Journal of Food and Drug Analysis

Volume 9 | Issue 4 Article 3

Evaluation of commercially available kits for detection of Escherichia coli O157

Journal of Food and Drug Analysis, Vol. 9, No. 4, 2001, Pages 207-214 藥物食品分析 第九卷 第四期

Evaluation of Commercially Available Kits for Detection of *Escherichia coli* O157

LIH-CHING CHIUEH*, FENQ-RU CHEN AND DANIEL YANG-CHIH SHIH

National Laboratories of Foods and Drugs, Department of Health, Executive Yuan
161-2, Kuen Yang Street, Nankang 115, Taipei, Taiwan, R. O. C.

(Received: April 25, 2000; Accepted: June 26, 2001)

ABSTRACT

In this study, four commercially available kits specific for *Escherichia coli* O157 and one PCR method were evaluated for their performance while applied to detect *E. coli* O157:H7 in foods. Using 69 pure strains, the specificity and sensitivity rates were both 100% as determined on the base of O157-antigen. While targeted on both O157 and H7 antigens, all tested kits showed 100% sensitivity rates, but specificity rates decreased to 89.6%. The detection limits of the four kits ranged from 10^3 to 10^4 CFU/mL. Both the specificity rate and sensitivity rate of the PCR method were 100%, with a detection limit of 10^3 CFU/test. This study indicated that these four commercial kits were designed only for O157 antigen of *E. coli*, suggesting that these kits are only useful for prescreening of O157 antigen of *E. coli*. Positive samples tested by the kits should be confirmed by other appropriate methods following isolation of suspect colonies. Inoculation tests revealed that one *E. coli* O157 cell in one gram sample could be detected following enrichment procedures. When the four kits were applied to examine market food samples, all gave different degrees of false-positive results. Hence, these kits can only be used for screening. No false-positive results were obtained by the PCR method.

Key words: *E. coli* O157, detection, kits

INTRODUCTION

The first case report of an *E. coli* O157: H7 outbreak occurred in 1982. Since then, food poisoning outbreaks caused by *E. coli* O157: H7 have become a common occurrence worldwide. The *E. coli* O157: H7 outbreak, which occurred in Japan in 1996 was the largest in scope [1]. *E. coli* O157: H7 was classified as an enterohemorrhagic *E. coli* (EHEC). It is also named as shiga toxin-producing *E. coli* (STEC), which was usually called verotoxin-producing *E. coli* (VTEC), since it is capable of producing shiga-like toxin (SLT). It has been confirmed that the STEC strains include more than 100 serotypes[2].

In addition to producing shiga-like toxin, the pathogenic mechanisms of EHEC might be related to an adherence factor and production of enterohemolysin[2]. Unlike other *E. coli* strains, *E. coli* O157: H7 lacks β-D-glucuronidase activity and is unable to ferment sorbitol except for some variants, which possess β-D-glucuronidase and are capable of fermenting sorbitol[3]. STEC is a pathogen that is transmissible between humans and animals. The major route of infection is via consuming undercooked ground beef. Cow and sheep faeces are considered as the sources of contaminants[2, 4]. The infection dosage of *E. coli* O157: H7 is less than 100 CFU. Children and old people are susceptible to be infected with *E. coli* O157: H7. A few serious symptoms of infection may accompany the following symptoms: hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP)[2].

Traditional methods for STEC inspection include five steps: enrichment culture, selective culture, biochemical identification, serotype confirmation test, and toxin test (5). It takes at least 2 days to get a negative test result and 1~3 more days to get a positive result. Because traditional test methods are time consuming, several rapid test methods have been thus developed. These include selective culture biochemical identification method[2], enzyme-linked immunosorbent assay[6, 7], DNA probe[7, 8], latex agglutination[9], immunoprecipitation[8, 10], immunomagnetic separation[7, 10], and PCR method[11, 12]. The above test methods can be classified into two groups. The first group is specific to the *E. coli* O157: H7 test and most of the test methods are designed for this purpose. For example, by using Petrifilm *E. coli* O157: H7, food samples with proper dilution are ready for testing. Culture media enrichment is the only step to carry out for obtaining a positive or negative result when EHEC-TEK, VIDAS, PATH-STIK *E. coli* O157: H7, VIP and Reveal methods are used. Several selective culture media were modified, such as Ranbow Agar O157, sorbitol MacConkey Agar (SMAC), cefixime potassium tellurite-sorbitol MacConkey Agar (CT-SMAC), Fluorocult HC, and Fluorocult *E. coli* O157: H7 Agar. RIM, Singlepath, and MicroScreen are the test kits designed for testing colonies isolated from the selective enrichment culture. The other group is used for STEC testing. These test kits, including Verotox-F, Premier EHEC, and Tox-Tech O157: H7, are used to test the toxin produced from STEC.

