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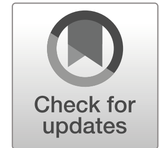
ORIGINAL ARTICLE



Rapid culture-based identification of Shiga toxin-producing *Escherichia coli* and *Shigella* spp./Enteroinvasive *E. coli* using the eazyplex® EHEC complete assay

Anika Penzel [1] · Katrin Schützler [1] · Jana Dröge [1] · Alexander Mellmann [2] · Ralf Ehricht [3] · Ines Engelmann [4] · Sascha D. Braun [3] · Benjamin T. Schleenvoigt [5] · Bettina Löffler [1] · Jürgen Rödel [1]

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Abstract

Shiga toxin–producing *Escherichia coli* (STEC) and *Shigella* spp./enteroinvasive *E. coli* (EIEC) are common diarrheagenic bacteria that cause sporadic diseases and outbreaks. Clinical manifestations vary from mild symptoms to severe complications. For microbiological diagnosis, culture confirmation of a positive stool screening PCR test is challenging because of time-consuming methods for isolation of strains, wide variety of STEC pathotypes, and increased emergence of non-classical strains with unusual serotypes. Therefore, molecular assays for the rapid identification of suspect colonies growing on selective media are very useful. In this study, the performance of the newly introduced eazyplex® EHEC assay based on loop-mediated isothermal amplification (LAMP) was evaluated using 18 representative STEC and *Shigella* strains and 31 isolates or positive-enrichment broths that were collected from clinical stool samples following screening by BD MAX™ EBP PCR. Results were compared to real-time PCR as a reference standard. Overall, sensitivities and specificities of the eazyplex® EHEC were as follows: 94.7% and 100% for Shiga toxin 1 (*stx1*), 100% and 100% for *stx2*, 93.3% and 97.1% for intimin (*eae*), 100% and 100% for enterohemolysin A (*ehlyA*), and 100% and 100% for invasion-associated plasmid antigen H (*ipaH*) as *Shigella* spp./EIEC target, respectively. Sample preparation for LAMP took only some minutes, and the time to result of the assay ranged from 8.5 to 13 min. This study shows that eazyplex® EHEC is a very fast and easy to perform molecular assay that provides reliable results as a culture confirmation assay for the diagnosis of STEC and *Shigella* spp./EIEC infections.

Keywords Shiga toxin · *E. coli* · *Shigella* · LAMP · Stool culture

Introduction

Screening PCRs for enteric bacterial pathogens offer a fast diagnosis or exclusion of bacterial diarrheal disease, except for *Salmonella*, because they can be isolated using highly sensitive enrichment culture techniques [1]. In this context, the extension of screening stool samples for Shiga toxin genes *(stx)* in all patients with community-acquired acute diarrhea regardless of bloody stool or suspicion of hemolytic uremic syndrome (HUS) has led to significantly increased numbers of Shiga toxin-producing *E. coli* (STEC) cases notifiable to public health offices [2, 3]. In Germany, notification criteria by the Infection Protection Act include not only the culture isolation of a *stx*-positive strain but also the detection of *stx* genes or Shiga toxin antigen in the stool sample when clinical symptoms occur. However, in cases of uncomplicated diarrhea, a positive *stx* PCR result does not have a clinical impact in every case because it provides no distinct evidence of the presence of an STEC [4, 5]. In regard to the *stx* subtype and the association with hemorrhagic colitis and HUS, STEC is classified as low or high virulent, the latter is designated as enterohemorrhagic *E. coli* (EHEC) [6]. The combination of

✉ Jürgen Rödel
juergen.roedel@med.uni-jena.de

[1] Institute of Medical Microbiology, Jena University Hospital, Jena, Germany

[2] Institute of Hygiene and National Consulting Laboratory for Hemolytic Uremic Syndrome (HUS), University Hospital Münster, Münster, Germany

