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COMPARISON OF RIDA®QUICK VEROTOXIN/O157 TEST, VEROCYTOTOXICTY ASSAY AND PCR IN DETECTION OF SHIGA TOXINS IN CULTURES OF HUMAN STOOL, ANIMAL FAECES AND MEAT PRODUCTS

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ABSTRACT

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Shiga toxins (Stx) are the major virulence factors of STEC, which cause diarrhea, life-threatening haemolytic uraemic syndrome (HUS) and acute renal failure in children. This work aimed to evaluate the new RIDA®QUICK Verotoxin/O157(R-Biopharm, Darmstadt, Germany), which detects Stx & *E. coli* serogroup O157 and to determine the extent of faecal contamination of meat products by STEC. A total 623 samples, including 214 faecal samples collected from sheep, 209 stool samples from human and 200 samples of different meat products were cultured on Trypticase Soya Broth and CT-Sorbitol MacConkey agar, samples were Biochemically identified as *Escherichia coli*. Further identifications were performed by serotyping and Vero cells cytotoxicity assay. PCR for specific VT1/VT2 genes and RIDA®QUICK Verotoxin/O157 were applied on VCA positive samples. The results of RIDA®QUICK revealed the detection of *STEC* in 14(93.33%), 6 (85.71%) and 13(86.66%) while PCR test detected 15(100%), 7(100%) and 15(100%) VCA positive samples from sheep, man and meat products, respectively. Overall RIDA®QUICK Verotoxin / O157 Combi test detected 33(89.18%) from 37 strains of STEC that were positive for VCA and PCR. The test was sensitive for the Stx1 (9/9 positive), Stx2 (18/22 positive) and Stx1& Stx2 (6/6). Moreover, The sensitivity of the assay for the *E. coli* O157 antigen was tested with 7 strains belonging to sheep (4 strains) and man (3strains), The RIDA Quick assay detected all of them. Furthermore, Strains belonging to the non-O157 serogroups did not react for the O157 antigen in the RIDA Quick assay indicating a specificity of 100%. In Conclusion: Fecal contamination of meat products is important route through which STEC pathogens enter the food chain and the new RIDA®QUICK Verotoxin/O157 is a highly sensitive, specific and rapid method to determine the presence of Stx and serogroup O157 in enrichment cultures.

Keywords: *E. coli, RIDA®QUICK Verotoxin O157, Egypt.*

INTRODUCTION

The Gram-negative bacterium, *Escherichia coli* is a prominent member of the bacterial microbiota of the environment and in the feces of many species of birds and mammals. Five categories of *E. coli* have been well associated with diarrhea in several epidemiological studies (Nataro & Kaper, 1998) enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and enterohemorrhagic *E. coli* (EHEC) which mostly regarded as Shiga toxin-producing *E. coli* (STEC).

The production of Shiga toxins (Verotoxins) is a characteristic trait of STEC. Shiga toxin-producing *Escherichia coli* (STEC), also called Verotoxin-producing *E. coli* (VTEC), were first described by Konowalchuk *et al.* (1977) by their cytotoxic activity on African green monkey kidney (Vero) cells (Konowalchuk *et al.*, 1977). STEC of serotype O157:H7 were linked to cases of Haemorrhagic Colitis (HC) and the consumption of STEC-contaminated meat of bovine origin for the first time in 1982 (Riley *et al.*, 1983; Karmali *et al.*, 2010).

EHEC are capable of initiating life threatening illnesses, particularly in those with immune deficiency, young children and the elderly. The main sources of infection are contaminated, raw or insufficiently heated foods of animal origin, e.g. meat and dairy products, Food-borne outbreaks of STEC

disease appear to be increasing and, when mass-produced and mass-distributed foods are concerned, can involve large numbers of people (Mohammed, 2010). The reservoir for EHEC is the feces of cattle, sheep and goats. These microorganisms can enter food during the processing of meat and dairy products if hygienic conditions are inadequate (Kudva *et al.*, 1997; Mohammed, 2012).