Since July 1996, we have been collecting raw meat, raw milk, and animal faeces, to inspect the status of STEC contamination. In the meantime, inspection and serotyping methods were also developed[13, 14]. In order to operate in coordi-

* Author for correspondence. Tel:02-26531273; Fax:02-26531268; E-mail:clc1025@nlfd.gov.tw

nation with joining APEC and the WTO that will increase international food circulation, a rapid STEC inspection method has to be developed to ensure food hygiene safety. Several commercial test kits were evaluated, and compared to the PCR method currently used in our laboratory for developing a test procedure, which can practically be applied for food inspection. Initially, the STEC strain and other reference strains were used as the test strains. After setting up a method, the food samples spiked with pure culture were then tested. Finally, a reliable method was set up and used to test the marketed food samples.

MATERIALS AND METHODS

I. *Materials*

(I) *Chemicals*

Novobiocin was purchased from Sigma (St. Louis, Missouri, USA). Cefixime, potassium tellurite, and glycerol were obtained from Merck (Darmstadt, Germany).

(II) *Instruments*

A microorganism auto-analyzer (model: Vitek Auto Microbic System) and VIDAS auto-analyzer (model: mini VIDAS) were purchased from bioMerieux Vitek, Inc. (Hazelwood, Missouri, USA). A PCR reactor with Programmable Thermal Controller, PTC-100 was made by MJ Research (Water Town, Massachusetts, USA).

(III) *PCR Primer and Reagents*

Three primers specific to shiga toxin gene and *eaeA* gene of EHEC (Table 1) were synthesized by TIB Molbiol (Berlin, Germany). DynaZyme DNA Polymerase kit was purchased from Finnzyme (Espoo, Finland).

(IV) *Test Kits*

The following test kits were used in this study: Reveal *E. coli* O157: H7 Test System (Neogen, Lansing, Mississippi, USA); VIDAS *E. coli* O157 (bioMerieux, Vitek, Inc., Hazelwood, Missouri, USA); PATH-STIK One Step Rapid *E. coli* O157 Test (Celsis Lumac, B. V. Landgraaf, The Netherlands); Visual Immunoprecipitate Assay (VIP) for EHEC (BioControl System, Inc. Bellevue, Washington, USA).

(V) *Pathogenic E. coli Serum and Gram Negative Identification Card*

Forty-three pathogenic *E. coli* O-antiserums and 22 H-antiserums were obtained from Denka Seiken (Tokyo, Japan). Gram negative identification card was supplied by Vitek Systems (Hazelwood, Missouri, USA).

(VI) *Culture Media and Agar*

The following culture media and agar were used in this study: Eosin methylene-blue lactose sucrose Agar (EMB), Tryptic Soy Agar (TSA) (Difco, Detroit, Michigan, USA), sorbitol MacConkey Agar (Oxoid, Hampshire, England), mTSB-Broth with novobiocin (20 mg/L), cefixime (50 μg/L) and potassium tellurite (2.5 mg/L)-MacConkey Broth (CT-MB), Fluorocult *E. coli* O157:H7 Agar (Merck, Darmstadt, Germany), and Agarose (Amresco, Solon, Ohio, USA).

(VII) *Test Strains*

In total, 69 test strains were used in this study. These include 20 strains of *E. coli* O157: H7 (CCRC13086, CCRC 13087, CCRC13088, CCRC13089, CCRC13090, CCRC13091, CCRC13092, CCRC13094, CCRC13095, CCRC13096, CCRC13097, CCRC13098, CCRC13099, CCRC15373, CCRC15374, CCRC15376, CCRC15377, CCRC15970, CCRC14824, CCRC14825) and *E. coli* O157: H7 CLC136B-2 isolated from sheep faeces in our laboratory; 14 strains of STEC and non- *E. coli* O157: H7 (CLCM213-2, CLC164A-6, CLC184A-6, CLC10B-1, CLC21C-11, CLC38A-19, CLC55B-17, CLC81C-10, CLC94C-4, CLC116A-4, CLC179B-16, CLC231C-3, CLC203C-6, CLC273C-4), which were isolated from faeces and raw milk of cow and sheep; 12 strains of non-STEC *E. coli* and 22 strains of others as listed in Table 2. *E. coli* CCRC 14824, which possesses *eaeA* and *hlyA* genes and is capable of producing SLT1 and SLT2, was used as reference strain of EHEC. They were obtained from the Culture Collection and Research Center at Food Industry Research & Development Institute or isolated in our laboratory.

(VIII) *Test Samples*

Twenty test samples of chicken and pork were collected from local markets in northern Taiwan during March to April 1999. Samples were kept in a refrigerator while shipping to the laboratory.

Table 1. Primers used in this study

Primer	Sequence 5'-3'	Gene	Amplicon (bp)	Reference
LP30	CAG TTA ATG TGG TGG CGA AGG	*slt1*	348	(12)
LP31	CAC CAG ACA ATG TAA CCG CTG			
LP43	ATC CTA TTC CCG GGA GTT TAC G	*slt2*	584	(12)
LP44	GCG TCA TCG TAT ACA CAG GAG C			
AE19	CAG GTC GTC GTG TCT GCT AAA	*eaeA*	1087	(15)
AE20	TCA GCG TGG TTG GAT CAA CCT			