[3] Leibniz Institute of Photonic Technology (IPHT), Jena, Germany

[4] Blink AG, Jena, Germany

[5] Institute of Infectious Diseases and Infection Control, Jena University Hospital, Jena, Germany

culture-independent and culture-dependent methods is recommended for the diagnosis of STEC infections and confirmation of the pathological relevance of a positive screening PCR [4, 5, 7]. The isolation of strains in culture is also important to estimate the distribution and frequency of non-O157 serotypes and recognize epidemiological changes [5, 8]. Identification of STEC in culture is challenging because except for O157 which is negative for β-glucuronidase and sorbitol fermentation, most STEC are not biochemically different from non-pathogenic *E. coli* and their colonies cannot easily be distinguished on differential media [9]. Similar difficulties occur in the diagnosis of *Shigella* spp. and EIEC. Both pathogens can cause mild diarrhea but also severe dysentery characterized by inflammatory lesions and ulceration of the intestinal epithelium [10]. Whereas *Shigella* can be easily differentiated by its different biochemical behavior on selective media and by serotyping to identify the 4 species, EIEC can exert different characteristics more similar to *Shigella* or non-pathogenic *E. coli* [11]. Serotypes of EIEC can not only overlap with *Shigella* but also with *E. coli* serotypes that are not included in standard polyclonal antisera [12]. Now the application of PCR to identify STEC and *Shigella* spp./EIEC isolates by their virulence factors in culture is the method of choice [13, 14]. An alternative molecular technique of interest is loop-mediated isothermal amplification (LAMP) that uses *bst* DNA polymerase with strand-displacing activity and offers high-speed amplification under isothermal conditions [15]. In comparison to PCR, LAMP is very fast and involves a low hands-on time, features that are advantageous for using this technique in rapid diagnostic tests [16]. This study was designed to evaluate the performance of the newly introduced eazyplex® EHEC complete LAMP assay (Amplex Diagnostics, Gars-Bahnhof, Germany) as a culture confirmation test for the diagnosis of STEC and *Shigella* spp./EIEC infections.

Material and methods

Bacterial strains and clinical samples

A total of 12 strains of STEC possessing different *stx* subtypes and 6 strains of *Shigella* spp. from the strain collections of the Institute of Medical Microbiology, Jena, and the Institute of Hygiene, Münster, were used as retrospective samples for the evaluation of the eazyplex® EHEC complete assay (Table 1).

As prospective samples, 16 isolates and 15 enrichment broths cultured from diarrheal stool specimens as part of the routine patient diagnostics at the Institute of Medical Microbiology, Jena, were analyzed (Tables 2 and 3). The isolates were collected between April 2017 and October 2018 from diarrheal stool samples tested positive for *stx* or *Shigella* spp./EIEC using the BD MAX™ Enteric Bacterial Panel (EBP) assay (BD, Heidelberg, Germany) as screening PCR. Formed stools and rectal swabs were excluded.

BD MAX™ enteric bacterial panel (EBP) PCR and bacterial culture

Unpreserved stool samples were tested with the BD MAX™ EBP assay within 8 h after receipt according to the manufacturer's protocol. This automated PCR test uses the following primer sets: *spaO* for *Salmonella* spp., *ipaH* for *Shigella* spp./EIEC, *tuf* for *Campylobacter jejuni* and *C. coli*, and *stx1/stx2* for STEC. *Stx1* and *stx2* are not differentiated from each other. Samples positive for *stx* were inoculated onto Brilliance *E. coli*/coliform chromogenic agar (Oxoid, Thermo Fisher Scientific, Wesel, Germany) and GN broth (BD). For isolation of *Shigella* Hektoen agar (BD), GN, and selenite broth (BD) were used. Identification of suspect colonies was performed using the RIDA®GENE EHEC/EPEC PCR, agglutination with polyclonal and monoclonal antisera (Sifin Diagnostics, Berlin, Germany) and biochemical differentiation by API20E (bioMerieux, Nürtingen, Germany).

RIDA®GENE EHEC/EPEC real-time PCR

RIDA®GENE EHEC/EPEC PCR (R-Biopharm, Darmstadt, Germany) targeting *stx1/stx2*, *eae*, and *ipaH* served as reference method for testing bacterial isolates. A single colony or 5 μl of GN broth were transferred into 1 ml of DEPC water. A total of 20 μl of internal control DNA were added as extraction control. The suspension was vortexed, heated at 95 °C for 10 min, and centrifuged at 12.000 rpm for 1 min. Five microliters of the supernatant was added to 20 μl of PCR mastermix. The test was run on a SmartCycler (Cepheid, purchased from BD) with the following cycling conditions: 95 °C for 1 min, 45 cycles at 95 °C for 10 s and 60 °C for 15 s.

