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Interlaboratory Evaluation of the U.S. Food and Drug Administration *Escherichia coli* Identification Microarray for Profiling Shiga Toxin–Producing *Escherichia coli*

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Abstract

The U.S. Food and Drug Administration *Escherichia coli* Identification (FDA-ECID) microarray provides rapid molecular characterization of *E. coli.* The effectiveness of the FDA-ECID for characterizing Shiga toxin–producing *E. coli* (STEC) was evaluated by three federal laboratories and one reference laboratory with a panel of 54 reference *E. coli* strains from the External Quality Assurance program. Strains were tested by FDA-ECID for molecular serotyping (O and H antigens), Shiga toxin subtyping, and the presence of the *ehxA* and *eae* genes for enterohemolysin and intimin, respectively. The FDA-ECID O typing was 96% reproducible among the four laboratories and 94% accurate compared with the reference External Quality Assurance data. Discrepancies were due to the absence of O41 target loci on the array and to two pairs of O types with identical target sequences. H typing was 96% reproducible and 100% accurate, with discrepancies due to two strains from one laboratory that were identified as mixed by FDA-ECID. Shiga toxin (Stx) type 1 subtyping was 100% reproducible and accurate, and Stx2 subtyping was 100% reproducible but only 64% accurate. FDA-ECID identified most Stx2 subtypes but had difficulty distinguishing among stx_{2a}, stx_{2c}, and stx_{2d} genes because of close similarities of these

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sequences. FDA-ECID was 100% effective for detecting *ehxA* and *eae* and accurately subtyped the *eae* alleles. This interlaboratory study revealed that FDA-ECID for STEC characterization was highly reproducible for molecular serotyping, *stx* and *eae* subtyping, and *ehxA* detection. However, the array was less useful for distinguishing among the highly homologous O antigen genes and the stx_{2a}, stx_{2c}, and stx_{2d} subtypes.

Keywords

Characterization; Microarray; Shiga toxin–producing *Escherichia coli*

Shiga toxin–producing *Escherichia coli* (STEC) strains are a large, complex group of bacteria with various serotypes and phenotypes. The sole characteristic for classification as STEC is the presence of the *stx* genes or the production of Shiga toxin (Stx), with the two main types designated Stx1 and Stx2. Stx1 has three known subtypes (1a, 1c, and 1d), and Stx2 has at least seven known subtypes (2a through 2g) *(*17*)*. Some Stx subtypes are produced mostly by STEC of environmental or animal origin and do not seem to adversely affect humans *(*8, 12*)*, but subtypes Stx1a, Stx2a, Stx2c, and Stx2d are most often associated with severe illnesses such as hemorrhagic colitis and hemolytic uremic syndrome *(*5, 16*)*. Researchers have identified more than 470 STEC serotypes *(*13*)*, and these can produce any one of the Stx subtypes alone or in combination with other subtypes. Therefore, determining the Stx subtype produced by STEC strains can be a complicated and extensive process *(*17*)*. The mechanism for STEC pathogenesis is complex, and the production of Stx alone without an adherence factor is deemed insufficient to cause hemolytic uremic syndrome. The most notable STEC adherence factor is the *eae*-encoded intimin protein. Intimin is also a virulence factor of enteropathogenic *E. coli*, and more than 40 *eae* alleles have been reported *(*14*)*. However, not all STEC possess *eae*. STEC strains that have caused hemolytic uremic syndrome but are *eae* negative, such as STEC serotypes O91:H21 and O113:H21, likely have other means of adherence that have yet to be identified *(*15*)*. Many STEC strains also possess a large virulence plasmid that contains the enterohemolysin-encoding gene *ehxA* *(*2*)*. Although *ehxA* can be present in non-STEC from environmental sources *(*1*)*, it is commonly found in many STEC strains that have caused infections. However, the role of enterohemolysin in STEC pathogenesis remains undetermined; therefore, enterohemolysin is regarded as a putative virulence factor.

The STEC pathotype is highly complex, both phenotypically and genotypically. Characterization of an isolate often entails the use of a large panel of PCR assays to identify the various virulence genes and their alleles or subtypes. *E. coli* has at least 183 somatic (O) and 53 flagellar (H) antigens, so serological typing of STEC is extremely time-consuming and labor intensive. Very few laboratories in the United States can perform complete *E. coli* serotyping analyses, which often can take a few weeks. Because over 50% of the STEC isolates from fresh produce yield only partial serotypes or are not typeable *(*4*)*, traditional serology is not a reliable technique for STEC characterization.

