EVALUATION OF THE VISUAL IMMUNOPRECIPITATE ASSAY (VIP™) FOR DETECTION OF *E.COLI* O157:H7 IN NATURALLY CONTAMINATED RAW MILK AND RAW MILK CHEESES

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SUMMARY

Visual Immunoprecipitate Assay (VIP™) (BioControl) is a single-step test that detects enterohemorrhagic E.coli O157: H7 in naturally contaminated food by forming an antigen-antibody-chromogen complex if the organism is present. This study evaluated the ability of the VIP™ Assay to detect E.coli O157: H7 in naturally contaminated raw milk and raw milk cheeses. A total of 200 samples of raw milk and fresh soft cheese (100 samples of each) were randomly collected from dairy venders in Kalyobia province and examined for the presence of E.coli O157: H7. Samples were examined using the VIP™ Assay side by side with the cultural method recommended by the FDA Bacteriological Analytical Manual (BAM) for isolating E.coli O157:H7 from food. By combining the results of the two methods used, 7 (3.5%) out of the 200 examined samples were found to have E.coli O157:H7. The organism isolated from 3 and 4% of the examined raw milk, and fresh soft cheese samples, respectively. Out of the 7 positive samples, VIP™ Assay detected the organism in only 3 (42.8%) samples. Moreover, the VIP™ Assay positively detected 4 samples, which could not be confirmed by cultural methods (false positive). On the other hand, the BAM cultural method alone detected the organism in 6 (85.7%) out of the 7 positive samples with only one sample being recorded as false negative. Interestingly, only two (28.6%) out of the 7 positive samples were found to be positive for E.coli O157:H7 by both the VIP™ Assay and the BAM cultural method. Furthermore, the acting of VIP™ Assay as a rapid method for detection of E.coli O157:H7 was not satisfying comparing to the cultural method used in this study. Moreover, the use of at least two protocols for detection of enterohemorrhagic E.coli is highly recommended to enhance the isolation of this organism from naturally contaminated milk and milk products.

INTRODUCTION

Enterohemorrhagic *E. coli* (EHEC) was first recognized as an important foodborne pathogen in 1982 (Riley et al., 1983). The organism causes hemorrhagic colitis, which may progress into hemolytic uremic syndrome, a more severe complication that can result in kidney failure and death (Karmali et al., 1983). Although there are many serotypes of EHEC, serotype O157:H7 has been most frequently implicated in foodborne diseases. The severe symptoms caused by *E.coli* O157:H7 combined with its apparent low infectious dose (<100 cells) qualify this organism to be among the most serious of known foodborne pathogens (Doyle et al., 1997).

Raw milk is proved to be the vehicle of 2.9% of the overall *E.coli* O157:H7 outbreaks (Doyle et al., 1997) with source of contamination is probably faecal material transferred into milk at milking (Padhye and Doyle, 1992). Although not currently recognized as a cause of mastitis in dairy cattle, *E.coli* O157:H7 can readily contaminate milk on the farm, with contamination rates of 4.2% and 2.0% reported for raw milk produced in the United states and Canada, respectively (D'Aoust, 1989). Moreover, consumption of raw milk and raw milk cheese have been documented as vehicles for several *E.coli* O157:H7 outbreaks (Bleem, 1994; Feng, 1995; Ryser, 2000; Soudah et al., 1996).

Cheese made from unpasteurized milk has also been implicated in two *E.coli* O157:H7 outbreaks in 1994 and 1997 in Scotland (Desmarchelier and Grau 1998). Raw milk cheese was also responsible for an outbreak in Wisconsin in 1998 in which fresh (held <60 days) cheese curds from a dairy plant was implicated as the source of infection (Durch et al., 2000). In May 1999, a group of children in Scotland were exposed to *E.coli* O157:H7 at school from eating cheese made from raw goat's milk (Ryser, 2000).

