Evaluation of a commercial assay for the detection of diarrhoeagenic *Escherichia coli* (DEC) in faecal specimens

UK Health Security Agency

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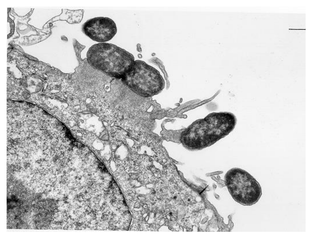
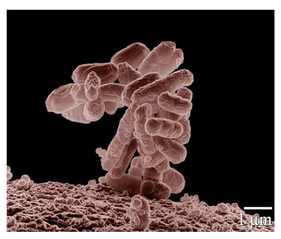
INTRODUCTION

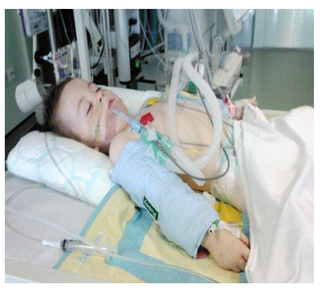
There are five pathotypes of diarrhoeagenic *Escherichia coli* (DEC) that cause gastrointestinal infections:

- Shiga toxin-producing *E. coli* (STEC)
- Enteropathogenic *E. coli* (EPEC)
- Enteroinvasive *E. coli* (EIEC)
- Enterotoxigenic *E. coli* (ETEC)
- Enteroaggregative *E.coli* (EAEC)





- Patients' faecal specimens are referred to the Gastrointestinal Bacteria Reference Unit (GBRU) at UKHSA for specialist PCR testing as this test is not widely available in front-line hospital laboratories.
- The public health and clinical significance of the different pathotypes of DEC is variable.
- STEC and EIEC causes severe symptoms, including dysentery, fever and vomiting, STEC can cause Haemolytic Uraemic Syndrome (HUS), a systemic condition that affects the kidneys and can be fatal.
- EPEC, EAEC, ETEC cause persistent, watery diarrhoea and abdominal pain, and these organisms are often the cause of traveller's diarrhoea.



Amplidiag Bacterial GE is a novel multiplex real-time PCR kit designed to detect the most common and important bacteria causing gastroenteritis.

- The current in-house PCR assays used in GBRU are not CE-IVD marked assays.
- The new *in vitro* diagnostic regulation (IVDR) monitors the manufacturing of industrial diagnostic assays focusing on the clinical validity and requires IVD assays to be CE marked.
- The Amplidiag Bacterial GE assay is a potential alternative to the current in-house assays.

The aim of the project is to evaluate the CE-IVD marked assay Amplidiag Bacterial GE and compare to the in-house PCR assay currently in use.

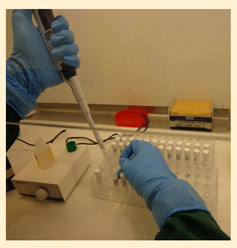
METHODS

We tested 233 faecal specimens referred to the GBRU for specialised PCR testing.

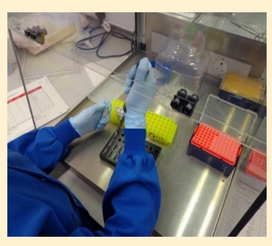
- Two types of real-time PCR instrument machines were used for the comparison in this project. Rotor-Gene Q (**Figure 1**) and Amplidiag Bacterial GE (**Figure 2**).

PCR

Figure 1. QIAGEN's real-time PCR cycler - Rotor-Gene Q.

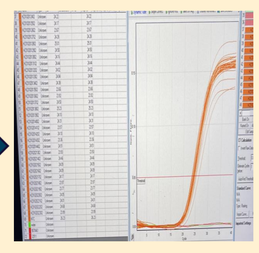


1. Instagene clinical extraction.



2. Boilates are only removed from CL3 to CL2 once they have been heat inactivated. After that, it will be processed at the CL2 PCR bench.

3. Microtubes loaded in a Rotor-Gene rotor and locking ring.



4. Takyon software used to analyse PCR results.

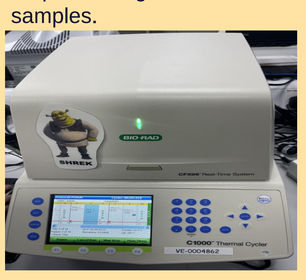
Figure 2. Mobidiag - Amplidiag Bacterial GE.

1. Nucleic acid extraction from faeces in eNAT tubes to the stage where the nucleic acid is ready for a PCR assay.

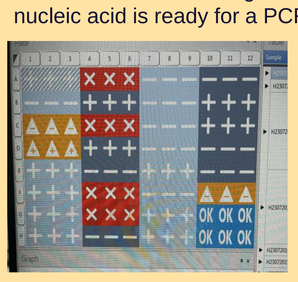
2. Amplidiag Easy Platform for processing *E. coli f*aeces samples.

3. The instrument also sets up the PCR plate, so that it can be immediately transferred to a thermal cycler.

4. Place the PCR plate in the centrifuge for 10 seconds.



5. Loaded PCR plate into a real-time PCR instrument using the Amplidiag PCR Manager software.



6. Amplidiag Analyzer used to analyse PCR results.

RESULTS

Amplidiag Bacterial GE consistent with the gold standard results.