The U.S. Food and Drug Administration *E. coli* Identification (FDA-ECID) microarray, which tests for 41,932 *E. coli* gene targets with a single assay, has been evaluated for its

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potential to quickly characterize STEC isolates. The FDA-ECID microarray assay was more effective and much faster than traditional serology for identifying serotypes of STEC isolates from fresh produce (10) and was an effective tool for strain characterization because it could be used to detect the virulence genes and alleles carried by STEC isolates in foods (11). A single-laboratory validation was performed by an FDA laboratory to verify the effectiveness of the FDA-ECID assay for use in molecular serotyping (3); however, the assay has not been evaluated by other laboratories outside of the FDA. In this study, we performed an interlaboratory evaluation of the FDA-ECID microarray assay. An identical panel of 54 *E. coli* reference strains was sent to laboratories at the Agricultural Research Service of the U.S. Department of Agriculture (USDA), the Centers for Disease Control and Prevention (CDC), and the *E. coli* Reference Center at the Pennsylvania State University. The results obtained by these three laboratories were compared with our results and with the known data for the reference strains to assess the performance and reproducibility of FDA-ECID assay for characterizing STEC strains.

MATERIALS AND METHODS

Bacteria.

The reference strains used in this study were obtained from the WHO Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella* (Copenhagen, Denmark). The Centre conducts a yearly international external quality assurance (EQA) evaluation funded by the European Centre for Disease Prevention and Control (Solna, Sweden), where panels of 10 to 15 reference strains are sent to participants worldwide to evaluate the performance characteristics of the assays used by the respective laboratories to identify and characterize STEC strains (18). For this interlaboratory study, we used the bacterial reference panels from the 2011 to 2014 EQA programs, including nine strains used as representatives for each of the known Stx subtypes, for a total of 54 strains. The strains sent were identified by EQA numbers, but the EQA data for each strain were not provided to the participating laboratories until the end of the study.

FDA-ECID.

Each participating laboratory used the FDA-ECID microarray assay as described previously (14). The R-Bioconductor software packages affy and made4 were used to extract robust multiarray summarized probe set intensities and MAS5 calls (presence and divergence) for each scanned array-generated cel file (6, 7). The custom R-script (R Foundation for Statistical Computing, Vienna, Austria) used by all the laboratories is available upon request.

RESULTS

The FDA-ECID data obtained by the four laboratories were examined for reproducibility for O and H typing, *stx* gene detection and subtyping, and detection of *eae* and *ehxA*. The results were also compared with the EQA data to determine their accuracy for identifying these gene targets.

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O typing.

With the FDA-ECID assay, an O type could be identified in 94% (51 of 54) of the EQA strains (Table 1). According to the EQA data, three nontypeable strains (II9, 481, and 3879) all belong to type O41, an O type not represented on the array. O typing results obtained by the four laboratories were in agreement for 96% (52 of 54) of the strains. The first discrepancy, strain 2745, was positive for both O121 and O166 according to the results obtained by the USDA but was identified by the other laboratories to be O166, consistent with the EQA data. The second discrepancy, strain D4159, was characterized as O128 by the CDC, but the other laboratories identified it as O78, consistent with the EQA data. The FDA-ECID assay also could not precisely determine the O types for strains 1258 (O124 or O164) and D3428 (O118 or O151), which the EQA data indicated were O124 and O118, respectively (Table 1).

H typing.

Three of the 54 strains were classified as H negative according to the EQA data. For these strains (481, 3393, and D3522), the FDA-ECID results from all four laboratories were in agreement and identified these types as H26, H8, and H19, respectively (Table 1). A comparison of the results obtained by the four laboratories with the EQA data revealed an agreement of 96% (49 of 51 strains). Two strains were typed by the USDA laboratory as having two H types: strain 2745 (H15 and H19) and strain 3336 (H11 and H26) (Table 1). However, the other laboratories identified strains 2745 and 3336 as H15 and H11, respectively, consistent with the EQA data. Excluding these two discrepancies, the FDA-ECID H typing results obtained by the four laboratories were the same as the EQA data for 100% (51 of 51) of the isolates tested.

stx$_1$ subtyping.