The drastic increase in the incidence of food infection caused by *E. coli* demands reliable and rapid methods of detection. In addition to traditional culture methods, immunological techniques are becoming more useful due to their improved specificity and sensitivity (Melton-Celsa *et al.*, 2002).RIDA®QUICK Verotoxins is an immunochromatographic screening test based on the immune flow principle.

The RIDA®QUICK Verotoxin O157 test is an immunochromatographic rapid test intended to be used in food analysing laboratories for the qualitative detection of Verotoxins (Shiga-like toxins) 1 and 2 from Verotoxinogenic *E.coli* (including *E. coli* O157:H7). This test has been validated and received AOAC (association of analytical communities) approval for detection of Verotoxins 1 and 2 from isolated Verotoxin-producing *E.coli* (including *E. coli* O157:H7). The test is also intended to be used in clinical laboratories for the qualitative identification of Verotoxins 1 and 2 (Shiga-like toxins 1 and 2) produced by *E. coli* isolated in cultures derived from clinical stool specimens and aids in the diagnosis of diseases caused by enterohemorrhagic *E. coli* infections.

MATERIALS and METHODS

Isolation of STEC from collected samples:
This study included 623 samples collected from 4 sheep flocks (214 faecal samples), human (209 stool samples) beside 200 samples from different meat products products (40 luncheon, 40 Beef burger, 40 Minced meat, 40 Fresh sausage and 40 Beef kofta. Samples were obtained from animals, patients or products in clean sterile containers then transported with the minimum of delay to the laboratory of animal hygiene and zoonoses department.

A small mass of faecal sample was injected into 5 ml of Tryptic Soy Broth while 25 g of meat product samples were added to 225 ml of Tryptic Soy Broth and incubated overnight at 37 °C. Subculture from Tryptic Soya broth was performed on Sorbitol MacConkey Agar (SMAC) with cefixime and tellurite (CT) to obtain the suspected colonies of the bacteria. The obtained colonies were prepared for VCA to detect STEC. Positive samples were confirmed to be STEC by serotyping and PCR reaction to determine the type of *Stx.* (Konowalchuk *et al.*, 1977).

Vero cell assay of the suspected *E. coli* strains
The cytotoxicity of the suspected *E. coli* isolates for vero cells was determined by using tissue culture supernatant and thereby detecting only high level of producers of these cytotoxins based on Konowalchuk *et al.* (1977).

This test was carried out in 96 well tissue culture plates. 90µL of sterile physiological saline was added to each of the test wells, while 50µL of the physiological saline was added to the negative control wells. 60 µL of the bacterial lysates was added to each well. 50µL of RPMI medium containing 10% calf serum, 2mM L-glutamin, 100 U penicillin/ml and 100 µg streptomycin /ml were added to each one of the test wells. A suspension of vero cells was prepared and 50 µL of this suspension was seeded in each well of the test wells. 50 µL of 1% SDS solution was added to each of the positive control wells. The plates were incubated at 37°C in 5% CO2 atmosphere, observed daily by using inverted microscope for detection of cell lysis and vacuolation.

Serotyping *E. coli* isolates
Serotyping of *E. coli* samples that were positive in Vero cell assay was performed in the (serology unit, Animal Health Research Institute, Dokki). When this reagent is mixed with *E. coli* strain which has antigens correspondent to the reagent, the antigen antibody reaction occurs to produce agglutination. This reaction is macroscopically observed to determine each serotype (Edwards and Ewing, 1972). Positive Vero cell assay *E. coli* strains were streaked on Agar media (nutrient agar medium, heart infusion (HI) agar medium, slant or plate medium), previously autoclaved at 121°C. Pure cultures of the selected *E. coli* strains were prepared for determination of their O-groups using slide agglutination test.