Eazyplex® EHEC complete LAMP assay

The eazyplex® EHEC is a qualitative assay for the detection of different intestinal pathovars of *E. coli* and their virulence factors. A single eazyplex® test strip contains six oligonucleotide primers in each filled cap and these provide the means for simultaneous, specific amplification of different genes in a single isothermal amplification reaction. A single colony or 5 μl of GN broth were suspended in 500 μl of resuspension and lysis fluid (RALF, Amplex Diagnostics) and boiled for 2 min. After centrifugation at 4000 rpm for 1 min, 25 μl of the supernatant was pipetted into each tube of the eazyplex® test strip containing the lyophilized master mix. The strip was gently knocked to remove air bubbles and loaded into the Genie II Mk2 machine (OptiGene Ltd., West Sussex, UK; purchased from Amplex Diagnostics). Tests were run at 65 °C for 25 min. Amplification was measured by real-time

Table 1 Performance of the eazyplex® EHEC complete assay for retrospective identification of STEC and *Shigella* strains

Strain/serovar	Virulence factors[a]	eazyplex® results, threshold time (min)							
		stx1	*stx2a-e, g*	*stx2f*	*eae*	*ehlyA*	*aggR*	*ipaH*	IC[b]
STEC O128:H-	*stx1c, ehlyA*	10.5	–	–	–	19	–	–	7.25
STEC O8:H-	*stx1d, ehlyA*	14.75	–	–	–	19	–	–	7.25
STEC O157	*stx2a, eae, ehlyA*	–	11.25	–	11.75	17	–	–	11.5
STEC O104:H4	*stx2a, aggR*	–	6.25	–	–	–	8.5	–	10.5
STEC O118:H12	*stx2b*	–	17.25	–	–	–	–	–	7.25
STEC O157:H-	*stx2c, eae, ehlyA*	–	8.25	–	8.5	11.75	–	–	8.25
STEC O91:H21	*stx2d, ehlyA*	–	4.75	–	–	24.5	–	–	9.5
STEC O8:H19	*stx2e*	–	13.5	–	–	–	–	–	9
STEC O128:H2	*stx2f, eae*	–	–	8.75	8.5	–	–	–	7.75
STEC O2:H25	*stx2g*	–	11.75	–	–	–	–	–	8
STEC O113:H4	*stx1c, stx2b*	13.5	12	–	–	–	–	–	9.5
STEC O181:H16	*stx1c, stx2d, ehlyA*	10.25	8.75	–	–	23.5	–	–	7.75
S. dysenteriae type 3	*ipaH*	–	–	–	–	–	–	6.25	8.25
S. flexneri	*ipaH*	–	–	–	–	–	–	7	9
S. flexneri	*ipaH*	–	–	–	–	–	–	7	8.75
S. sonnei	*ipaH*	–	–	–	–	–	–	7.5	10.75
S. sonnei	*ipaH*	–	–	–	–	–	–	7.5	9.5
S. sonnei	*ipaH*	–	–	–	–	–	–	7	9.25

[a] *stx*, Shiga toxin; *eae*, intimin; *ehlyA*, enterohemolysin A; *aggR*, transcriptional activator of AAF; *ipaH*, invasion plasmid antigen H

[b] IC, inhibition control

fluorescence detection using a DNA intercalating dye. Data interpretation was automatically performed by the integrated eazyReport™ software.