The FDA-ECID data from all four laboratories identified 31 strains as negative for the Stx1 gene (*stx*$_1$), in agreement with the EQA data (Table 2). The 23 *stx*$_1$-positive strains possessed various known allelic subtypes (*stx*$_{1a}$, *stx*$_{1c}$, and *stx*$_{1d}$), all of which were correctly identified by all four laboratories (Table 2). The USDA laboratory identified one additional strain, 3336, as carrying *stx*$_{1d}$, but the other laboratories determined that this strain was *stx*$_1$ negative, consistent with the EQA data (Table 2). Excluding the USDA result for strain 3336, which appears to be a mixed culture, the overall FDA-ECID *stx*$_1$ subtyping results obtained by the four laboratories were in 100% agreement and consistent with the EQA data.

stx$_2$ subtyping.

The FDA-ECID subtyping data for the Stx2 gene (*stx*$_2$) obtained by all four laboratories were in agreement for 98% (53 of 54) of the strains, including 21 strains characterized as *stx*$_2$ negative. The lone discrepant result was for strain D4159, which the CDC laboratory identified as *stx*$_{2f}$ positive but the other three laboratories characterized as *stx*$_2$ negative, consistent with the EQA data. The FDA-ECID assay consistently made it difficult to distinguish among the *stx*$_{2a}$, *stx*$_{2c}$, and *stx*$_{2d}$ subtypes. These alleles have a high degree of sequence homology, especially *stx*$_{2c}$ and *stx*$_{2d}$, which can differ by as few as two polymorphic sites (17). For strains with *stx*$_{2a}$ alone, the FDA-ECID results from all four

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laboratories correctly identified the subtype as *stx*$_{2a}$ (Table 2). However, for strains with *stx*$_{2c}$ and/or *stx*$_{2d}$ or with *stx*$_{2a}$ in combination with either *stx*$_{2c}$ or *stx*$_{2d}$, the array results from all four laboratories indicated all three *stx*$_2$ subtypes in these strains (Table 2). The FDA-ECID *stx*$_2$ subtyping data matched that of the EQA data for 64% (21 of 33) of the isolates, with all 12 of the discrepancies due to strains with *stx*$_{2c}$ and/or *stx*$_{2d}$.

eae and *ehxA*.

Analysis of the FDA-ECID *eae* results indicated that all four laboratories were in complete agreement on the presence or absence of this gene, with the exception of the USDA result for strain 2745 and the CDC result for strain D4159 (Table 3). The combined FDA-ECID results from the four laboratories matched the EQA data for 53 (98%) of the 54 strains. According to the EQA data, strain D3522 is expected to be *eae* positive, but the FDA-ECID results from all four laboratories indicated that this strain is *eae* negative. The EQA data indicated only the presence or absence of *eae*, but the FDA-ECID assay is capable of allele determination. The *eae* alleles identified with the FDA-ECID assay among the EQA strains are α2, β1, γ1, γ2, ε1, and ι1 (Table 3). Analysis for the presence of *ehxA* revealed that the FDA-ECID results from all four laboratories were in 100% agreement (Table 3). Of the 54 strains tested, 26 strains had *ehxA*, and this result is consistent with the EQA data.

DISCUSSION

The efficiency of the FDA-ECID microarray assay for characterizing STEC strains was evaluated by public health laboratories from three federal government agencies and the *E. coli* Reference Center at the Pennsylvania State University. The FDA-ECID results obtained for the analysis of 54 EQA *E. coli* reference strains were examined for reproducibility (data compared among the four laboratories) and accuracy (compared with the EQA data) for identifying O and H types and Shiga toxin gene subtypes and determining the presence or absence of *eae* and *ehxA* (Table 4).