As *E.coli* O157: H7 strains have emerged as significant foodborne pathogens, a variety of approaches to isolation and identification have been developed, together with a large range of bacteriological media. An enrichment/isolation procedure using the Modified Trypticase Soy Broth (mTSB) with Tellurite-Cefixime-Sorbitol MacConkey (TC SMAC) medium was recently introduced for detecting O157:H7 in foods. Both the enrichment and the selective media contain several antibiotics, which effectively suppress the growth of normal flora other than *E.coli* O157: H7 (Hitchins et al., 2001). Moreover, Comparative analysis of the TC SMAC procedure with some other media used for isolation of *E.coli* O157:H7 from naturally contaminated foods showed that the TC SMAC procedure was the best in the recovery of *E.coli* O157: H7 bacteria (Weagant et al., 1995). Enrichment and culture of *E.coli* O157: H7 from food is time consuming and labor intensive. Therefore considerable effort has been focused on the development of rapid methods for screening of the organism in naturally contaminated foods. VIPTM is one of the immunologically based assays, which can be applied specifically on any serotype through immunopercipitation (Roszak et al., 1984).

The objectives of this research were (1) To evaluate the VIP^{TM} assay as a rapid method for screening *E.coli* O157:H7 in naturally contaminated raw milk and

raw milk cheeses and compare it to the BAM cultural method. (2) To determine the incidence of *E.coli* O157:H7 in raw milk and raw milk cheeses.

MATERIAL AND METHODS

Figure (1) shows a diagrammatic scheme for the procedures used in this study for detection of *E.coli* O157:H7 in raw milk and raw milk cheeses as following:

I- Sampling:
Two hundred samples of raw milk and fresh soft cheese (100 samples of each) were randomly collected from dairy venders in Kalyobia province. All samples were transported to the laboratory in a cooler where they were hold at 4°C until examined for the presence of *E. coli* O157:H7 within 24 h of collection.

II. A- BAM Cultural method for isolation of Enterohemorrhagic *E. coli* O157:H7 (Hitchins et al., 2001):

II. A. 1- Enrichment:
Twenty-five ml or g of sample were inoculated into 225 ml of Entrohemorrahagic *E. coli* Enrichment Broth (EEB). This enrichment broth is consisted of Modified Trypticase Soy Broth (mTSB) with the following filter-sterilized antibiotics that added after autoclaving (0.05 mg/liter Cefixime, 10.00 mg/liter Cefsulodin, and 8.00 mg/liter Vancomycin). Milk samples were well shaken before inoculating into the enrichment broth, while cheese samples were blended for 2 minutes with the enrichment broth before incubation. Samples were then incubated at 37 °C for 24 hours.

II. A. 2- Isolation:
A loopful of the incubated EEB was streaked onto Tellurite-Cefixime-Sorbitol MacConkey (TC SMAC) agar plates. The Agar medium is the same as Sorbitol MacConkey agar but with the following filter- sterilized additives after autoclaving and tempering (2.50 mg/liter of Potassium tellurite and 0.05 mg/liter of Cefixime). Agar plates were incubated at 37°C for 24 hours. Sorbitol-fermenting normal flora bacteria appear as pink to red colonies. Typical O157: H7 colonies are neutral/gray with a smoky center and 1-2 mm in diameter.

II. A. 3- Identification and serology:
Several typical O157:H7 colonies from TC SMAC were picked and streaked onto Trypticase Soy Agar with 0.6% Yeast Extract (TSAYE) slants and incubated at 37°C overnight. Isolates were screened by spotting growth from TSAYE slants to a filter wetted with Kovac's reagent (spot indole test). EHEC isolates are indole-positive. If indole-positive, O157 antigen was tested with commercial O157 antiserum (Difco, Detroit, MI). If positive, H7 antigen was then tested using commercial H7 antiserum (Difco, Detroit, MI). The serological identification was applied according to the manufacturer instructions.

II. B- Visual Immunoprecipitate Assay (VIP^{TM}) (BioControl, Bellevue, WA.):
II. B. 1- Enrichment:

Twenty-five ml or g of sample were inoculated into 225 ml of Modified Trypticase Soy Broth with Novobiocin (mTSB+n). Samples were then well mixed and incubated at 37°C for 24 hours.

II. B. 2- Sample analysis:

Enrichment broth bottles were shacked and allowed to settle the food particles. Then, 0.1 ml of the broth was inoculated into the sample addition well of the VIP™ unit. The VIP™ unit was incubated at ambient temperature for 10 minutes. Test sample well was observed, presence of distinct line indicates a presumptive positive sample while absence of the line considered negative. Presumptive positive samples were confirmed by streaking a loopful of the mTSB+n onto TC SMAC agar plates. The plates were incubated at 37°C for 24 hours, suspected colonies were then biochemically and serologically confirmed as *E.coli* O157:H7.