E. coli subtyping by microarray and sequencing

Because our standard real-time PCR did not determine *stx* subtypes and *ehlyA*, the clinical STEC and EIEC isolates were subjected to microarray-based analysis using the *E. coli* PanType AS2 genotyping kit (Alere Technologies, Abbott, Jena, Germany) to identify the serovar (O:H), the *stx* subtype, and additional virulence factors. Microarray analysis was performed as previously described [17]. The EIEC isolate was further characterized by whole-genome sequencing (WGS). Genomic DNA was isolated from an overnight culture using the NucleoSpin® Microbial DNA kit (Macherey-Nagel, Düren, Germany). The Nanopore Oxford MinION platform was used for WGS. Briefly, no size selection was performed and the DNA library was generated using the native barcoding expansion kit EXP-NBD103 and the Nanopore sequencing kit SQK-LSK109 (Oxford Nanopore Technologies, Oxford, UK) according to manufacturer's instructions. The used flow cell FLO-MIN106 (R9-Version) was primed by the flow cell priming kit EXP-FLP001 (Oxford Nanopore, Oxford, UK). The protocol named "1D Native barcoding genomic DNA" was used in version NBE_9065_v109_revB_23May2018 (last update, 03/09/2018). The Albacore basecaller (Oxford Nanopore Technologies, Oxford, UK) translated the minion raw data (FAST5) into short-quality tagged sequence reads (FASTQ). After barcode trimming using Porechop (https://github.com/rrwick/Porechop), canu (https://github.com/marbl/canu) was used to assemble the short reads to nine contigs. All contigs were analyzed by abricate (https://github.com/tseemann/abricate). After nanopolishing (https://github.com/jts/nanopolish), the corrected sequence data were used for a direct comparison to the outbreak strain O96:H19 from Italy in 2007 (LIT) by MUMmer4 software (https://mummer4.github.io/).

Statistical analysis

The qualitative performance of the eazyplex® EHEC complete assay in comparison to the reference method was assessed by calculating the specificity, sensitivity, and accuracy (percent positive agreement).

Results and discussion

Retrospective evaluation using characterized strains

For a first evaluation of the eazyplex® EHEC assay, a collection of representative strains covering all test targets including

Table 2 Characteristics of prospectively tested STEC, EIEC, and *Shigella* isolates

Isolate/serovar	PCR result[a]	Stx subtype[b]	Adhesins, toxins[b]	eazyplex® results, threshold time (min)							
				stx1	*stx2a-e, g*	*stx2f*	*eae*	*ehlyA*	*aggR*	*ipaH*	IC[c]
STEC O91:H14 (1)	*stx1*	*stx1a*	*lpfA*	10.5	–	–	–	–	–	–	14.25
STEC O91:H14 (2)	*stx1*	*stx1a*	*ehlyA, lpfA*	15.75	–	–	21	13.25	–	–	13.75
STEC O91:H14 (3)	*stx1*	*stx1a*	*ehlyA, lpfA*	6.5	–	–	–	21.75	–	–	9
STEC O91:H14 (4)	*stx1*	*stx1a*	*ehlyA, lpfA*	7	–	–	–	8.25	–	–	12.25
STEC O103:H2	*stx1, eae*	*stx1a*	*eae, tir, ehlyA,*	6.25	–	–	8	12.75	–	–	8.25
STEC OR:H19	*stx1*	*stx1c*	*ehlyA, senB*	10	–	–	–	6	–	–	5
STEC O157:H7	*stx1, stx2, eae*	*stx1a, stx2a*	*eae, tir, ehlyA, astA, toxB*	7	8.5	–	9.25	15.5	–	–	8.75
STEC O26:H11	*stx2,eae*	*stx2a*	*eae, tir, lpfA, astA, ehlyA,*	–	7	–	9	15.75	–	–	8.75
STEC OR:H2	*stx2, eae*	*stx2a*	*eae, tir, ehlyA*	–	9	–	12.75	10	–	–	9.5
STEC OR:H8	*stx2*	*stx2b*	*ehlyA, senB*	–	10.5	–	–	6.75	–	–	11.5
STEC O2:H6	*stx2*	*stx2b*	*prfB, sfaS, cnf1*	–	11	–	–	–	–	–	8.75
STEC O117:H4	*stx2*	*stx2b*	*ehlyA, lpfA, astA, subA*	–	9.25	–	–	6.5	–	–	10.25
STEC O146:H28	*stx2*	*stx2b*	*ehlyA, lpfA, astA*	–	7.75	–	–	6.25	–	–	9.5
EIEC O96:H19	*ipaH*	–	*lpfA.ipaD, senB, virF*	–	–	–	–	–	–	6.5	9
S. flexneri	*ipaH*	–	N.D.	–	–	–	–	–	–	6.75	9
S. sonnei	*ipaH*	–	N.D.	–	–	–	–	–	–	20.5	10.25

[a] *stx*, Shiga toxin; *eae*, intimin; *aggR*, transcriptional activator of AAF; *ipaH*, invasion plasmid antigen H

