Evaluation of the *EasyScreen™ C. difficile* Detection Kit for *tcdA* and *tcdB*

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Introduction and Purpose:

More severe *Clostridium difficile* infection (CDI) which originated in North America in the early 2000s has now spread world-wide and it has become vital for a early definitive diagnosis of CDI to made. This has been a driving force behind rapid advances in laboratory-based technologies particularly nucleic acid amplification tests (NAATs) which promise rapid turn around times. This study evaluated the recently developed *EasyScreen*™ Detection Kit (Fig.1) that uses 3base™ chemistry in which all cytosine bases are converted to uracil to improve the efficiency of RT PCR by reducing the temperature variability in multiplex reactions making it possible to amplify both toxin A and B genes simultaneously for the identification of toxigenic *C. difficile*. The *EasyScreen*™ Detection Kit may be used on a variety of RT PCR platforms after either manual or automated extraction.

Fig.1. *EasyScreen C. difficile* Detection Kit.

Methods:

Faecal samples

304 stool samples from the Enteric Laboratory of PathWest Laboratory Medicine (WA) and all samples assayed for glutamate dehydrogenase (GDH) using Wampole® CHEK™-60 (TechLab). All positives tested with the BD GeneOhm Cdiff assay. All specimens stored at -20°C until assayed with the *EasyScreen™ C. difficile* Detection Kit.

C. difficile culture, identification and ribotyping

Stool samples cultured on ChromID *C. difficile* agar before storage. *C. difficile* isolates were identified phenotypically[1], ribotyped[2] and, if required, toxin profiled by PCR [3,4].

EasyScreen™ C difficile assay

All faecal samples were manually extracted following manufacturer's instructions and assayed on a Roche Lightcycler® LC480 following Genetic Signatures protocol (~2 h) (Fig. 2). Inhibited samples were diluted either 1/5 or 1/12.5 and re-analysed.

Resolution of false positives and negatives

Growth of toxigenic *C. difficile* on ChromID *C. difficile* agar was a true positive. False positives results were resolved by enrichment culture in Robertson's Cooked Meat (RCM) medium containing gentamicin (4 mg/L), cefoxitin (8 mg/L) and cycloserine (200 mg/L) for 7 days before plating onto cycloserine cefoxitin fructose agar containing 0.1% sodium taurocholate (CCFA) after alcohol shock. False negative results were resolved by repeated faecal extraction and re-testing.

EasyScreen™ C. difficile Reflex assay

All positives with the *EasyScreen*™ Detection Kit were retested with the *EasyScreen*™ *C. difficile* Reflex Kit which targets the Δ117 deletion in the *tcdC* gene, the threonine to isoleucine substitution in the gyrase A gene responsible for fluoroquinolone resistance and the binary toxin gene (*cdtA*) to look for the presence of epidemic strains of ribotype (RT) 027 and 078.

Results:

EasyScreen™ C. difficile Detection assay

304 stool samples with 34 (11.2%) inhibited. The initial high inhibition rate was thought to be due to manual extraction. After dilution only 2 (0.6%) were inhibited (Table 1).

Table 1: Sensitivity, specificity, PPV and NPV unchanged by resolution of false positives and false negatives.

	Initial Results	After Resolution
True positives	32	33
True negatives	263	265
False positives	4	3
False negatives	3	3
Inhibited	2	0
Sensitivity	91.4	91.7
Specificity	98.5	98.9
PPV	88.9	91.7
NPV	98.9	98.9

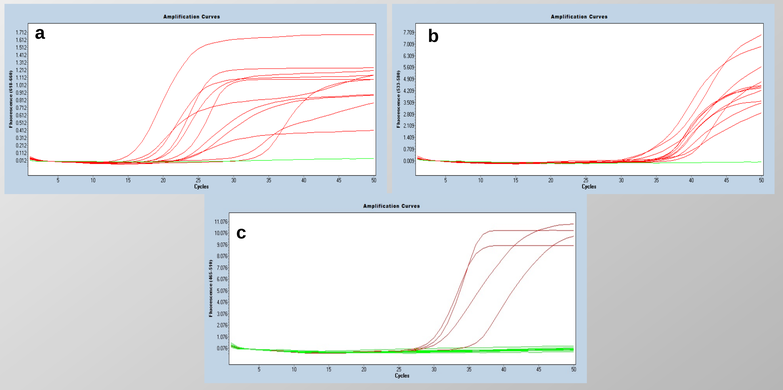

Fig.2. Amplification curves that represent (a) extraction control, (b) internal positive control and (c) detection of toxin genes in *EasyScreen™ C. difficile* Detection Kit.

Results:

EasyScreen™ C. difficile Reflex

Positive faecal extracts tested for the presence of possible 'epidemic strains' RT 027 and RT 078. One extract was positive for binary toxin and the deletion in *tcdC*. This sample grew RT 244.

Results:

Ribotyping

Major ribotype was 014/020 group with 16 (44%) isolates. Another 4 isolates were RT 002 and all other isolates belonged to various RTs. 4 were not able to be named with our reference collection. All false negatives were 014/020 group.

GDH positive samples

79% agreement between BD GeneOhm and *EasyScreen*™ with Cohen's kappa value at 0.543 (moderate agreement) (Table 2).

Table 2: A subset of 52 GDH positive stools assayed with BD GeneOhm and *EasyScreen™ C. difficile* were compared.

	EasyScreen	BD GeneOhm
True positives	30	27
True negatives	19	12
False positives	1	9
False negatives	2	4
Sensitivity	93.7	87.1
Specificity	95.0	57.1
PPV	96.8	75.0
NPV	90.5	75.0

Conclusions:

- **Sensitivity (91.7%) and specificity (98.9%) of *EasyScreen™ C. difficile* Identification Kit were comparable with other commercial NAAT systems**
- **All false negatives were RT 014/020**
- **The *EasyScreen™ C. difficile* Reflex Kit detected *cdtA* and the Δ117 deletion in *tcdC* of RT244 strains**

Disclosure: Genetic Signatures (Randwick, NSW, Australia) supplied kits used in this study, and technical advice and assistance.

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