Three discrepancies were found when comparing the data from the four laboratories. First, O166:H15 strain 2745 was characterized by the USDA laboratory as positive for both O166 and O121. The USDA H type results for this strain were also mixed, listing the strain as positive for H15 and H19. The observation of two O types and two H types obtained for the same sample suggests that the strain 2745 analyzed by the USDA was most likely a mixed culture with the contaminant being an O121:H19 strain, possibly strain 2266 from the same EQA reference set. For strains that have a combination of *stx*$_{2a}$, *stx*$_{2c}$ and/or *stx*$_{2d}$, the probe sets on the array cannot differentiate among the alleles. Because strain 2266 contains *stx*$_{2a}$ and strain 2745 has *stx*$_{2d}$, no contamination is readily apparent. However, further evidence for contamination of the USDA strain 2745 with strain 2266 in indicated by the *ehxA* and *eae* data. For *ehxA*, the hybridization signal intensity for the USDA strain 2745 run was marginal such that the stringent normalization criteria used resulted in a negative result but could also be interpreted as a weak positive result under less stringent conditions. Strain 2745 is supposed to be *eae* negative, but in the USDA analysis, the strain was positive for ε1, which is the allele found in strain 2266. The second discrepancy, strain 3336, was analyzed by the USDA and is likely a mixture of O26:H11 (the real strain 3336) and

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O41:H26 (the contaminant, possibly strain 3879 from the same EQA set). As mentioned previously, O41 is not represented on the array, so a mixed culture of O26:H11 and O41:H26 would give a single O type but two H types on the array, the situation observed with the USDA results for strain 3336. Third, strain D4159 was identified by the CDC as O128:H2 with *stx*$_{2f}$ and β1 *eae*, but the other three laboratories identified this strain as O78:H2 and negative for both *stx*$_2$ and *eae*, which is consistent with the EQA data. These results suggest that the results attributable to strain D4159 by the CDC analysis are actually for a different strain, most likely either strain CC3 or D3546, both of which are O128:H2 with *stx*$_{2f}$ and β1 *eae*.

The FDA-ECID O typing results were 100% reproducible (51 of 51 strains) when the contamination and strain mix-up issues were not included in the calculation. Compared with the EQA data, the FDA-ECID O typing results were in complete agreement for 51 of the 54 strains. Three strains in the O41 serogroup were not typeable because targets for this O type are not represented on the array. The FDA-ECID array was designed when sequence data were available for only 152 of the 183 known O types. Another apparent O typing discrepancy involved strains 1258 and D3428, which were typed by the array as O124-O164 and O118-O151, respectively. The EQA data lists strain 1258 as O124 and strain D3428 as O118. Although the array results seem to be mixed, they were not treated as discrepant. The discriminatory ability of the FDA-ECID probe sets is limited to those O antigens for which the degree of homology is below 98%. Several O types are known to possess highly similar O antigen gene clusters (9), making it difficult if not impossible to distinguish among them with this array. This is the case for both the O124-O164 and O118-O151 pairs of O types. Within each pair, the sequences of the *wzx* and *wzy* loci used as targets on the array are identical or nearly identical.

The reproducibility of FDA-ECID H typing results was determined to be 100% (52 of 52 strains), with 100% accuracy (51 of 51 strains) compared with the EQA data. The calculations for reproducibility excluded the USDA data on the mixed-culture strains 2745 and 3336. An advantage of the FDA-ECID assay over traditional serology is the ability to characterize nonmotile (H negative) strains. For example, 3 of the 54 EQA strains are classified as H negative, but the FDA-ECID assay identified the specific H type for all three strains. By omitting these outliers, both the reproducibility and accuracy of FDA-ECID assay for H typing was 100%. The FDA-ECID array contains probe sets for all 53 known *E. coli* H type genes, and others have reported 96% efficiency for H typing using the FDA-ECID microarray (10, 11).

The reproducibility and accuracy of FDA-ECID assay for *stx*$_1$ detection were 98 and 100%, respectively. Strain 3336 was listed by the USDA laboratory as having *stx*$_{1d}$, but this appears to be a false-positive result due to contamination because the other three laboratories and the EQA data indicated that this strain is *stx*$_1$ negative. The *stx*$_1$ subtyping data obtained by all four laboratories for the 23 *stx*$_1$-positive strains were in 100% agreement and consistent with the EQA data, indicating that the FDA-ECID assay effectively identified all the known *stx*$_1$ subtypes. These results are in agreement with that of a previous study (11), in which the FDA-ECID *stx*$_1$ subtyping results were comparable to those obtained with the standard *stx* subtyping protocol utilizing conventional PCR (17).

