Diagnostic Microbiology and Infectious Disease 86 (2016) 35–39

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Evaluation of the Alere SHIGA TOXIN QUIK CHEK™ in comparison to multiplex Shiga toxin PCR

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ARTICLE INFO

Article history:
Received 14 January 2016
Received in revised form 21 April 2016
Accepted 23 May 2016
Available online 27 May 2016

Keywords:
Shiga toxin-producing *Escherichia coli*
Enzyme immunoassay
Alere SHIGA TOXIN QUIK CHEK™

ABSTRACT

Shiga toxin-producing *Escherichia coli* (STEC) causes gastrointestinal outbreaks worldwide and due to its low infectious dose highly sensitive diagnostics are needed. In this study, the performance of the enzyme immunoassay SHIGA TOXIN QUIK CHEK (STQC) (Alere, TechLab) for the detection of Stx1 and Stx2 was evaluated directly from fecal samples, from culture on agar (SMAC(−CT)) and from broth enrichment (mTSB) in comparison to our in-house multiplex PCR. The STQC could not detect the Stx2f subtype, but detected all other subtypes with an analytical sensitivity varying between 10^8 and 10^4 CFU/ml. The SMAC(−CT) assay had the best performance with an overall sensitivity of 93.3%; broth and direct fecal testing had sensitivities of 74.1% and only 46.7%, respectively. All methods were 100% specific. Because of the unacceptably low sensitivity we do not recommend applying the STQC directly on stools, but only after overnight culture.

1. Introduction

Shiga toxin- or verocytotoxin-producing *E. coli* (STEC/VTEC) infections are the cause of sporadic and epidemic watery or bloody diarrhea worldwide. In some individuals it may progress to more severe disease such as hemorrhagic colitis (HC) and the life-threatening hemolytic uremic syndrome (HUS) (Buvens and Piérard, 2012; Piérard et al., 2012). STEC's main pathogenic mechanism is production of Shiga toxins (Stx) also called verocytotoxins (Vtx), which cause cell death by blocking the protein synthesis. The Stx family can be divided in two major antigenically distinct types, Stx1 and Stx2, which show distinct immunogenic and genetic properties. Besides the Stx produced by *Shigella* species, 3 subtypes for Stx1 (Stx1a, stx1c and Stx1d) and 7 subtypes for Stx2 (Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f, and Stx2g) have been described in STEC. STEC strains can produce either only one Stx or a combination of different Stx subtypes. The Stx-coding genes (*stx*) are carried by bacteriophages that can be easily lost or acquired by horizontal transfer (Scheutz et al., 2012; Tozzoli and Scheutz, 2014). Determination of the Stx variants is clinically relevant as some Stx2 subtypes seem to be more often associated with severe human illness, especially when they are present in combination with the *eae* virulence gene coding for intimin (Brandal et al., 2015; EFSA Panel on Biological Hazards (BIOHAZ), 2013; Ethelberg et al., 2004; Persson et al., 2007).

Early and accurate diagnosis of STEC infection is important in order to improve the overall patient outcome and to be able to respond to outbreak situations in an effective and timely manner (Gould et al., 2009). For epidemiological surveillance in Belgium, fecal samples from patients with suspected STEC infections as well as strains can be referred to the National Reference Centre (NRC) for STEC/VTEC for diagnosis and extensive isolate typing (Muyldermans et al., 2012). STEC infections are mainly sporadic, but a few small outbreaks have been reported over the years (Braeye et al., 2014; De Schrijver et al., 2008; Piérard and De Rauw, 2016). Since the official establishment of the NRC in 2011, an average of 99 STEC strains have been isolated from patients annually, the majority of which belonged to the O157:H7/H- serotype. O26 is the most common serogroup among the non-O157 STEC, followed by O111, O103, O145 and O146. O-serogroups 45 and 121, also considered part of the most pathogenic non-O157 serotypes (Gould et al., 2009), were rarely detected in Belgian patients (Piérard and De Rauw, 2016).

At the NRC all fecal samples and isolates are screened with an in-house PCR assay for the detection of *stx* genes after culture on selective agar. As routine clinical laboratories seldom use molecular detection in the routine screening of human stools for STEC; rapid, easy to perform, yet sensitive tests are needed. Different enzyme immunoassays (EIAs) for the detection of Stx are commercially available but most of them require an additional overnight enrichment step extending the turnaround time.