Detection of *Stx1*, *Stx2* and *eae* genes in STEC isolated from faecal samples using Multiplex PCR:
The multiplex PCR was performed as described by Brian *et al.* (1992) at the laboratory of infectious diseases and Internal medicine, faculty of Veterinary Medicine, Sadat city University.

Genomic DNA extraction:
Chromosomal DNA was isolated from STEC isolates using protienase K extraction. Pure cultures of bacteria in the trypticase soya broth were streaked on MacConkey s agar plates. The inoculated plates were incubated at 37°C. A single colony from the plate was subcultured into 300 ml of TSB and incubated for 24 hours at 37°C. After incubation, bacteria were collected by centrifugation at 13000 rpm for 2 minutes and the sediment was suspended in equal volume of Tris-EDTA buffer. Furthermore, 100µl of lysozyme solution (10 mg/L), 100 µl of protienase K enzyme (0.3 mg/L) and 1% dodecyl sulphate were added. The DNA lysate was extracted once with

chloroform/isoamyl alcohol (24:1, ratio by volume), then extracted with phenol/ chloroform/isoamyl alcohol (25:24:1, ratio by volume), the aqueous phase was mixed with isopropanol alcohol and incubated at -20C for 30 minutes. The precipitated DNA was spooled out, rinsed in 70 % ethanol and dissolves in 0.5 ml of Tris EDTA buffer.

PCR Amplifiction:
Polymerase chain reaction (PCR) was performed in a thermal cycler model (Biometra). The PCR reaction mix (50 µL) for each sample was consistent of: 10 µL extracted DNA, 3 µL primers mix (0.5 µL from every primer); 1 µL deoxynucleoside triphosphate (dNTP-mix); 5 µL 10x buffer; 1 µL Taq-DNA polymerase enzyme (5000 U/ml) and 30 µL ultra pure deionized water. The reaction mixture was overlaid with mineral oil and was incubated in the thermal cycler as follows: The first initial cycle: 94°C for 4 minute (initial denaturation). The consequent 35cycles: 94°C for one minute (denturation), 60°C for one minute (annealing) and 72°C for one minute and 30 seconds (extension). The final extension step at 72°C for 7 minutes then kept at 4°C (hold temperature) (Brian *et al.*, 1992).

Table 1: Showing Primer sequences for *stx1, stx2* and *eae* genes.

Gene	Primer sequence	Predicted size	Reference
Stx1	5'-AAATCGCCATTCGTTGACTACTTCT-3' 5'-TGCCATTCTGGCAACTCGCGATGCA- 3'	366 bp	**Brian *et al.* (1992)**
Stx2	5'-CAGTCGTCACTCACTGGTTTCATCA-3' 5'-GGATATTCTCCCCACTCTGACACC-3'	282 bp	**Brian *et al.* (1992)**
Eae	5 'AGGCTTCGTCACTGTG 3 ' 5'CCATCGTCACCAGAGGA 3'	579 bp	**China *et al.* (1996)**

Detection of amplified products:
10 µl from each PCR amplification were electrophoresed on agarose gel and stained with ethidium bromide solution (0.5 µg/ml), visualized under an ultraviolet transilluminator and photographed. Visible bands of appropriate size of 366 bp (*Stx1*), 282 bp (*Stx2*) and 579 bp (*eae*) were considered positive. Molecular mass markers (100 bp ladder) were electrophoresed simultaneously.

RIDA®QUICK Verotoxin / O157 Combi test (dipsticks):
For in vitro diagnostic use. The RIDA®QUICK Verotoxin / O157 Combi test (RBiopharm, Darmstat, Germany) is an immunochromatographic rapid test for qualitative determination of Vero toxins (shiga toxins) and of Escherichia coli serotype O157 in enrichement cultures.