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The FDA-ECID *stx*$_2$ subtyping results obtained by the four laboratories were in 98% agreement and were correlated 100% with the EQA data for identifying *stx*$_{2b}$, *stx*$_{2e}$, *stx*$_{2f}$, and *stx*$_{2g}$. The FDA-ECID assay was correctly and consistently identified *stx*$_{2a}$ in strains with *stx*$_{2a}$ alone. However, the array had some difficulty discriminating between *stx*$_{2c}$ and *stx*$_{2d}$ in strains that had these subtypes alone or in combination with *stx*$_{2a}$. The inability of FDA-ECID to differentiate between *stx*$_{2c}$ and *stx*$_{2d}$ has been reported previously (11). The sequences of *stx*$_{2a}$, *stx*$_{2c}$, and *stx*$_{2d}$ are very similar, and *stx*$_{2c}$ and *stx*$_{2d}$ are nearly identical (17). Although the FDA-ECID assay cannot accurately resolve members of the *stx*$_{2a}$, *stx*$_{2c}$, and *stx*$_{2d}$ complex, it does not misclassify them as another *stx*$_2$ subtype. Compared with the EQA data, the accuracy of the FDA-ECID *stx*$_2$ subtyping results was 64% (21 of 33 strains), and all the discrepant results were attributable to the 12 strains with *stx*$_{2c}$, *stx*$_{2d}$, or combinations including these subtypes.

The FDA-ECID assay was very effective for detecting *eae* and *ehxA*, with 100% reproducibility and 98% accuracy for *eae* and 100% reproducibility and 100% accuracy for *ehxA*. This assay also identified the various *eae* alleles with 100% reproducibility. The accuracy of *eae* subtyping could not be determined because the EQA database did not include allelic data; however, the *eae* allele data from all four laboratories were consistent. The 98% *eae* accuracy was due to the results for strain D3522. According to the EQA data, D3522 is *eae* positive, but the FDA-ECID results from all four laboratories indicated that this strain is *eae* negative. This discrepancy could reflect an error in the EQA data, and the accuracy of the *eae* status of strain D3522 in the EQA database remains to be determined.

In conclusion, the results of this interlaboratory study indicate that the FDA-ECID microarray assay is a simple and fast alternative for characterizing selected traits of STEC strains with a high degree of reproducibility. Compared with the EQA database, the FDA-ECID results were accurate for *ehxA* detection, *eae* detection and subtyping, and *stx*$_1$ detection and subtyping. The assay was also able to reliably identify the more distinct *stx*$_2$ subtypes but had some difficulty with *stx*$_{2a}$, *stx*$_{2c}$, and *stx*$_{2d}$. The FDA-ECID assay was highly accurate for O and H serotyping, and the pangenomic platform of the array enabled the detection of mixed strain cultures. The FDA-ECID microarray is a useful tool for stratifying strain identification and assessing the pathogenic potential of STEC and other *E. coli* pathotypes in food and clinical diagnostic laboratories.

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TABLE 1.

FDA-ECID molecular serotyping results obtained by various laboratories[a]

		O type					H type				
Set	**EQA no.**	**EQA**	**CDC**	**ECRC**	**FDA**	**USDA**	**EQA**	**CDC**	**ECRC**	**FDA**	**USDA**
EQA-3	AA1	174	174	174	174	174	8	8	8	8	8
	BB2	55	55	55	55	55	7	7	7	7	7
	CC3	128	128	128	128	128	2	2	2	2	2
	DD4	177	177	177	177	177	25	25	25	25	25
	EE5	111	111	111	111	111	8	8	8	8	8
	FF6	113	113	113	113	113	4	4	4	4	4
	GG7	103	103	103	103	103	2	2	2	2	2
	HH8	26	26	26	26	26	11	11	11	11	11
	II9	41	**NT**	**NT**	**NT**	**NT**	26	26	26	26	26
	JJ10	157	157	157	157	157	7	7	7	7	7
EQA-4	426	146	146	146	146	146	21	21	21	21	21
	481	41	**NT**	**NT**	**NT**	**NT**	–	**26**	**26**	**26**	**26**
	949	157	157	157	157	157	7	7	7	7	7
	1111	113	113	113	113	113	4	4	4	4	4
	1214	157	157	157	157	157	7	7	7	7	7
	1258	124	**124/164**	**124/164**	**124/164**	**124/164**	30	30	30	30	30
	1559	78	78	78	78	78	11	11	11	11	11
	1871	177	177	177	177	177	25	25	25	25	25
	1941	111	111	111	111	111	8	8	8	8	8
	2112	128	128	128	128	128	2	2	2	2	2
	2160	26	26	26	26	26	11	11	11	11	11
	2266	121	121	121	121	121	19	19	19	19	19
	2710	104	104	104	104	104	4	4	4	4	4
	2745	166	166	166	166	**121 & 166**	15	15	15	15	**15 & 19**
	2968	103	103	103	103	103	2	2	2	2	2
EQA-5	D4141	145	145	145	145	145	34	34	34	34	34
	D4142	121	121	121	121	121	19	19	19	19	19