Journal of Food and Drug Analysis, Vol. 9, No. 4, 2001

II. *Methods*

(I) *Test for the Specificity and Sensitivity of Kits and PCR Method*

Sixty-nine test strains were streak cultured in EMB or TSA media and incubated at 37˚C overnight. One colony was then transferred to TSA medium, incubated at 37˚C overnight, transferred to a 0.45% saline solution, and adjusted to a concentration of McFarland:2 suspension. Above suspension (0.5 mL) was inoculated to a mTSB+novobiocin enrichment culture media (9 mL, once enrichment broth) and incubated at 42˚C for 6 hrs. One mL of above broth was then inoculated to a CT-MB enrichment media (9 mL, twice enrichment broth). Both mTSB+novobiocin and CT-MB media were incubated at 37˚C for 14 and 18 hrs, respectively. The test kits were kept at room temperature for 30 min prior to test. After finishing enrichment procedure, the enrichment broth (100 μL) was then reacted with kits. All the test procedures were followed according to the operation manual. The reaction times were as follows: Reveal, 15~20 min; PATHSTIK, 5 min; VIP, 10 min. By using VIDAS kit, the twice enrichment broth (1 mL) was kept at 100˚C for 15 min and 500 μL of the resulting broth was then placed in a mini VIDAS auto-analyzer for reaction. PCR reaction was conducted as follows. The once enrichment broth (0.5 mL) was centrifuged. The precipitate was then washed with sterile water twice and re-suspended in 300 μL sterile water. Twenty μL of suspension was then sampled for PCR reaction where AE19 and AE20 were used as primers.

(II) *Test for Detection Limit of Kits and PCR Method*

One colony of *E. coli* O157: H7 (*E. coli* CLC136-B2) was transferred to a mTSB enrichment broth, inoculated at 37˚C overnight, and then made a series dilution with 0.85% saline solution. The dilution solutions ranged from 10^3 to 10^6 CFU/mL were tested for detection limit of kits and PCR method.

(III) *Application of Kits and PCR Method on Spiked Samples*

Five test samples (egg, ground chicken meat, beef slice, ground pork, and mutton slice) were stirred, well mixed, partitioned into a sterile bag with 25 g of each, and immediately quick-frozen. After irradiated with γ-ray (10 kGy), the test samples were spiked with 1 mL of strain mixture and 225 mL of mTSB+novobiocin enrichment broth. Four strain mixtures were conducted in this study. Two of them contained equal concentration of five *E. coli* O157: H7 strains (CCRC 14824 , CCRC 14825 , CCRC 13087 , CCRC 13088 , CCRC 13094), which were mixed with test samples and enrichment broth to make a final concentration of 10^0 and 10^2 CFU/g samples. The other two strain mixtures containing equal concentration of five non-O157 *E. coli* (CCRC14917 , CLC94C-4 , CLC116A-4 , *Citrobacter freundii* M264-2 , *Enterobacter cloacae* 137D-16) was diluted to 10^2 and 10^4 CFU/g samples. Five detection methods were conducted after culture enrichment as described.

(IV) *Isolation and Identification of E. coli O157 from Commercial Samples*

Test samples (25g) mixed with 225 mL of mTSB+novobiocin enrichment culture[5] were incubated at 42˚C for 6 hrs (referred to as once enrichment culture). One mL of resulting broth was transferred into a 9-mL CT-MB and TSB enrichment culture (referred to as twice enrichment culture) and incubated at 37˚C for 18 hrs. The once enrichment culture was also incubated at 37˚C for 14 hrs. Above twice TSB enrichment cultures (0.5 mL) were then tested by PCR reaction (including *slt1*, *slt2*, and *eaeA* genes). Either the once or twice enrichment culture was tested by commercial kits. The once or twice enrichment culture of test samples, which showed a positive result as tested by kits or the PCR method, were further streak cultured on sorbitol MacConkey and EMB media at 37˚C overnight. The strain colonies with typical characteristics were then transferred to TSA culture medium and incubated at 37˚C overnight for further *slt* and *eaeA* gene-PCR reaction, biochemical and serotype identification. The once enrichment culture of test samples with negative results was also cultured as described above. The colonies with typical characteristics were also selected for further confirmation.

(V) *Confirmation of Pathogenic Gene in STEC*

A PCR method[12, 15] was carried out for pathogenic gene confirmation. The primers listed in Table 1 were used in this study. PCR reagent was prepared by mixing 63.8 μL of water with DynaZyme DNA Polymerase kit, which was composed of 10 μL of 10-folds buffer solution containing 1.5 mM Mg^{2+}, 1.5 μL of dNTPs (200 μM), DNA polymerase (0.5 unit), and 1 μL of each primer (100 μM). PCR reaction was performed by transferring one loop of colony to a microcentrifugation tube which contained 300 μL of sterile water, placed in boiling water for 10 min. After cooling, 20 μL of resulting solution was transferred to another centrifugation tube where the PCR reagent and one drop of mineral oil were then added. The centrifugation tube was then incubated in a PCR thermocycler under the following program: 94˚C for 4 min followed by 94˚C for the another 1 min, 60˚C for 2 min, and finally 72˚C for 2 min (in total, 35 cycles of above program was performed). The PCR products were analyzed using a 2% agarose gel electrophoresis. If the enrichment culture broth was to be tested, the following procedure was required. The culture broth (0.5 mL) was centrifuged. The precipitate was washed with sterile water twice and re-suspended in 300 μL sterile water. Twenty μL of which was then tested by PCR reaction.

