 samples were determined to be PP using the VIDAS®/GENE-UP® STEC Top7 detection method. By comparison, in-house PCR screening of the samples classified 77 (77%) of the 100 samples as potential positives. When the in-house PCR screening is compared to the bioMérieux GENE-UP® STEC Top7 results the bioMérieux system results in a reduction of 49% of potential positives requiring cultural confirmation.

4.3 Detection of confirmed samples

The bioMérieux screening method (ESPT1/GENE-UP®) detected 16/17 (94%) of the confirmed samples and the in-house PCR screening method detected 17/17 (100%) of the confirmed samples. Attempts were made to isolate STEC using the ESPT2 protocol from the 17 confirmed (DAWR method) manufacturing beef enrichment broths. All 17 STEC were recovered using the ESPT2 protocol and included the isolation of an O26 STEC from sample BM52 which was not

identified as PP by the bioMérieux screening method. Comparative quantitative analysis of *eae* and O26 concentrations in BM52 indicated that the original sample contained 4.85 and 5.06 $\log_{10}$ gene copies/mL of *eae* and O26, respectively. Importantly, analysis of the ESPT1 DNA preparation for BM52 determined that it contained 1.50 and 3.19 $\log_{10}$ gene copies/mL of *eae* and O26, respectively. BM52 was not considered a PP by the bioMérieux screening method as *eae* was not detected in this sample, presumably due to the very low concentration of *eae* following ESPT1 preparation. The low concentration of *eae* in BM52 was overcome during the application of ESPT2 and the culture confirmation process. It is also noted that the enrichment broths used in this study are different from the broth used during the validation of the bioMérieux screening method and BM52 may have tested positive had the sample been enriched in the recommended broth.

4.4 Concentration of *eae* and O-antigen genes

The mean concentration of *eae* and O-antigen genes for all samples, GENE-UP® PPs, and in-house PCR PPs are shown in Table 1. Quantitative data can be useful for understanding the relative performance of STEC test systems. Although the concentrations of *eae* and O26 in PPs generated using the bioMérieux screening method are elevated compared to the overall or in-house PCR data, in general there appears to be minimal differences between the performance of GENE-UP® and in-house PCR's.

Table 1: Mean ($\log_{10}$ gene copies / mL) concentration of *eae* and O-antigen genes for all samples, GENE-UP® PPs, and in-house PCR PPs

	O26	**O111**	**O45**	**O121**	**O103**	**O145**	**O157**	***eae***
Overall [1]	5.38*	5.42	3.66	4.58	5.03	5.27	3.82	6.15
GENE-UP® PPs [2]	6.22	5.48	3.70	5.03	5.37	ND#	4.14	6.78
In-house PCR PPs [3]	5.38	5.42	3.67	4.57	5.04	5.27	3.84	6.29

* All counts are $\log_{10}$ gene copies / mL; ND – not detected

[1] in-house PCR preparations containing the gene of interest (regardless of PP status).

[2]In-house PCR preparations containing the gene (only includes samples deemed PP by GENE-UP®)

[3]In-house PCR preparations containing the gene (only includes samples deemed PP by in-house PCR)

5 Conclusions/Recommendations

- The use of the VIDAS®/GENE-UP® STEC Top7 test method resulted in a reduction of 49% of potential positives requiring cultural confirmation when compared to the in-house screening PCR results.

- The results of this study indicate that they may be correlated with a previous study (see https://www.mla.com.au/research-and-development/search-rd-reports/final-report-details/Product-Integrity/Molecular-characterisation-of-bacteria-for-continued-market-access/3473) which identified 82% of 100 samples as PP using the in-house PCR method. The bioMérieux screening method utilised in the current study concluded that 28% of samples were PP with this subset containing 16/17 (94%) of confirmed positives. These data compare favourably with the screening systems assessed previously.

- STEC test methods that utilise additional gene targets and/or an STEC concentration step reduce the number of potential positives requiring culture confirmation. The VIDAS® component of the GENE-UP® STEC Top7 test method uses a phage capture technique as an STEC concentration step which assists in reducing the number of potential positives that Australian beef processors send for confirmation. Such reductions could provide economic benefits to Australian beef processors.

- ESPT2 with CHROMID®EHEC agar, CHROMID® coli Agar, and SMAC-CT Agar was successfully used to isolate Top7 STEC from all samples previously confirmed using a DAWR approved confirmation method. Further studies comparing the effectiveness of the ESPT2-based confirmation method with the DAWR approved confirmation should be considered.

6 References

1. Perelle S, Dilasser F, Grout J, Fach P. Detection by 5'-nuclease PCR of Shiga-toxin producing *Escherichia coli* O26, O55, O91, O103, O111, O113, O145 and O157:H7, associated with the world's most frequent clinical cases. Mol Cell Probes. 2004;18(3):185-92. PubMed PMID: 15135453.