[b] Determined by microarray (Alere). *ehlyA*, enterohemolysin A; *lpfA*, long polar fimbriae; tir; *senB*, enterotoxin TieB protein; *astA*, heat stable enterotoxin 1; *prfB*; P-related fimbriae regulatory gene; *sfaS*, S fimbriae minor subunit; *cnf1*, cytotoxic necrotizing factor; *ipaD*, IpaD invasion; *virF*, transcriptional regulator of *virB* and *icsA; subA,* subtilase cytotoxin subunit A

[c] IC, inhibition control

stx types of high-virulent (*stx 2a*, *c*, and *d*) and low-virulent (*stx 1*, *2b*, *2e*, *2f*, and *2g*) STEC was used [3]. All strains were correctly identified regarding the *stx* types 1 and 2, adhesion intimin (*eae*), and enterohemolysin A (*ehlyA*) (Table 1). The assay also correctly identified the German outbreak strain O104 from 2011 possessing *stx2a* and aggregative adherence fimbriae (AAF) which are adhesins of enteroaggregative *E. coli* (EAEC) [18]. The eazyplex® EHEC assay contains primers targeting *aggr* encoding a regulatory protein for AAF expression [19].

The *stx2f* has been included in the assay as a specific target because of its low-sequence homology to other *stx2* subtypes [20]. The *stx2f* gene of strain O128:H2 was detected without cross-reactivity to the common *stx2* target detecting only *stx2a-e and g* (Table 1). It should be noted that *stx2f* is not covered by all commercial PCRs, e.g., BD MAX™. Although *stx2f*-positive STEC is rarely isolated, outbreaks have already been reported [21]. Enterohemolysin A (*ehlyA*) is a characteristic virulence factor especially for classical EHEC strains and the gene was correctly identified in all cases [22]. For identification of *Shigella* spp./EIEC, the eazyplex® EHEC contains primers for *ipaH*, a multicopy gene that is encoded chromosomally and on the large invasion virulence plasmid and therefore commonly used as target in diagnostic PCR tests [11, 23]. Several strains of *S. dysenteriae*, *S. flexneri*, and *S. sonnei* were correctly identified (Table 1).

Prospective evaluation of clinical sample isolates

Clinical samples were stool specimens from patients with diarrhea or suspicion of HUS that were screened by BD MAX™ PCR for bacterial pathogens as part of routine diagnostics. A total of 4996 specimens tested between April 2017 and October 2018 yielded 130 *Campylobacter jejuni*/*C. coli*, 58 *stx*, 35 *Salmonella* spp., and 8 *Shigella* spp./EIEC-positive results. The overall positive rate was 4.7%, excluding 82 invalid test results. The positive rate for *stx* was 1.2%. From 27 out of 58 *stx*-positive samples, a STEC could be isolated in culture or detected in GN enrichment broth, amounting to a culture confirmation rate of 46.6% (Tables 2 and 3). Four out of 8 *Shigella* spp./EIEC-positive samples were also positive in culture. The discrepancy between PCR and culture may be due to several reasons. BD MAX™ has been shown to be significantly more sensitive than culture [24]. All but one of the isolates were non-O157 *E. coli* and showed growth characteristics identical to non-pathogenic *E. coli* on Brilliance *E. coli*/coliform agar by positive reactions for both β-galactosidase and β-glucuronidase. It should also be noted

Table 3 Characteristics of positive GN enrichment broth cultures

Broth number	Reference PCR results[a]	eazyplex® results, threshold time (min)							
		stx1	*stx2a-e, g*	*stx2f*	*eae*	*ehlyA*	*aggR*	*ipaH*	IC[b]
1	*stx1*	15.25	–	–	–	–	–	–	10.5
2	*stx1*	16	–	–	–	10	–	–	11
3	*stx1*	11	–	–	–	10.5	–	–	9
4	*stx1*	12.75	–	–		12.75	–	–	12.75
5	*stx1*	12.25	–	–	–	–	–	–	8.75
6	*stx1, eae*	12	–	–	15.5	24.5	–	–	10.5
7	*stx1, eae*	10.5	–	–	11.75	12.5	–	–	8
8	*stx1, eae*	–	–	–	–	8.75	–	–	9.25
9	*stx2*	–	12.75	–	–	–	–	–	9.75
10	*stx2, eae*	–	22	–	14.75	–	–	–	10
11	*stx2, eae*	–	17.75	–	12.75	9.5	–	–	8.75
12	*stx2, eae*	–	13.25	–	12.25	9.5	–	–	10.75
13	*stx2, eae*	–	23.75	–	12.5	10	–	–	9.5
14	*stx2, eae*	–	20	–	14.75	–	–	–	9.75
15	*ipaH*	–	–	–	–	–	–	7.25	10.25