(VI) *Biochemical and Serotype Tests*

Biochemical test was performed using a Gram negative

identification card and analyzed using Vitek microbial auto-analyzer. The test strains identified to be *E. coli*, as analyzed by using Vitek microbial auto-analyzer, were sero-typed by using 43 commercially available O-antiserums of pathogenic *E. coli*.

(VII) *Culture Preservation*

The cultures, which were confirmed to be *E. coli* strains, were frozen preserved at -70˚C. Prior to preservation, the colonies in TSA culture medium were transferred into a TSB culture vial containing 20% glycerol.

(VIII) *Definition of Specificity and Sensitivity*

1. Specificity to O157 antigen

Specificity (O157 antigen) = NS(–)/[NS(–)+NS(+)] × 100%.

Where NS(–) is the number of negative reaction on *E. coli* O157 and NS(+) is the number of false positive reaction on *E. coli* O157.

2. Specificity to O157 and H7 antigens

Specificity (O157 and H7 antigens) = NS(–)/[NS(–)+ NS(+)] × 100%.

Where NS(–) is the number of negative reaction on *E. coli* O157: H7; NS(+) is the number of false positive reaction on *E. coli* O157: H7.

3. Sensitivity to O157 antigen

Sensitivity (O157 antigen) = S(+)/[S(+)+S(–)]×100%.

Where S(+) is the number of positive reaction on *E. coli* O157 and S(–) is the number of false negative reaction on *E. coli* O157.

Table 2. Results of 69 strains tested by 4 commercially available kits and PCR method

Strains	No. of strains	No. of positive				
		Reveal	VIDAS	Path-Stik	VIP	PCR
E. coli O157:H7	21 [a]	21	21	21	21	21
E. coli O157:H? CLC 41-3	1 [b]	1	1	1	1	0
E. coli O157:NM CLC C14-3	1 [b]	1	1	1	1	0
E. coli O157:NM CLC C35-3	1 [b]	1	1	1	1	0
E. coli O157:H41 CLC P7-1	1 [b]	1	1	1	1	0
E. coli O157:H45 CLC 159B-1	1 [b]	1	1	1	1	0
non-*E. coli* O157:H7 STEC	14 [b]	0	0	0	0	0
E. coli O25:NM CCRC 15370	1	0	0	0	0	0
E. coli O78:H11 CCRC 15372	1	0	0	0	0	0
E. coli O78:H12 CCRC 15371	1	0	0	0	0	0
E. coli O124:NM CCRC 15375	1	0	0	0	0	0
E. coli O26 CCRC 14917	1	0	0	0	0	0
E. coli O111 CCRC 14918	1	0	0	0	0	0
E. coli O1:H7 CCRC 10675	1	0	0	0	0	0
Enterobacter aerogenes CLC M213-12	1 [b]	0	0	0	0	0
Enterobacter cloacae CLC 137D-16	1 [b]	0	0	0	0	0
Pseudomonas aeruginosa ATCC [c] 27853	1	0	0	0	0	0
Pseudomonas aeruginosa CLC 203C-7	1 [b]	0	0	0	0	0
Klebsiella pneumoniae CLC 192A-3	1 [b]	0	0	0	0	0
Citrobacter freundii CLC M246-1	1 [b]	0	0	0	0	0
Citrobacter freundii CLC M264-2	1 [b]	0	0	0	0	0
Bacillus cereus CLC01	1 [b]	0	0	0	0	0
Bacillus thuringiensis ATCC 10792	1	0	0	0	0	0
Listeria monocytogenes ATCC 19115	1	0	0	0	0	0
Staphylococcus aureus ATCC 12606	1	0	0	0	0	0
Vibrio cholerae O145-6Vc	1	0	0	0	0	0
Yersinia enterocolitica 2635	1	0	0	0	0	0
Yersinia enterocolitica 964	1	0	0	0	0	0
Vibrio parahaemolyticus CLC01	1 [b]	0	0	0	0	0
Salmonella typhimurium CCRC 12947	1	0	0	0	0	0
Salmonella typhimurium CCRC 10747	1	0	0	0	0	0
Shigella spp.-Lin	1 [b]	0	0	0	0	0
Shigella dysenteriae CCRC 13983	1	0	0	0	0	0
Shigella sonnei CCRC 15966	1	0	0	0	0	0
Aeromonas hydrophila CCRC 13880	1	0	0	0	0	0
Proteus mirabilis-Tsai	1 [b]	0	0	0	0	

[a]: From Culture Collection & Research Center (CCRC, Hsinchu, Taiwan) or isolated by National Laboratories of Foods and Drugs (NLFD, Taipei, Taiwan),[b]: Isolated by NLFD,[c]: From American Type Culture Collection (ATCC, Manassas, Virginia).

Journal of Food and Drug Analysis, Vol. 9, No. 4, 2001

4. Sensitivity to O157 and H7 antigens

Sensitivity (O157 and H7 antigens) = S(+)/[S(+)+S(–)] ×100%.