The aim of this study was to evaluate the performance of the SHIGA TOXIN QUIK CHEK (STQC) test (Alere™, TechLab®), a rapid membrane EIA for the simultaneous detection and differentiation of Stx1 and Stx2 directly from fecal samples, and to compare it to our well-validated in-house conventional multiplex PCR.

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http://dx.doi.org/10.1016/j.diagmicrobio.2016.05.016
0732-8893/

2. Materials and methods

2.1. Analytical sensitivity for Stx subtypes

A selection of *E. coli* reference strains and a clinical isolate of *Shigella dysenteriae* type I, representing all Stx1 and Stx2 subtypes, were used to determine the analytical sensitivity of STQC for the different toxin variants (Table 1). The strains were grown overnight (35 °C) on sorbitol MacConkey agar biplates enriched with/without cefixime and tellurite (SMAC(−CT)) and a suspension of 0.5 McFarland in nutrient broth (NUB) was prepared. Two ten-fold serial dilutions were made of each strain starting from the 0.5 McFarland suspensions. First, a ten-fold 1:10000–1:10000000 dilution series (dilutions −4 to −7) was made in NUB, of which 100 μl were grown on SMAC agar plates overnight (35 °C) in order to count the colony forming units (CFU). A second ten-fold dilution series was made in a 0.5 McFarland solution of the Stx-negative *E. coli* ATCC 25922 to mimic a background of commensal *E. coli*. Hundred microliters (100 μl) of the 1:1–1:1000 dilution series (dilutions 0 to −3) in a background of *E. coli* ATCC 25922 were grown on SMAC agar overnight (35 °C) and another 100 μl were added to a tryptic soy broth modified with bile salt N° 3, novobiocin, vancomycin, rifampicin and K-tellurite (mTSB; produced in-house) and incubated for 24 h (42 °C). The protocol for the selective STEC O157 enrichment with mTSB broth was adapted from Possé et al. (Possé et al., 2008). STQC analysis was performed with the plate method and the broth method according to the manufacturer's instructions starting from the bacterial growth on the SMAC agar plates and the mTSB's respectively. Our in-house multiplex PCR was performed as described previously starting from the bacterial growth on the SMAC agar (Buvens et al., 2012). Briefly, a suspension of the bacterial colonies in NUB was used as a template in a PCR using the Qiagen Multiplex PCR Mastermix (Qiagen, Venlo, the Netherlands) and 3 sets of primers for the amplification of the *stx1*, *stx2* and *stx2f* genes (Paton and Paton, 1998; Schmidt et al., 2000). Thermal cycler conditions consist of an initialization step of 15 min at 94 °C followed by 35 cycles of amplification (25 s at 94 °C, 76 s at 65 °C, 64 s at 72 °C).

2.2. Screening of human fecal samples and characterization of STEC isolates

The stool samples used in this study were collected from patients with gastrointestinal symptoms or HUS and were analyzed at the NRC STEC/VTEC located at the Laboratory of Microbiology of the university hospital UZ Brussel. During a period of nine months in 2013 (February-October) fresh fecal samples positive for the presence of *stx* genes as well as randomly selected *stx*-negative stools that were submitted to our laboratory the same day were analyzed with the STQC according to the package insert using 3 test procedures: one directly on feces (direct method), one after 24h incubation in mTSB, routinely used in our laboratory for selective enrichment of STEC O157 in fecal samples from HUS patients (broth method), and one after overnight culture on SMAC(−CT) agar (plate method).

Isolation of a STEC strain from the *stx*-positive samples was attempted as described before (Buvens et al., 2012). If no isolate was found, the sample was reported as PCR-positive without further testing. Biochemical identification was applied for confirmation of the *E. coli* species. Slide agglutination was performed using the O26, O103, O111, O121, O145, O157 and the K9 (used for O104) antisera (Statens Serum Institut, Copenhagen, Denmark). H7 antiserum sorbitol fermentation medium was used for the identification of the H7 flagellar antigen (Farmer and Davis, 1985). All non-agglutinating strains were sent to the Statens Serum Institut in Copenhagen for full serotyping. STEC isolates were further characterized by molecular detection of the virulence genes *eaeA*, *hlyA*, *aaiC* and *aggR* as described before (Boisen et al., 2012; De Rauw et al., 2014; Paton and Paton, 1998). A PCR-based method described by Scheutz et al. (2012) was applied to determine the *stx* subtypes.