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Set	EQA no.	O type					H type				
		EQA	CDC	ECRC	FDA	USDA	EQA	CDC	ECRC	FDA	USDA
	D4149	166	166	166	166	166	15	15	15	15	15
	D4151	91	91	91	91	91	14	14	14	14	14
	D4152	55	55	55	55	55	7	7	7	7	7
	D4155	103	103	103	103	103	2	2	2	2	2
	D4156	26	26	26	26	26	11	11	11	11	11
	D4158	157	157	157	157	157	7	7	7	7	7
	D4159	78	**128**	78	78	78	2	2	2	2	2
	D4160	111	111	111	111	111	8	8	8	8	8
EQA-6	3336	26	26	26	26	26	11	11	11	11	**11 & 26**
	3393	111	111	111	111	111	–	**8**	**8**	**8**	**8**
	3431	166	166	166	166	166	15	15	15	15	15
	3570	63	63	63	63	63	6	6	6	6	6
	3744	104	104	104	104	104	7	7	7	7	7
	3844	157	157	157	157	157	7	7	7	7	7
	3879	41	**NT**	**NT**	**NT**	**NT**	26	26	26	26	26
	3987	157	157	157	157	157	7	7	7	7	7
	3994	174	174	174	174	174	21	21	21	21	21
	4131	121	121	121	121	121	19	19	19	19	19
STX reference	D2653	157	157	157	157	157	7	7	7	7	7
	D3428	118	**118/151**	**118/151**	**118/151**	**118/151**	12	12	12	12	12
	D3431	157	157	157	157	157	7	7	7	7	7
	D3509	2	2	2	2	2	25	25	25	25	25
	D3522	8	8	8	8	8	−	**19**	**19**	**19**	**19**
	D3546	128	128	128	128	128	2	2	2	2	2
	D3602	174	174	174	174	174	8	8	8	8	8
	D3648	139	139	139	139	139	1	1	1	1	1
	D4134	166	166	166	166	166	15	15	15	15	15

[a]EQA, External Quality Assurance program; CDC, Centers for Disease Control and Prevention; ECRC, *E. coli* Reference Center; FDA, U.S. Food and Drug Administration; USDA, U.S. Department of Agriculture. Differences from EQA data are bold. NT, nontypeable; −, negative.

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TABLE 2.

FDA-ECID stx subtyping results obtained by various laboratories[a]

		stx$_1$					*stx*$_2$				
Set	**EQA no.**	**EQA**	**CDC**	**ECRC**	**FDA**	**USDA**	**EQA**	**CDC**	**ECRC**	**FDA**	**USDA**
EQA-3	AA1	c	c	c	c	c	b	b	b	b	b
	BB2	a	a	a	a	a	–	–	–	–	–
	CC3	–	–	–	–	–	f	f	f	f	f
	DD4	–	–	–	–	–	c, d	**acd**	**acd**	**acd**	**acd**
	EE5	a	a	a	a	a	a	a	a	a	a
	FF6	c	c	c	c	c	b	b	b	b	b
	GG7	a	a	a	a	a	–	–	–	–	–
	HH8	a	a	a	a	a	–	–	–	–	–
	II9	d	d	d	d	d	–	–	–	–	–
	JJ10	–	–	–	–	–	c	**acd**	**acd**	**acd**	**acd**
EQA-4	426	–	–	–	–	–	d	**acd**	**acd**	**acd**	**acd**
	481	d	d	d	d	d	–	–	–	–	–
	949	–	–	–	–	–	a	a	a	a	a
	1111	c	c	c	c	c	b	b	b	b	b
	1214	–	–	–	–	–	a, c	**acd**	**acd**	**acd**	**acd**
	1258	–	–	–	–	–	–	–	–	–	–
	1559	–	–	–	–	–	–	–	–	–	–
	1871	–	–	–	–	–	–	–	–	–	–
	1941	a	a	a	a	a	–	–	–	–	–
	2112	c	c	c	c	c	–	–	–	–	–
	2160	–	–	–	–	–	a	a	a	a	a
	2266	–	–	–	–	–	a	a	a	a	a
	2710	–	–	–	–	–	–	–	–	–	–
	2745	–	–	–	–	–	d	**acd**	**acd**	**acd**	**acd**
	2968	a	a	a	a	a	–	–	–	–	–
EQA-5	D4141	–	–	–	–	–	f	f	f	f	f
	D4142	–	–	–	–	–	a	a	a	a	a