Where S(+) is the number of positive reaction on *E. coli* O157:H7; S(–) is the number of false negative reaction on *E. coli* O157:H7.

RESULTS AND DISCUSSION

I. *Specificity, Sensitivity, and Detection Limit of Kits and PCR Method*

A pure culture was used to evaluate the specificity, sensitivity, and detection limit of kits. Sixty-nine strains of *E. coli* were inoculated in the enrichment culture for both specificity and sensitivity tests. Four test kits showed 100% specificity to O157 antigen, and 89.6% specificity to both O157 and H7 antigens. The sensitivity of all test kits was 100%. Because the research and development of commercial kits is confidential, the constitution of commercial kits for detection of *E. coli* O157 is not available. In this study, the following strains: *E. coli* O157:H7, *E. coli* O157:H41, *E. coli* O157:H45, *E. coli* O157:H7 and non-*E. coli* O157:H7 STEC (Table 2) were selected to resolve the antibody constitution in these kits. Results showed that these kits were specific to O157 antigen, H7 antigen, or Shiga toxin. Of the 4 test kits, two of them (VIDAS and PATH-STIK) were specific to *E. coli* O157 according to the label; while the others (VIP and Reveal) were not clearly labeled for *E. coli* O157 or *E. coli* O157: H7. The results for specificity test in this study revealed that an O157 antigen-antibody reaction was observed but no H7 antigen included as using the above 4 kits. This indicates that a positive result would appear as the samples contain *E. coli* O157 whether including H7 antigen or not. A further test is required to confirm if H7 antigen exists in samples. Some *E. coli* O157 (non-H7) strains such as *E. coli* O157: H3, O157: H12, O157: H16, O157: H38, O157: H43, and O157: H45, which are non-pathogenic strains and not capable of inducing food poisoning, are commonly found in food samples according to Feng[16]. Above strains and some non-*E. coli* strains can cause a false-positive reaction. The cross-reaction caused by *Salmonella giessen* (PATH-STIK)[17], by *Salmonella* serogroup N and *Citrobacter* (VIDAS)[18], and by *Salmonella* spp. (VIP)[19] have been reported. However, a negative result was observed when *Citrobacter freundii* and *Salmonella* spp. were tested in our study. No false negative reaction was found in this study indicating the sensitivity was 100%. Both specificity and sensitivity by using PCR method were 100%. The primers, AE19 and AE20, used in this study were specific to the 3'-terminor of *eaeA* gene in *E. coli* O157: H7[15]. Thus, only if the *E. coli* strains containing O157: H7 serotype with *eaeA* gene could produce PCR fragment. Most of the pathogenic *E. coli* O157: H7 strains contain *slt*, *eaeA*, and *hlyA* genes, which are considered as the pathogenic genes in EHEC[2]. A few PCR primers, which are specific to O157-antigen[20], H7-antigen[21], *slt* gene[12], *eaeA* gene[15], *hlyA* gene[22], or *uidA* (β-glucuronidase) gene[12], are currently developed for *E. coli* O157: H7 testing. Therefore, the purpose of detecting several genes at the same time can be achieved by selecting different primers for PCR testing.

The detection limits performed by using several concentrations of strains diluted from pure enrichment culture, were 10^4 CFU/mL for Reveal, VIP, and VIDAS, 10^3 CFU/mL for PATH-STIK, and 10^3 CFU/test (10^4 CFU/mL) for PCR method. According to the makers, the detection limits for VIDAS, Reveal, and PATH-STIK were 10^4 CFU/mL[18], 10^4-10^5 CFU/mL[23], and 5×10^5-5×10^6 CFU/mL[17], respectively. A similar result for detection limit of VIDAS and Reveal kits between the studies from the makers and our laboratory was observed. The data for detection limit of PATH-STIK generated from makers was higher than that from our laboratory. This could be due to the difference in test samples. Those test samples used by makers were the strain culture from food which might contain interference which reduces the detection ability. The detection limit for the PCR method was reportedly capable of reaching as low as 10^0 CFU/test[24], which was more sensitive than the result of our test (10^3 CFU/test). The different test strains could result in a difference in detection limit. According to the literature, a pure culture strain was used for detection limit test that may give the lower detection limit. The enrichment culture used in our study may have interference which could affect the test result. In practical terms, the enrichment culture is recommended for real sample testing when the PCR method is used because it usually can give a better result[20]. The culture enrichment step has been routinely used for *E. coli* O157:H7 testing. Theoretically, this step can increase the strain concentration up to 10^4 CFU/mL. Therefore, the detection limit of the PCR method or commercial kits could meet the requirement of real sample testing.