[a] *stx*, Shiga toxin; *eae*, intimin; *ipaH*, invasion plasmid antigen H; *ehlyA*, enterohemolysin A, was not included in the reference PCR

[b] IC, inhibition control

that a positive *stx* PCR results does not necessarily implicate the presence of a STEC because the occurrence of free *stx*-carrying phages in stool specimens from healthy donors has been described [25]. STEC can also lose *stx* genes during the isolation procedure [26, 27]. This occurred at least in one case of this study in which a patient suffering from HUS was tested positive for *stx* by BD MAX™ screening PCR but isolates were only positive for *eae* and *ehlyA*.

There is an ongoing discussion on the clinical relevance of a positive *stx* PCR with negative culture in cases without clinical complications [4, 5]. In Germany, as in many other countries, not only is it obligated to report the isolation of STEC in culture to the public health offices but also a culture-independent positive *stx* test. Introduction of broad screening *stx* PCR can lead to a significant increase in notifications of STEC infections, resulting in increased workload and costs for hospitals and laboratories [3]. However, clinical significance of diagnostic reports is often questionable when the STEC cannot be classified as high or low virulent, e.g., in the absence of a culture isolate. High-virulent STEC can cause hemorrhagic colitis and HUS and include strains typically possessing *stx2a*, *2c*, *2d*, or *1a* (the latter included in children < 5 years with bloody diarrhea) [3]. Low-virulent STEC harboring *stx1*, *2b*, *2e*, *2f*, or *2g* can be present in asymptomatic carriers, cause diarrhea, and have also been reported to be associated with long-term gastrointestinal symptoms [28]. In this study, out of a total of 13 STEC isolates 10 were classified as low virulent (Table 2). Most of the low-virulent isolates were of serotype O91:H14 harboring *stx1a* which belongs to the most common *eae*-negative STEC types [29, 30]. The three high-virulent isolates were *stx2a*-positive, as subtyped by microarray, and associated with complicated disease (Table 2). The O157:H7 was isolated from a 5-year-old child with hemorrhagic colitis, the O26:H11 isolate from a 5-year-old child suffering from HUS, and the OR:H2 from a 39-year-old female patient with hemorrhagic colitis and coinfection by *Clostridioides difficile*. All low-virulent STEC were collected from patients with uncomplicated diarrhea. The *stx* subtypes and additional virulence factors were determined by microarray analysis. The results of the eazyplex® EHEC assay for identification and characterization of the culture isolates were in accordance with PCR and microarray with only one exception of a false-positive eazyplex® result for eae [isolate O91:H14 (2)] (Table 2). All isolates harbored additional virulence factors such as adhesins and toxins, as shown by microarray analysis (Table 2). From 15 specimens, STEC infection was confirmed by positive GN enrichment broth but no isolate could be recovered from single colonies (Table 3).

Out of a total of 8 *ipaH* PCR- positive stool samples, 2 specimens yielded *Shigella* isolates, one specimen was culture-positive for EIEC and one specimen was only confirmed by a positive enrichment broth (Tables 2 and 3). Both reference PCR and eazyplex® LAMP correctly identified the isolates by a positive *ipaH* result (Table 2). The EIEC was further characterized by Alere microarray and tested positive for *ipaD* as a marker gene for the invasion plasmid pINV and for *virF*, a regulator of plasmid-encoded virulence genes in *Shigella* and EIEC (Table 2). Nanopore MinION sequencing

Table 4 Overall performance of the eazyplex® EHEC complete assay for identification of STEC in culture