Modified Tryptic Soya broth (mTSB) containing Mitomycin C (Klie *et al.*, 1997) was inoculated with the test strain and incubated at 37°C for 18 to 24 hours for the production of shiga toxins (Mitomycin C serve as enhancer to stimulate Stx production and release in the culture fluid. after growth, the samples was centrifuged (1500g for 15 minutes) and 1ml of the supernatant fluid is diluted 1:1 with a sample buffer. The mixture was used directly for the test.

The RIDA-QUICK test stick was placed into the test sample and the reaction occurs by the lateral flow of specific antibodies bound to coloured latex particles. Antibodies bind themselves to specific antigens (Stx and/or O157 LPS) if present in the sample and the antigen-antibody complex flows via the membrane to specific collection bands where they are fixed by immobilized, specific antibodies.

RESULTS

Table 2: Comparison of the results of cultivation on SMAC medium with VCA, multiplex polymerase chain reaction (PCR) and RIDA®QUICKVerotoxin / O157 Combi test for detection of STEC infection in sheep, human beings and meat products.

Subject	No. of examined samples	No. of +ve colonies on SMAC medium	No. of samples +ve VCA.	No. of samples tested by PCR and were +ve VCA.	RIDA®QUICKVerotoxin / O157 Combi test
Sheep	214	29	15 (51.7 %)	15 (100 %)	14(93.33%)
Human	209	22	7 (31.81%)	7 (100 %)	6 (85.71%)
Meat product samples	200	26	15(57.69%)	15 (100%)	13(86.66%)
Total	623	77	37(48.05%)	37 (100 %)	33(89.18%)

Table 3: Serogroups of verotoxin- producing *E. coli* isolated from sheep and man.

Serogroup	No.	%
O157	7	31.81
O103	3	13.63
O26	1	4.54
O111	2	9.09
O126	1	4.54
O8	2	9.09
O168	1	4.54
O20	3	13.63
O1	1	4.54
O78	1	4.54
Total	22	100

Table 4: Serogroups of verotoxin- producing *E. coli* isolated from meat.

Serogroup	No.	%
O111	4	26.6%
O26	3	20.0%
O91	2	13.3%
O103	3	20.0%
O145	2	13.3%
O86	1	0.66%
Total	15	100%

Table 5: Occurrence of some virulence genes in serogroups of Shiga toxin-producing *E. coli* (STEC) isolated from sheep.

Sero-types	No. of examined isolates	Stx1 alone		Stx2 alone		Stx1&Stx2		Eae	
		NO.	%	NO.	%	No.	%	No.	%
O157	4	0.0	0.0	2	50	2	50	3	75
O103	2	2	100	0.0	0.0	0.0	0.0	0.0	0.0
O20	3	0.0	0.0	3	100	0.0	0.0	0.0	0.0
Other STEC	6	1	16.6	5	83.3	0.0	0.0	4	66.6
Total	15	3	20	10	66.6	2	13.3	7	46.6

Table 6: Occurrence of some virulence genes in serogroups of Shiga toxin-producing *E. coli* (STEC) from man.

Sero-types	No. of examined isolates	Stx1 alone		Stx2 alone		Stx1&Stx2		Eae	
		NO.	%	NO.	%	No.	%	No.	%
O157	3	1	33.3	2	66.6	0.0	0.0	1	33.3
O103	1	1	100	0.0	0.0	0.0	0.0	0.0	0.0
Others	3	1	33.3	2	66.6	0.0	0.0	2	66.6
Total	7	3	42.8	4	57.1	0.0	0.0	3	42.8

Table 7: Occurrence of some virulence genes in serogroups of Shiga toxin-producing *E. coli* (STEC) isolated from meat products.