II. *Testing the Commercial Kits and PCR Method by Food Samples Spiked with Pure Culture*

Five test samples (egg, ground chicken meat, beef slice, ground pork, and mutton slice) individually spiked with 4 strain mixtures. Five *E. coli* O157: H7 strains were mixed in proportion to make 2 concentrations of mixtures (10^0 and 10^2 CFU/g sample). Five non- *E. coli* O157: H7 strains were mixed in proportion to make 2 concentrations of mixtures (10^2 and 10^4 CFU/g sample). Result showed that no false negative and no false positive for the test groups of *E. coli* O157: H7 strains and non-*E. coli* O157: H7 strains, respectively, revealing the detection limit of sample could reach as low as 10^0 CFU/g. According to the VIDAS data supplied by the maker, there was no false negative reaction occurred when 65 cheese samples spiked with ≥7 CFU/25g *E. coli* O157: H7 were tested[25]. The detection limit of sample conducted by Reveal was 100 CFU/25g[23]. The poisoning dose of *E. coli* O157: H7 is less than 100 CFU[2]. Thus, in theoretical, the detection limit lower than 100 CFU/25g could meet the inspection requirement.

III. *Application of Commercial Kits and PCR Method on Isolation of E. coli O157 from Marketed Samples*

Twenty samples each of chicken and pork were enriched and tested by the traditional method, PCR method, and commercial kits. Results showed that 6 (30%), 4 (20%), and 2 (10%) chicken samples as tested by Reveal, VIDAS, and PATH-STIK, respectively, appeared to be false positive; while no false positive reaction was found as tested by VIP kits and the PCR method. Results also showed that 4 (20%), 2 (10%), and 1 (5%) pork samples were found to be false positive as tested by Reveal and PATH-STIK, VIDAS, and VIP kits; while no false positive reaction was found as tested by the PCR method (Table 3). It was reported that the Reveal kit could result in less than 1% false positive after testing 16,000 ground beef samples[23]. The false positive ratios by VIP kit testing were reported to be 1% on apple juice, 0% on milk, 5-8% on ice cream, 7-8% on ground beef, and 8-25% on ground poultry meat[19]. The test samples used in this study were obtained from local traditional markets and may be contaminated with various microorganisms resulting in high ratios of false positive reaction when tested by VIDAS, Reveal, and PATH-STIK kits. However, the VIP kit showed fewer false positive reactions as compared to the data supplied by the maker. The primers, AE19 and AE 20, used for PCR testing were shown highly specific to *E. coli* O157: H7[15], and no false positive reaction was found as using above primers for PCR testing. In this study, the PCR method was also used to test the Shiga toxin gene. Results indicated that no PCR toxin gene product was observed. *E. coli* O157 or STEC strains could not be isolated from 40 test samples. However, by using traditional methods[5], the following strains could be isolated from pork samples: *E. coli* O8, *E. coli* O124, *E. coli* O153, *Hafnia alvei*, and *Citrobacter freundii*. The following strains could be isolated from chicken samples: *E. coli* O6, *E. coli* O8, *E. coli* O20, *E. coli* O29, *E. coli* O153, *Hafnia alvei*, and *Citrobacter freundii*. The strains *Enterobacter*, *Proteus*, and *Hafnia* accompanied with *E. coli* O157: H7 can possibly be isolated from milk cultured in a Sorbital MacConkey medium, because those strains carry similar characteristics to *E. coli* O157: H7, which is non-sorbitol fermenting and lacking of β-D-glucuronidase activity[26].

Table 3. Results of false-positives of marketed meat samples tested by 4 commercially available kits and PCR method

Sample	False-positive rate of commercially available kits %				
	Reveal	VIDAS	PATH-STIK	VIP	PCR
Chicken	30 (6/20)	20 (4/20)	10 (2/20)	0 (0/20)	0 (0/20)
Pork	20 (4/20)	10 (2/20)	20 (4/20)	5 (1/20)	0 (0/20)

IV. *Evaluation of the Expenses, Labor Cost, Operation Procedure of Kits and PCR Method*

In addition to accuracy, the expense, labor cost, operation procedure, and time are factors which are necessary to take into consideration for selection of test methods. A comparison among those commercial kits and the PCR method is listed in Table 4 based on the results of our study. A mini VIDAS instrument is required to run the VIDAS kit and a PCR reactor is necessary for PCR reaction. An incubator is the only equipment needed for operation of the other 3 test kits. The costs of each test were the purchase price and could be a general reference. Among them, PCR is the most economic method in terms of the cost of each test. The times necessary for reaction are as follows: PATH-STIK (5 min), VIP (10 min), Reveal (15-20 min), VIDAS (45 min), PCR (4-5 hrs). The operation procedures for Reveal, VIP, and PATH-STIK are quite straightforward. Results can be read upon adding strain culture with test kits. To perform VIDAS test, samples have to be incubated at 100˚C for 15 min, placed in a instrument and reacted for 45 min. The result can be read automatically by instrument. The capacity of each batch for reaction is 12 test samples. The operation procedure of PCR reaction is complicated. In addition to 4-5 hrs PCR reaction, other procedures such as sample pretreatment and electrophoresis and dying after reaction are required. Four commercial kits tested in this study are only specific to *E. coli*

Table 4. Comparison of four commercially available kits and PCR method

Factor	Kits				
	Reveal	VIDAS	PATH-STIK	VIP	PCR
Instrument	Incubactor	Incubactor mini VIDAS	Incubactor	Incubactor	Incubactor PCR thermocycler
Cost / Test (NT)	680[a]	400[a]	280[a]	300[a]	20[a]
Time	15-20 min	45 min	5 min	10 min	4-5 hr
Procedure	Easy	Moderate	Easy	Easy	Complex
Specificity rate (%) (O157 antigen)	100	100	100	100	100
Specificity rate (%) (O157&H7 antigen)	89.6	89.6	89.6	89.6	100
Sensitivity rate (%)	100	100	100	100	100
Detection limit [b](CFU/g)	10^0 CFU/g	10^0 CFU/g	10^0 CFU/g	10^0 CFU/g	10^0 CFU/g
Detection limit [c](CFU/mL)	10^4	10^4	10^3	10^4	10^4

[a]: This price is intended for general reference only.