		Reference PCR		eazyplex® performance		
		Positive	Negative	Sensitivity, % (CI[a])	Specificity, % (CI[a])	Accuracy, % (CI[a])
eazyplex®						
stx1	Positive	18	0	94.7 (74–99.9)	100 (88.4–100)	98 (89.2–100)
	Negative	1	30			
stx2[b]	Positive	23	0	100 (85.2–100)	100 (87.2–100)	100 (92.9–100)
	Negative	0	27			
eae	Positive	14	1	93.3 (68.1–99.8)	97.1 (84.7–99.9)	95.9 (86–99.5)
	Negative	1	33			
ehlyA	Positive	25	0	100 (86.3–100)	100 (85.8–100)	100 (92.8–100)
	Negative	0	24			
ipaH	Positive	10	0	100 (69.2–100)	100 (91–100)	100 (92.8–100)
	Negative	0	39			

[a] CI, 95% confidence interval

[b] Including *stx2a-e*, *g*, and *stx2f*

of the isolate revealed 99.37% identity with EIEC O96:H19 (NCBI GenBank number CP011416.1), a new emerging strain that has caused two large food-borne outbreaks of diarrhea in Italy (2012) and the UK (2014) with each > 100 cases [31–33]. In this study, the strain was isolated from a traveler returning from Tunisia in October 2018 and suffering from diarrhea, abdominal pain, and fever up to 38.5 °C. It is important to note that EIEC O96:H19 grows in lactose-positive colonies in contrast to most EIEC and *Shigella* and may easily be overlooked.

Performance of the eazyplex® EHEC assay

Analytical performance of eazyplex® LAMP was calculated by combining data from retrospective analysis of representative strains and prospective testing of isolates and GN broths collected from clinical samples. Both standard routine PCR and Alere microarray were defined as reference. Data on the sensitivity, specificity, and accuracy of the eazyplex® parameters are summarized in Table 4. LAMP showed a reliable accuracy compared to PCR. In all cases, *stx2* was correctly identified and there was only one false-negative result for *stx1* and *eae* from enrichment broth number 8 (Table 3). Data validation for *aggR* could not be performed because of a sample size of 1.

Table 5 Time to result of the eazyplex® EHEC assay

Target gene	*n*	Threshold time [min; mean values (SD)]
stx1	18	11.25 (3.25)
stx2[a]	23	12 (5)
eae	15	12.25 (3.5)
ehlyA	25	13 (5.5)
ipaH	10	8.5 (4.25)
IC[b]	49	9.5 (1.75)

[a] Including *stx2a-e*, *g*, and *stx2f*

[b] IC, inhibition control

A great advantage of the eazyplex® real-time LAMP technology is the easy handling and fast time to result. The mean threshold time for fluorescence intensity values of specific targets ranged from 8.5 to 13 min. IC was detected at a mean time of 9.5 min (Table 5).

Conclusions

The combination of an initial screening PCR for *stx* and *ipaH* with subsequent culture has been recommended for identifying STEC and *Shigella* spp./EIEC infections [34–36]. It is suggested to perform Shiga toxin testing from overnight enrichment cultures or primary isolation agars [37]. Both PCR and rapid antigen tests may be suitable for identifying STEC in culture but antigen tests have lower sensitivities [38]. There is a need for rapid, low-tech, and cost-effective assays to enable simple characterization of STEC isolates [7]. This study demonstrates that the eazyplex® EHEC LAMP assay is a suitable alternative molecular test for the characterization of culture isolates as well as testing of enrichment broths. The test can easily be integrated into the daily workflow because there is no need for DNA extraction, the hands-on time is low, and results are available within half an hour including sample preparation.

A limitation of this study is that the assay was not evaluated for direct detection of pathogens in stool samples. Although the consumable costs of eazyplex® LAMP for a single parameter are comparable to those of PCR the costs for a complete

test strip covering seven specific targets are relatively high. Therefore, it can be suggested to implement the eazyplex® EHEC assay as a culture confirmation test and to use a sensitive *stx* PCR as in initial screening test. Nevertheless, it would be of interest to further investigate whether LAMP reaches sufficient sensitivity for its application as a culture-independent rapid diagnostic test. To estimate the pathogenic potential of isolated STEC and to avoid unnecessary follow-up workload it would be attractive if a rapid LAMP assay would allow the differentiation of high- and low-virulent STEC by separate identification of *stx 2a*, *c*, and *d*.

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Compliance with ethical standards

Ethical approval The study protocol for the evaluation of the eazyplex® EHEC complete assay for clinical samples was reviewed and approved by the ethics committee of the Jena University Hospital (5548-05/18).

Conflict of interest The authors declare that they have no conflict of interest.

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