- 233 routine clinical stool samples have been evaluated to date; 191 (82%) samples had concordant results by Amplidiag Bacterial GE compared to the in-house gold standard
- From the concordant results of 191 samples, 127 were negative for all pathogenic target genes for both methods
- In accordance with the two methods, 64 (60.4%) samples were positive

Discrepancies

- Of the 233 samples, there were 42 (18%) discrepant results
- 21 were Amplidiag positive/Instagene negative
- 4 were Instagene positive/Amplidiag negative
- Amplidiag and Instagene both detected 17 samples as positive, but the two methods detected different genes

Discrepancies – Target genes

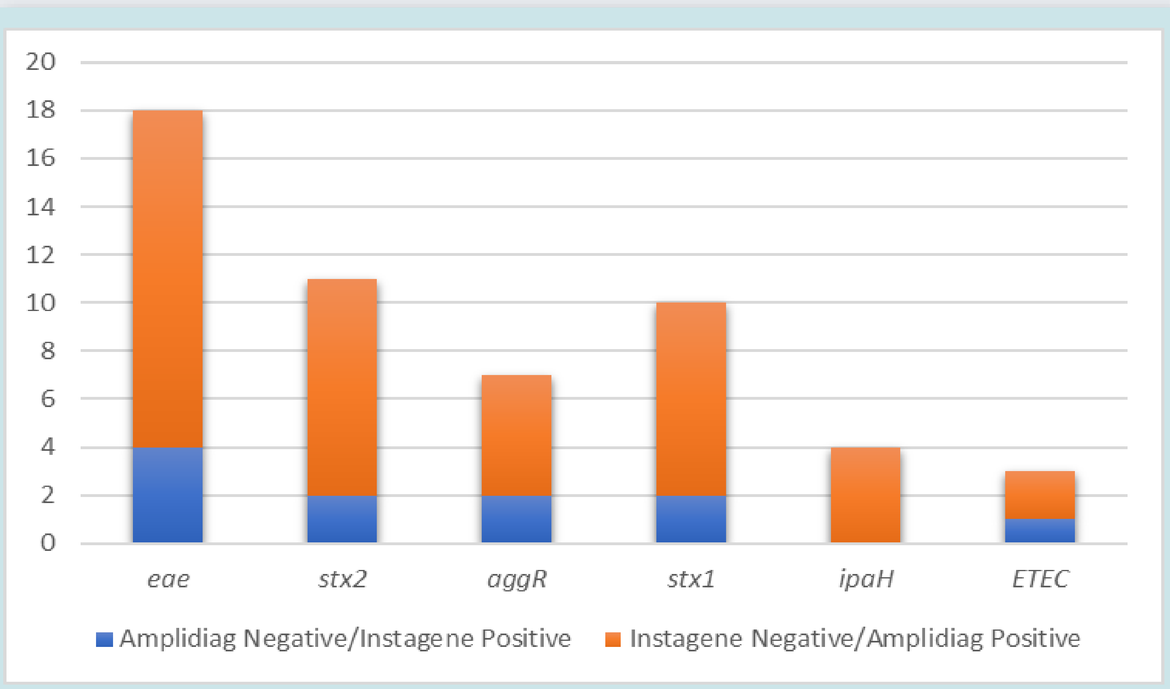


Figure 3. shows the total number of target genes that have amplified in either the gold standard or the Amplidiag Bacterial GE.

- There were 42 target genes overall among Instagene negative/Amplidiag positive (Figure 3).
- Moreover, 11 genes are targets for Amplidiag negative/Instagene positive.
- The number of discrepant results varied by target with the most discrepant results observed with *eae* (n=18), STEC *stx1* (n=10), and STEC *stx2* (n=11)
- ETEC (n=3), *ipaH* (n=4), and a*ggR* (n=7) had the fewest number of discrepancies

DISCUSSION

- The results of this study show that the Amplidiag Bacterial GE multiplex real-time PCR kit performs well in the qualitative identification and detection of common bacterial pathogens linked to gastroenteritis.
- This is evident in the fact that the Amplidiag Bacterial GE multiplex real-time PCR kit had 82% matching results with the in-house assay.
- Unlike standard stool culture, the benefits of implementing the Amplidiag Bacterial GE multiplex real-time PCR kit into routine use includes simplicity and less hands-on time.
- On the other hand, the Amplidiag has a limitation in how many samples can be processed in a normal working day (~60 samples).

CONCLUSIONS

- The study demonstrated that the Amplidiag assay may allow for a more precise detection of the target gene *stx1 & stx2* . It has been established that STEC *stx1* (n=2) and STEC *stx2* (n=2) were recognised as Amplidiag Negative/Instagene Positive, whereas STEC *stx1* (n=9) and STEC *stx2* (n=8) were identified as by Instagene Negative/Amplidiag Positive. Due to their propensity to spread gastrointestinal sickness and HUS, a serious life-threatening systemic condition, STEC are a significant public health problem. If we had used the Instagene assay, we would not have been able to detect these target genes.
- This study has shown that Amplidiag Bacterial GE offers significant clinical and laboratory process benefits when used as a screening test for stool samples.
- The test detects non-culturable pathogens and can quickly identify eight of the most common and major enteric bacterial pathogens from the same sample, allowing us to diagnose patients more effectively while lowering the safety risk associated with handling STEC enrichment broths.
- Amplidiag Bacterial GE has been identified as a good candidate for replacement of the in-house PCR assay for detection of GI pathogens directly in stool specimens, and may be implemented following further validation.

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