[b]: The detection limit of sample, that means one *E. coli* O157 cell in one gram sample could be detected following enrichment procedures.

[c]: The detection limits of four kits and the PCR method.

O157 antigen but unable to differentiate H7 flagellum antigen. While the PCR method, which is specific to *eae*A gene of *E. coli* O157: H7, is capable of distinguishing *E. coli* O157: H7 from *E. coli* O157[15]. Therefore, only the strains carry the *eae*A gene can get the positive result. This drawback can be overcome by using multiple primers specific to the genes other than *eae*A for PCR testing. No false negative reaction occurred when the above 5 methods were tested, showing 100% sensitivity was reached. The detection limits for commercial kits and test samples were 10^3-10^4 CFU/mL and 10^0 CFU/g, respectively, which all meet the inspection requirement.

In summary, the 4 commercial kits tested in this study are designed for testing a single serotype of *E. coli* O157 strains and can only be used to initially test if the samples contaminated with *E. coli* which contains the serotype O157. Further isolation and confirmation steps are required to confirm if the samples are contaminated with strain *E. coli* O157: H7. So far, the commercial kits are labeled to contain 2 monoclonal antibodies, which are specific to *E. coli* O157: H7 and NM (non-motile) without producing a cross-reaction with *E. coli* O157: H12, *E. coli* O157: H3, *Salmonella* group N, *Citrobacter*, and *Yersinia*[27]. The above product information was not yet obtained in test kits evaluation, but it is certain that the test samples with positive reaction should be performing the following test: strain isolation and confirmation. The PCR method used in this study showed excellent specificity, but is complicated in operation and extra equipment is required to perform this test. It is not suitable to be used in a quality control department or laboratory of general food processors. There were no significant differences among 4 commercial kits in specificity, sensitivity, and detection limit. In consideration of equipment investment and operation time, VIP, PATH-STIK, and Reveal kits are better choices since they are easy to operate and fast. When it comes to the application on real sample testing, 4 commercial kits all showed different levels of false positive reaction. The further product research and development by makers are suggested to improve the capability of testing.

ACKNOWLEDGEMENTS

This research was supported by the Council of Agriculture grant no. 88-KEJI-3.2-LIANG-01(5-5)-2. We would like to thank Dr. C. W. Chen for his translation work.

REFERENCES

1. Izumiya, H., Terajima, J., Wada, A., Inagaki, Y., Itoh, K. I., Tamura, K. and Watanabe, H. 1997. Molecular typing of enterohemorrhagic *Escherichia coli* serotype O157:H7 isolates in Japan using pulse-field gel electrophoresis. J. Clin. Microbiol. 35: 1675-1680.
2. Advisory committee on the microbiological safety of food working group on verocytotoxin-producing *Escherichia coli*: report on verocytotoxin-producing *Escherichia coli*. 1995. p. 15, 32-34, 64-65, 95-98, 120. HMSO, England.
3. Feng, P. 1995. *Escherichia coli* serotype O157:H7 novel vehicles infection and emergence of phenotypic variants. Emerg. Infect. Dis. 1: 47-52.
4. Borczyk, A. A., Karmali, M. A., Lior, H. and Duncan, L. M. 1987. Bovine reservoir for verotoxin-producing *E. coli* O157:H7. Lancet i: 98.
5. Hitchins, A. D., Feng, P., Watkins, W. D., Rippey, S. R. and Chandler, L. A. 1998. *Escherichia coli* and the coliform bacteria. In "Bacteriological Analytical Manual". 8th ed. pp. 4.01-4.29. Food and Drug Administration. Washington D. C., U.S.A.
6. Johnson, J. L., Rose, B. E., Sharar, A. K., Ransom, G. M., Lattuada, C. P. and Mcnamara, A. M. 1995. Methods used for detection and recovery of *E. coli* O157:H7 associated with a food-borne disease outbreak. J. Food Prot. 58: 597-603.
7. Feng, P. 1997. Impact of molecular biology on the detection of foodborne pathogens. Molecular Biotechnology 7: 267-278.
8. Beutin, L., Geier, D., Steinrick, H., Zimmermann, S. and Schentz, F. 1993. Prevalence and some properties of verotoxin (shiga-like toxin)-producing *E. coli* in seven different species of healthy domestic animals. J. Clin. Microbiol. 31: 2483-2488.
9. Sowers, E. G., Wells, J. G. and Strockbine, N. A. 1996. Evaluation of commercial latex reagents for identification of O157 and H7 antigens of *Escherichia coli*. J. Clin. Microbiol. 34: 1286-1289.
10. Baumgartner, A. and Grand, M. 1995. Detection of verotoxin-producing *E. coli* in minced beef and raw hamburgers: comparison of polymerase chain reaction (PCR) and immunomagnetic beads. Archiv fuer Lebensmittelhygiene 46: 127-130.
11. Gannon, V. P., King, R. K., Kim, J. Y. and Thomas, E. J. G. 1992. Rapid and sensitive method for detection of shiga-like toxin-producing *E. coli* in ground beef using the polymerase chain reaction. Appl. Environ. Microbiol. 58: 3809-3815.
12. Cebula, T. A., Payne, W. L. and Feng, P. 1995. Simultaneous identification of strains of *Escherichia coli* serotype O157:H7 and their shiga-like toxin type by mismatch amplification mutation assay-multiplex PCR. J. Clin. Microbiol. 33: 248-250.
13. Chiueh, L. C., Tsai, J. H., Chu, S. Y. and Shih, D. Y. C. 1999. Prevalence of *E. coli*, enterovirulent *E. coli* and verotoxin-producing *E. coli* in retail raw beef and lamb in Northern Taiwan. J. Chin. Agric. Chem. Soc. 37: 246-254.
14. Chiueh, L. C. and Shih, D. Y. C. 1999. Isolation, characterization and genetic relationships of shiga-toxin producing *E. coli* in Taiwan. unpublished data.
15. Gannon, V. P. J., Rashed, M., King, R. K. and Thomas, E. J. G. 1993. Detection and characterization of the *eae* gene of shiga-like toxin-producing *Escherichia coli* using polymerase chain reaction. J. Clin. Microbiol. 31:1268-1274.