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Set	EQA no.	*stx*$_1$					*stx*$_2$				
		EQA	**CDC**	**ECRC**	**FDA**	**USDA**	**EQA**	**CDC**	**ECRC**	**FDA**	**USDA**
	D4149	–	–	–	–	–	d	**acd**	**acd**	**acd**	**acd**
	D4151	–	–	–	–	–	b	b	b	b	b
	D4152	a	a	a	a	a	–	–	–	–	–
	D4155	a	a	a	a	a	–	–	–	–	–
	D4156	a	a	a	a	a	–	–	–	–	–
	D4158	–	–	–	–	–	a, c	**acd**	**acd**	**acd**	**acd**
	D4159	–	–	–	–	–	–	**f**	–	–	–
	D4160	a	a	a	a	a	–	–	–	–	–
EQA-6	3336	–	–	–	–	**d**	a	a	a	a	a
	3393	a	a	a	a	a	–	–	–	–	–
	3431	–	–	–	–	–	d	**acd**	**acd**	**acd**	**acd**
	3570	–	–	–	–	–	f	f	f	f	f
	3744	c	c	c	c	c	–	–	–	–	–
	3844	–	–	–	–	–	a, c	**acd**	**acd**	**acd**	**acd**
	3879	d	d	d	d	d	–	–	–	–	–
	3987	a	a	a	a	a	a	a	a	a	a
	3994	–	–	–	–	–	b, c	**b, acd**	**b, acd**	**b, acd**	**b, acd**
	4131	–	–	–	–	–	a	a	a	a	a
STX reference	D2653	a	a	a	a	a	a	a	a	a	a
	D3428	–	–	–	–	–	b	b	b	b	b
	D3431	–	–	–	–	–	c	**acd**	**acd**	**acd**	**acd**
	D3509	–	–	–	–	–	g	g	g	g	g
	D3522	d	d	d	d	d	–	–	–	–	–
	D3546	–	–	–	–	–	f	f	f	f	f
	D3602	c	c	c	c	c	b	b	d	b	b
	D3648	–	–	–	–	–	e	e	e	e	e
	D4134	–	–	–	–	–	d	**acd**	**acd**	**acd**	**acd**

[a]EQA, External Quality Assurance program; CDC, Centers for Disease Control and Prevention; ECRC, *E. coli* Reference Center; FDA, U.S. Food and Drug Administration; USDA, U.S. Department of Agriculture. Differences from EQA data are bold. −, negative.

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TABLE 3.

FDA-ECID eae *subtyping and ehxA results obtained by various laboratories*[a]

		eae					*ehxA*				
Set	**EQA no**	**EQA**	**CDC**	**ECRC**	**FDA**	**USDA**	**EQA**	**CDC**	**ECRC**	**FDA**	**USDA**
EQA-3	AA1	−	−	−	−	−	−	−	−	−	−
	BB2	+	γ1	γ1	γ1	γ1	−	−	−	−	−
	CC3	+	β1	β1	β1	β1	−	−	−	−	−
	DD4	+	β1	β1	β1	β1	+	+	+	+	+
	EE5	+	γ2	γ2	γ2	γ2	+	+	+	+	+
	FF6	−	−	−	−	−	+	+	+	+	+
	GG7	+	ε1	ε1	ε1	ε1	+	+	+	+	+
	HH8	+	β1	β1	β1	β1	−	−	−	−	−
	II9	−	−	−	−	−	−	−	−	−	−
	JJ10	+	γ1	γ1	γ1	γ1	+	+	+	+	+
EQA-4	426	−	−	−	−	−	−	−	−	−	−
	481	−	−	−	−	−	−	−	−	−	−
	949	+	γ1	γ1	γ1	γ1	+	+	+	+	+
	1111	−	−	−	−	−	+	+	+	+	+
	1214	+	γ1	γ1	γ1	γ1	+	+	+	+	+
	1258	−	−	−	−	−	−	−	−	−	−
	1559	−	−	−	−	−	−	−	−	−	−
	1871	+	β1	β1	β1	β1	+	+	+	+	+
	1941	+	γ2	γ2	γ2	γ2	+	+	+	+	+
	2112	−	−	−	−	−	−	−	−	−	−
	2160	+	β1	β1	β1	β1	+	+	+	+	+
	2266	+	ε1	ε1	ε1	ε1	+	+	+	+	+
	2710	−	−	−	−	−	−	−	−	−	−
	2745	−	−	−	−	ε1	−	−	−	−	−
	2968	+	ε1	ε1	ε1	ε1	+	+	+	+	+
EQA-5	D4141	+	ι1	ι1	ι1	ι1	−	−	−	−	−
	D4142	+	ε1	ε1	ε1	ε1	+	+	+	+	+