RESULTS & DISCUSSION

E.coli O157:H7 was isolated from 7 (3.5%) out of the 200 examined samples (Table 1) by using two protocols for isolating the organism. Four % of the examined Fresh soft cheese samples were reported to have *E.coli* O157:H7, while 3% of the examined raw milk samples were found to be positive for *E.coli* O157:H7. By using the VIP™ assay alone, *E.coli* O157:H7 was screened in only 3 (1.5%) samples. The VIP detected the organism in 2% and 1% of the examined raw milk and fresh soft cheese samples, respectively. On the other hand, the BAM cultural method could isolate the organism from 6 (3.0%) out of the 200 samples examined. Acting much better than the VIP™ assay, the BAM cultural method detected the organism in 3% of both the raw milk and fresh soft cheese samples.

Out of the 7 positive samples resulted from combining the results of the two protocols, the VIP™ Assay detected the organism in only 3 (42.9%) samples. On the other hand, the assay failed to detect the organism in 4 samples, which recorded to be positive by cultural method (false negative) (Figure 3). These 4 false negative samples representing 1 raw milk and 3 fresh soft cheese samples (Figure 2). This means that the assay missed the organism in 57.1% of the positive samples. Moreover, the assay yielded 4 false positive samples which could not be identified by the cultural method.

By using the BAM cultural method, *E.coli* O157:H7 was isolated from 6 (85.8%) out of the 7 positive samples. The cultural protocol failed to detect the organism in only one fresh soft cheese sample which recorded to be positive by the VIP™ assay (Figure 4).

From the data mentioned above, it is clear that the acting of the VIP™ as a rapid method for detecting *E.coli* O157:H7 was not satisfying comparing to the BAM cultural method. The low specificity of this Immuno-assay may be attributed to the antibodies used in the assay. Moreover, other O157 serotypes (e.g. O157:H16; O157:H43) that are not enterohemorrhagic strains may also react with O157 antibody (Martin et al., 1994 and Okrend et al., 1990).

The incidence of *E.coli* O157:H7 in raw milk samples examined in this study (3%) is comparable to the findings of Wells et al. (1991) who reported that *E.coli* O157:H7 could be isolated from raw milk with incidences ranged from 2% to 5%. On the other hand, the incidence was lower than those reported by Padhye and Doyle (1991) and Abdul-Raof et al. (1996) who recovered the organism in 10 and 6% of the examined samples, respectively. The raw milk incidence obtained in this study was higher than that obtained by many researchers (Altekruse et al., 1998; Hancok et al.1994; Headrick et al., 1998; Steele et al., 1997) who isolated the organism with percentage ranged from 0.87% to 2.0% of raw milk samples examined. The high incidence obtained in this study may be attributed to the use of more than one protocol for detecting *E.coli* O157:H7 in the same sample. Moreover, the wide variation of *E.coli* O157:H7 incidence in raw milk depends upon many factors, including the geographical factor and the sanitary conditions under which milk is produced and handled (Eley, 1996).

Fresh soft cheeses have been implicated in several *E.coli* O157:H7 outbreaks. During the period of 1973 to 1992, 32 cheese-associated outbreaks were attributed to contamination before distribution (Altekruse et al., 1998). These findings raise serious concerns regarding the adequacy of the mandatory 60-day holding period at greater than or equal to 1.7°C for complete inactivation of some pathogenic microorganisms including *E.coli* O157:H7 in cheeses that can be legally prepared from raw milk. In this study *E.coli* O157:H7 was isolated from 4% of the examined fresh soft cheese samples. This finding is considered comparable to that reported by Ryser (2000) who stated that infections with hemorrhagic colitis reported in early 1999 in Scotland were attributed to consumption of soft cheese that was made from unpasteurized cows' milk. The organism was isolated from 3.4% of the examined cheese samples. On the other hand, the incidence reported in this study considered higher comparing to the low incidence (1. 0%) obtained by Boor (1996). However, because this organism is acid tolerant, raw milk cheeses and soft-ripened cheeses could conceivably pose public health concerns if prepared or aged improperly (Dineen et al., 1998).