Sero-types	No. of examined isolates	Stx1 alone		Stx2 alone		Stx1&Stx2		Eae	
		NO.	%	NO.	%	No.	%	No.	%
O111	4	0.0	0.0	2	50	2	50	2	50
O26	3	1	33.3	2	66.7	0.0	0.0	0.0	0.0
O91	2	0.0	0.0	2	100	0.0	0.0	0.0	0.0
O103	3	1	33.3	1	33.3	1	33.3	2	66.6
O145	2	0.0	0.0	1	50	1	50	0.0	0.0
O86	1	1	100	0	0.0	0.0	0.0	1	100
Total	15	3	20.0	8	53.2	4	26.6	5	33.3

Table 8: The sensitivity and specificity of the RIDA®QUICK Verotoxin / O157 Combi test for detection of samples containing Stx1 and/or stx2 and the O157 antigen:

Sample type		No. of samples	Positive	sensitivity	Specificity
Acc. to Stx	Stx1	9	9	100%	100%
	stx2	22	18	81.86%	100%
	Stx1&stx2	6	6	100%	100%
Acc. to O157 antigen	O157	7	7	100%	100%
	Non- O157	30	0.0	-	100%

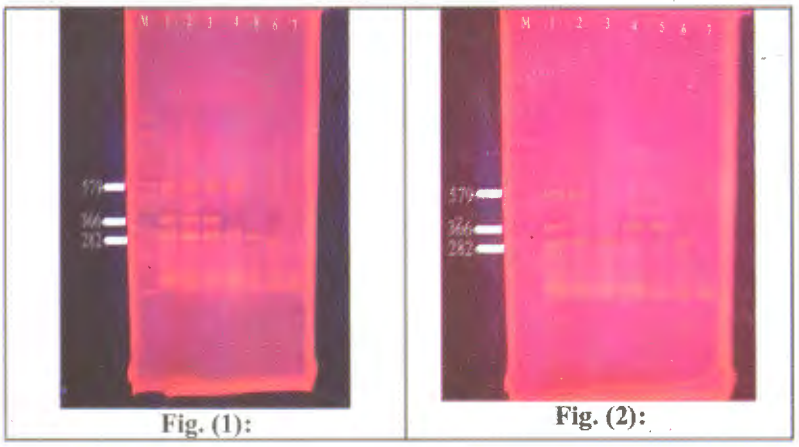

Fig. (1): Fig. (2):

Fig. (1): Ethidium bromide stained Agarose gel showing the specific amplification products with specific size (366, 282 and 579 bp) primers specific for *Stx1, Stx2* and *eae* genes respectively for STEC isolates from sheep. **Lane (M):** MW marker = 100 bp DNA ladder (Promega). **Lane (1):** Positive Control *(E. coli O157H7* provided by Animal Health research Institute, Egypt). **Lane (2- 6):** *E. coli* isolates from sheep.**Lane (6): Lane (7):** Negative Control.

Fig. (2): Ethidium bromide stained Agarose gel showing the specific amplification products with specific size (366, 282 and 579 bp) primers specific for *Stx1, Stx2* and *eae* genes respectively for STEC isolates from man. **Lane (M):** MW marker = 100 bp DNA ladder (Promega). **Lane (1):** Positive Control. **Lane 2-6** *E. coli* isolates from man. **Lane 7:** Negative Control.