2.3. Statistical analysis

The qualitative test performance of STQC in comparison to multiplex *stx* PCR was assessed by calculating the specificity, sensitivity and Kappa statistic for the 3 different methods (direct fecal, SMAC(−CT), mTSB). The kappa statistic assesses to which degree both methods agree (interpretation Viera and Garrett, 2005). The Fisher exact test was used to assess the significance of the differences in sensitivity between the results of this study and the results described in the paper of Chui et al., 2015. All statistics were calculated using the Analyse-it software v2.26 (Analyse-it Software Ltd, Leeds, United Kingdom). A probability (P) value of ≤.05 was considered statistical significant.

3. Results

3.1. Analytical sensitivities

Analytical sensitivities are shown in Table 1. The multiplex PCR detected all *stx* subtypes with a sensitivity varying between 10^6 and 10^4 CFU/ml. Using STQC, Stx2b could not be detected by the plate method in strain DG131/3 which possessed Stx1c as well as Stx2b and it could not be detected after mTSB enrichment in STEC EH250 which only possessed Stx2b. Likewise Stx2e and Stx2g were not detected by the broth method. Detection of Stx2f was not possible regardless of the method used. After inoculation on SMAC the sensitivity of STQC was comparable to PCR for Stx1a, Stx1c, Stx2a and Stx2g, and lower for Stx1d, Stx2b, Stx2c, Stx2d, and Stx2e. Additional Stx2b- and Stx2c-producing strains

Table 1
Analytical sensitivities of the STQC test and the multiplex *stx* PCR.

Strain (reference)	Serotype	Stx subtype	Lowest detectable dilution (CFU/ml)[a]		
			STQC		PCR
			Plate (SMAC)	Broth (mTSB)	
STEC H19 (Konowalchuk et al., 1977)	Unknown; Non-O157	Stx1a	$3.0x10^4$	$3.0x10^4$	$3.0x10^4$
STEC DG131/3 (Paton et al., 1995)	O174:H8	Stx1c, Stx2b	Stx1c: $7.5x10^4$ Stx2b: ND	Stx1c: $7.5x10^7$ Stx2b: $7.5x10^7$	Stx1c: $7.5x10^4$ Stx2b: $7.5x10^4$
STEC MH1813 (Burk et al., 2003)	O8:Hrough	Stx1d	$1.0x10^7$	$1.0x10^5$	$1.0x10^6$
STEC 94C (Paton et al., 1995)	O48:H21	Stx1a, Stx2a	Stx1a: $5.9x10^4$ Stx2a: $5.9x10^4$	Stx1a: $5.9x10^5$ Stx2a: $5.9x10^4$	Stx1a: $5.9x10^4$ Stx2a: $5.9x10^4$
STEC EH250 (Piérard et al., 1998)	O118:H12	Stx2b	$6.6x10^6$	ND	$6.6x10^4$
STEC E32511 (Schmitt et al., 1991)	O157:H-	Stx2c	$7.3x10^7$	$7.3x10^6$	$7.3x10^4$
STEC C165-02 (Persson et al., 2007)	O73:H18	Stx2d	$2.0x10^8$	$2.0x10^8$	$2.0x10^6$
STEC S1191 (Weinstein et al., 1988)	O139:H1	Stx2e	$8.9x10^7$	ND	$8.9x10^5$
STEC T4/97 (Schmidt et al., 2000)	O128ac:H2	Stx2f	ND	ND	$1.0x10^5$
STEC 7v (Leung et al., 2003)	O2:H25	Stx2g	$1.3x10^6$	ND	$1.3x10^6$
S. dysenteriae type I EH791 (clinical isolate)	/	Stx	Stx1: $1.2x10^6$	Stx1: $1.2x10^8$	Stx1: $1.2x10^6$

[a] ND: not detected from the undiluted suspension; $>10^7$ CFU/ml