16. Feng, P. 1997. Current research on strain *E. coli* O157:H7 in FDA. personal communication.
17. PATH-STIK One Step Rapid *E. coli* O157 Test, Celsis Lumac.
18. VIDAS *E. coli* O157, bioMerieux.
19. VIP for EHEC, BioControl.
20. Paton, A. W. and Paton, J. C. 1998. Detection and characterization of shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *stx1*, *stx2*, *eaeA*, enterohemorrhagic *E. coli hlyA*, *rfb*O111, and *rfb*O157. J. Clin. Microbiol. 36: 598-602.
21. Gannon, V. P. J., D'souza, S., Graham, T., King, R. K., Rahn, K. and Read, S. 1997. Use of the flagellar H7 gene as a target in multiplex PCR assays and improved specificity in identification of enterohemorrhagic *Escherichia coli* strains. J. Clin. Microbiol. 35: 656-662.
22. Schmidt, H., Beutin, L. and Karch, H. 1995. Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157:H7 strain EDL933. Infect. Immun. 63: 1055-1061.
23. Reveal *E. coli* O157:H7 Test System, Neogen.
24. Olive, D. M. 1989. Detection of enterotoxigenic *Escherichia coli* after polymerase chain reaction amplification with a thermostable DNA polymerase. J. Clin. Microbiol. 27: 261-265.
25. Cohen, A. E. and Kerdahi, K. F. 1996. Evaluation of rapid and automated enzyme-linked fluorescent immunoassay for detecting *Escherichia coli* serogroup O157 in cheese. J. of AOAC International. 79: 858-860.
26. Padhye, N. V. and Doyle, M. P. 1991. Rapid procedure for detecting enterohemorrhagic *Escherichia coli* O157:H7 in food. Appl. Environ. Microbiol. 57:2693-2698.
27. ImmunoCard STAT! *E. coli* O157:H7 Clinical, Meridian Diagnostics.

O157型大腸桿菌商業化檢測套組之評估

闕麗卿* 陳鳳茹 施養志

行政院衛生署藥物食品檢驗局
台北市南港區昆陽街161-2號

（收稿：April 25, 2000 ；接受：June 26, 2001）

摘 要

本研究選用四種*E. coli* O157商業化檢測套組及PCR方法，進行食品中*E. coli* O157:H7檢測方法評估。以69株菌之純菌培養測試套組之專一度、敏感度，其結果針對O157抗原，專一度及敏感度皆為100%，若同時針對O157及H7抗原，全部測試套組之敏感度均為100%，專一度則降為89.6%，四種套組之最低檢測量為10^3至10^4 CFU/mL。PCR方法之專一度、敏感度則均為100%，最低檢測量為10^3 CFU/test。由本實驗得知，此四種商業化套組乃針對*E. coli* O157抗原設計之套組，僅能初步測試檢體是否污染O157血清型之*E. coli*，至於最後確認呈陽性反應結果之檢體，必須再配合其他方法做進一步菌株分離及確認。純菌添加檢體試驗，每克檢體中僅污染單一*E. coli* O157菌量，即可經由增菌步驟檢測出。四種套組實際應用於市售檢體之檢測，均出現不同比率之偽陽性反應，故祇能作為食品檢體之初篩方法，PCR方法則皆未出現偽陽性。

關鍵詞：O157型大腸桿菌，偵測，套組