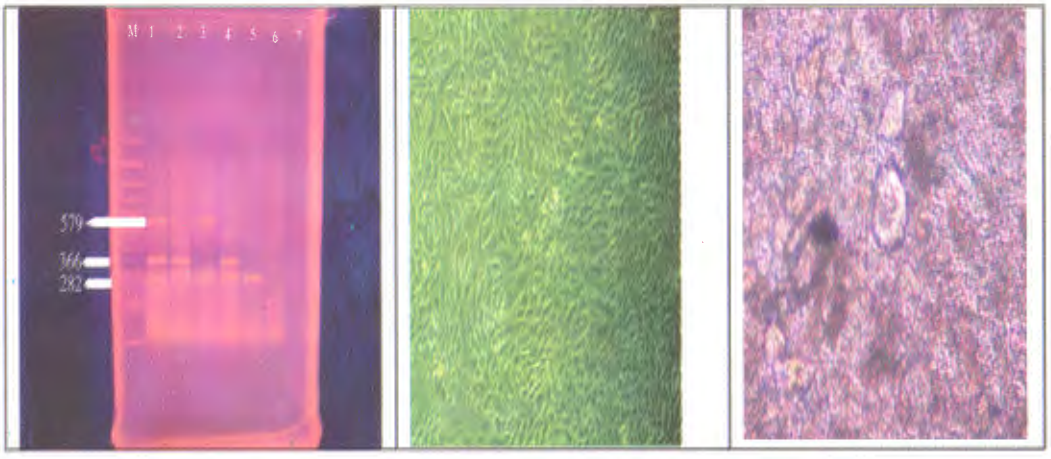

Fig. (3): Ethidium bromide stained Agarose gel showing the specific amplification products with specific size (366, 282 and 579 bp) primers specific for *Stx1, Stx2* and *eae* genes respectively for STEC isolates from meat products. **Lane (M):** MW marker = 100 bp DNA ladder (Promega). **Lane (1):** Positive Control. **Lane (2-5)** isolates from meat products. **Lane (6):** Negative Control.

Fig. (4): Intact vero cell monlayer incubated with control medium.

Fig. (5): Cytotoxic effect of shiga toxin cotaining bacterial lysate of STEC on vero cells.

DISCUSSION

Among the *E. coli* human pathogens, Verotoxin (Shiga-like toxin) forming strains (VTEC) have gained special importance in recent years. The group of enterohaemorrhagic *E. coli* (EHEC) with its highly pathogenic serovars 0157:H7, 026, 0103, 0111, 0145, and other strains are of particular concern. Production of Verotoxins is the most common criteria for the detection of this group of bacteria and can be classified into two main categories Verotoxin 1 (VT1, SLT1, Stx1) and Verotoxin 2. EHEC strains may produce either VT1 or VT2 only or both VT1 and VT2 simultaneously (Akiba *et al.*, 2005).

Healthy cattle and sheep are recognized as a major natural reservoir of EHEC and other STEC strains. There are more than 100 serotypes of STEC which have been also isolated from other animals such as goats, pigs, goats, deer, horses, dogs and birds (Kudva *et al.*, 1997 and Gyles 2007).

The results recorded in Table (2) showed that from 623 samples collected from sheep (214 samples), human (209 samples) and meat products (200 samples), 77(12.06 %) samples yielded positive culture SMAC-CT. Further identifications of the isolated colonies were performed by Vero cells cytotoxicity assay which revealed that 37 of *E. coli* isolates (48.05 %) were verotoxin producing *E. coli*. The results obtained in this study agreed with Ramotar *et al.* (1995) who reported that SMAC was positive for only 30 % of verocytotoxin-positive samples. On other hand, another study indicated that SMAC resulted in the isolation of *E. coli* O157 from 80 % of fecal samples which were positive for *Stx* by direct cytotoxicity (Ritchie *et al.*, 1992).

In the present study, the most common serogroups of STEC in sheep and man were recorded in table (4). They were O157 (31.82 %), O103 (13.63 %), O20 (13.63 %) and O111 (9.09 %), O8 (9.09 %); O26 (4.54 %); O78 (4.54 %); O126 (4.54 %); O168 (4.55 %); and O1 (4.55 %). While in meat products (luncheon, Beef burger, Minced meat, Fresh sausage and Beef kofta) O111 (26.6%, O26 (20.0 %); O91 (13.3 %); O103 (20.0 %); O145(13.3%) and O86 (0.66%) were the most prevalent (Table, 3). These results are in agreement with Mohammed, (2012) who demonsrated that non O157 STEC were the most prevalent in meat products sold at Mansoura city, Egypt.

Fecal contamination of meat and milk are important routes through which these pathogens enter the food chain,together with the contamination of vegetables with animal manure. There are many vectors that can be used to transfer *E. coli* on and/or into meat products. The feces of the animal can be transferred through hides, carcass, contaminated equipment, non proper hygienic practices, airborne contamination, rodents, insects, and other carrier animals (Mohammed, 2012).