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Set	EQA no	*eae*					*ehxA*				
		EQA	**CDC**	**ECRC**	**FDA**	**USDA**	**EQA**	**CDC**	**ECRC**	**FDA**	**USDA**
	D4149	−	−	−	−	−	−	−	−	−	−
	D4151	−	−	−	−	−	+	+	+	+	+
	D4152	+	γ1	γ1	γ1	γ1	−	−	−	−	−
	D4155	+	ε1	ε1	ε1	ε1	+	+	+	+	+
	D4156	+	β1	β1	β1	β1	−	−	−	−	−
	D4158	+	γ1	γ1	γ1	γ1	+	+	+	+	+
	D4159	−	β1	−	−	−	−	−	−	−	−
	D4160	+	γ2	γ2	γ2	γ2	+	+	+	+	+
EQA-6	3336	+	β1	β1	β1	β1	+	+	+	+	+
	3393	+	γ2	γ2	γ2	γ2	+	+	+	+	+
	3431	−	−	−	−	−	−	−	−	−	−
	3570	+	α2	α2	α2	α2	−	−	−	−	−
	3744	−	−	−	−	−	−	−	−	−	−
	3844	+	γ1	γ1	γ1	γ1	+	+	+	+	+
	3879	−	−	−	−	−	−	−	−	−	−
	3987	+	γ1	γ1	γ1	γ1	+	+	+	+	+
	3994	−	−	−	−	−	−	−	−	−	−
	4131	+	ε1	ε1	ε1	ε1	+	+	+	+	+
STX reference	D2653	+	γ1	γ1	γ1	γ1	+	+	+	+	+
	D3428	−	−	−	−	−	−	−	−	−	−
	D3431	+	γ1	γ1	γ1	γ1	+	+	+	+	+
	D3509	−	−	−	−	−	+	+	+	+	+
	D3522	+	−	−	−	−	−	−	−	−	−
	D3546	+	β1	β1	β1	β1	−	−	−	−	−
	D3602	−	−	−	−	−	−	−	−	−	−
	D3648	−	−	−	−	−	−	−	−	−	−
	D4134	−	−	−	−	−	−	−	−	−	−

[a]EQA, External Quality Assurance program; CDC, Centers for Disease Control and Prevention; ECRC, *E. coli* Reference Center; FDA, U.S. Food and Drug Administration; USDA, U.S. Department of Agriculture; −, negative; +, positive.

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TABLE 4.

Summary of FDA-ECID assay reproducibility among laboratories and accuracy versus EQA data

Locus	Assay	% reproducibility (no. of strains/total strains)	% accuracy (no. of strains/total strains)
O	Serotyping	96 (52/54)	94 (51/54)
H	Serotyping	96 (52/54)	100 (51/51)
stx$_1$	Detection	98 (53/54)	100 (54/54)
	Subtyping	100 (23/23)	100 (23/23)
stx$_2$	Detection	98 (53/54)	100 (54/54)
	Subtyping	100 (33/33)	64 (21/33)
eae	Detection	98 (53/54)	98 (53/54)
	Subtyping	100 (30/30)	ND[a]
ehxA	Detection	100 (54/54)	100 (54/54)

[a]ND, not determined; the EQA database did not contain *eae* allelic information, so accuracy could not be determined.