The use of PCR with specific primers for *Stx1, Stx2* and *eae* genes revealed the presence or absence of these genes in the tested isolates. The obtained results recorded in Tables (5,6&7) revealed that isolates of O26, O103 and one isolate O157 from human; O103 (2 isolates), O126 from sheep as well as O26, O103 and O86 from meat products had only *Stx1* while, *E. coli* O78, O111 O168, O8 (2 isolates), O20 (3 isolates) and O157 (2 isolates) from sheep; O1, O111, 2 isolates O157 from human as well as O111,O26, O91,O103, O145 from meat products had only *Stx2*. On the other hand, isolates that possessed both *Stx1* and *Stx2* were 2 isolates O157 from sheep and O111, O103&O145 from meat products Previous studies have shown that the virulence of STEC for humans may be related to the type of Shiga toxin which is produced by the bacteria. Of the different Shiga toxins, Stx2 (stx2) was found to be related with high virulence and was significantly associated with STEC strains from BD and HUS patients (Paton and Paton, 1998).

Concerning diagnosis of STEC by detection of Shiga toxins, a number of indicator media for the identification of some STEC strains and types have been developed, as well as immunological, genetic and cell culture toxicity assays for Stx detection. The detection of Shiga toxins is the only way to identify all members of the STEC group which consists of strains with different phenotypes and serotypes (Bettelheim and Beutin, 2003).

The Vero cell toxicity assay (VCA) is used as a gold standard for detection of Stx production as it is the most sensitive. However, its specificity must be confirmed in neutralization assays with antisera directed against Stx. As the VCA is laborious, time consuming and demands a specifically designed laboratory and specially trained personnel there is a need for STEC detection systems which are rapid, reliable, standardized and easy to employ (Paton and Paton 2003).

The data presented in Table (2) illustrated that vero cells cytotoxicity assay showed that 37 of *E. coli* isolates out of 77(48.05 %) isolates were verotoxin producing *E. coli*. In PCR study, 37 (100 %) of the 37strains were confirmed to be STEC whereas 22 strains (63.63%) were positive for the VT2 gene, 9 (27.27%) were positive for the VT1 gene and 6 (9.09%) isolates were positive for both genes. In comparison to Vero cells cytotoxicity, the sensitivity of PCR were 100 %. PCR and RIDA®QUICK Verotoxin / O157 Combi test were compared with Vero cytotoxicity assay for a number of reasons. Firstly, The profound sensitivity of Vero cells to *Stx* which was first observed by Konowalchuk *et al.* (1977), Secondly, the cytotoxicity for this cell line remains the "gold standard" for confirmation of putative STX-producing isolates (Byomi, 1995).

The sensitivity of the RIDA®QUICK Verotoxin / O157 Combi test for detection of samples containing Stx1 and/or stx2 and the O157 antigen was illustrated in Table (8). The sensitivity is the probability that the assay will be positive when the infection is present. The specificity is the probability that the assay will be negative when the infection is absent. They were calculated according to (Agnamey *et al.*, 2011).

We have evaluated the RIDA Quick test for its sensitivity with all the known variants of Stx1 and/or Stx2. For the evaluation, we employed 37 STEC strains of different STEC isolates from sheep, human and different meat sources. The RIDA Quick was compared for its sensitivity with the PCR and Vero cell cytotoxicity test which was used as a gold-

standard for toxin activity. The thirty seven strains of STEC that were positive for VCA and PCR were tested by RIDA®QUICK Verotoxin / O157 Combi test and the results showed that 33(89.18%) of them were positive (table, 2 and table,6). The results were very good for the Stx1 (9/9 positive), Stx2 (19/22 positive) and Stx1& Stx2 (6/6). Nearly similar results were obtained by (Melton-Celsa *et al.,* 2002) who detected 5 of 7 positive Stx2 strains (71.4 %).

Detection of the O157 antigen by RIDA®QUICK Verotoxin / O157 Combi test was demonstrated in Table (8). The sensitivity of the assay for the *E. coli* O157 antigen was tested with 7 strains belonging to sheep (4 strains) and man (3strains). The RIDA Quick assay detected all *E. coli* O157 strains tested. Strains belonging to the non-O157 serogroups did not react for the O157 antigen in the RIDA®Quick assay indicating a specificity of 100%. These results were in agreement with Park *et al.* (2003) who described That immunochromatographic test of STEC demonstrated a sensitivity of 100% for the detection of clinical isolates without any cross-reactivity with other enteric organisms

In Conclusion: Sheep are important reservoir of STEC to man besides, Faecal contamination of meat products by animal manure is important route through which STEC pathogens enter the food chain and the newly developed RIDA®QUICK Verotoxin/O157 test is a highly sensitive, specific when compared to PCR and VCA. Moreover, it is more rapid method to determine the presence of Stx and serogroup O157 in enrichment cultures. Further studies are needed to investigate the test performance in comparison to Stx targeting ELISA.

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مقارنة استخدام بعض الاختبارات الحديثة في الكشف عن الميكروب القولونى المفرز لتوكسين شيجا في الحيوانات والانسان ومنتجات اللحوم

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نظرا للأهمية الصحية للميكروب القولونى المفرز لتوكسين شيجا ودوره في حدوث الاسهال في بعض الحيوانات والانسان اجريت هذه الدراسة علي عدد ٦٢٣ عينة شملت روث الاغنام (٢١٤ عينة) وبراز الانسان (٢٠٩ عينة) بالاضافة الي ٢٠٠ عينة من منتجات اللحوم المختلفة. تم فحص العينات معمليا لعزل الميكروب القولوني المفرز لتوكسين شيجا أولا بطريقة العزل علي الوسط المخصص (SMAC-Media) والذى أسفر عن وجود ٧٧ عينة ايجابية للميكروب القولوني تم تأكيدها باستخدام الطرق البيوكيميائية. كذلك تم اختبار العينات الايجابية باستخدام vero cell assay (VCA) لتحديد العترات المفرزة لتوكسين شيجا. بعد ذلك تم تصنيف العينات الايجابية لاختبار VCA سيرولوجيا وكذلك تم تأكيدها باستخدام تفاعل البلمرة المتسلسل المتعدد (Multiplex PCR) لتحديد نوع الجين المسئول عن افراز سموم شيجا. هذا وقد أسفر فحص العينات عن تواجد 37 عينة ايجابية لاختبار VCA (١٥ من الاغنام و ٧ من الانسان و١٥ من المنتجات) تم تأكيدهم جميعا بالاختبارات السيرولوجية وكذلك باستخدام تفاعل البلمرة المتسلسل المتعدد (Multiplex PCR) بنسبة ١٠٠% . تم مقارنة اختبار الكروماتوجرافي المناعي بالاختبارات السابقة وذلك بفحص ٣٧ عينة ايجابية واسفر الفحص عن كشف ٣٣ عينة منهم مما يعني ان له حساسية تعادل (89.18%) وهذا يؤكد كفاءة وسرعة الاختبار في تشخيص الميكروب القولونى المفرز لتوكسين شيجا. كما أسفر استخدام تفاعل البلمرة المتسلسل المتعدد (Multiplex PCR) بواسطة بادئات لجينات شيجا توكسين١ *Stx1* وشيجا توكسين٢ *Stx2* و *eae* عن وجود او غياب هذه الجينات في العينات المعزولة. وكانت أكثر المعزولات من الأغنام والانسان والمنتجات هي O26,O157 , O103, O20 وغيرها مما يقوي من دور الأغنام واللحوم الملوثة في نقل العدوي للانسان. O111,.