Current evidence indicates that *E.coli* O157:H7 is not unduly heat resistant and is readily destroyed in milk by minimum pasteurization (71.7°C/15 sec) (Law, 2000). So Pasteurization of milk and prevention of post-pasteurization contamination provides consumer protection. On the other hand, preventing temperature-abuse during storage and handling of raw milk and cheese is another important issue since *E.coli* O157:H7 is unable to grow in milk held at 10°C (Kasrazadeh and Genigeorgis, 1995). Nonetheless, substantial growth can occur in temperature-abused milk, with this pathogen exhibiting generation times of 7.2 and 1.5 hours at 12°C and 20°C, respectively (Heuvelink et al., 1998).

Control steps of *E.coli* O157:H7 in milk and milk products extend from the farm to the plate. Care should be taken during the milking process to reduce the possibility of fecal contamination of raw milk. Hands should always be washed prior to milking and ideally thorought the milking process. Regardless of the rarity of dairy products associated foodborne disease, the importance of dairy products in human diets requires systems that ensure dairy product safety. The presence of *E.coli*

O157: H7 in raw milk and raw milk cheeses led us insist to questioning the Government's decision not to ban the sale of unpasteurized milk and unpasteurized milk cheeses in Egypt.

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Table (1) Incidence of *E.coli* O157:H7 in raw milk and raw milk cheeses using VIP™ assay and BAM cultural method

Sample	No. of samples	VIP™ assay		BAM cultural M.		VIP + BAM	
		No.	%	No.	%	No.	%
Raw milk	100	2	2	3	3	3	3
Fresh Soft cheese	100	1	1	3	3	4	4
Total	200	3	1.5	6	3	7	3.5

Figure (1) Diagrammatic scheme for the detection of *E.coli* O157:H7 in raw milk and raw milk cheeses

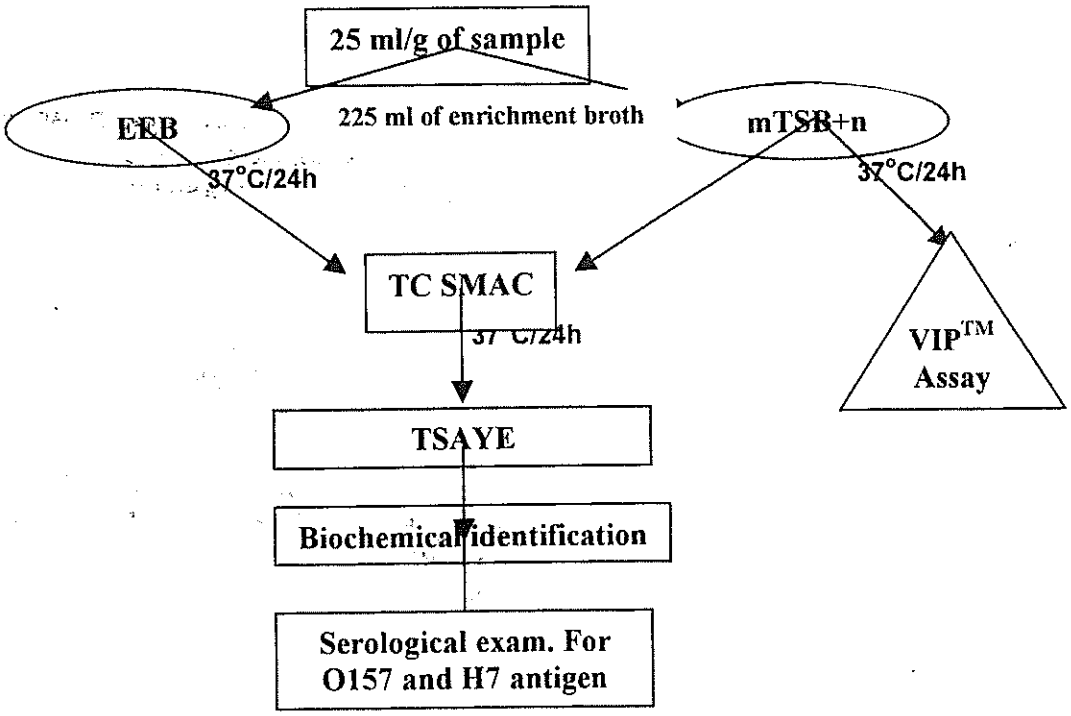
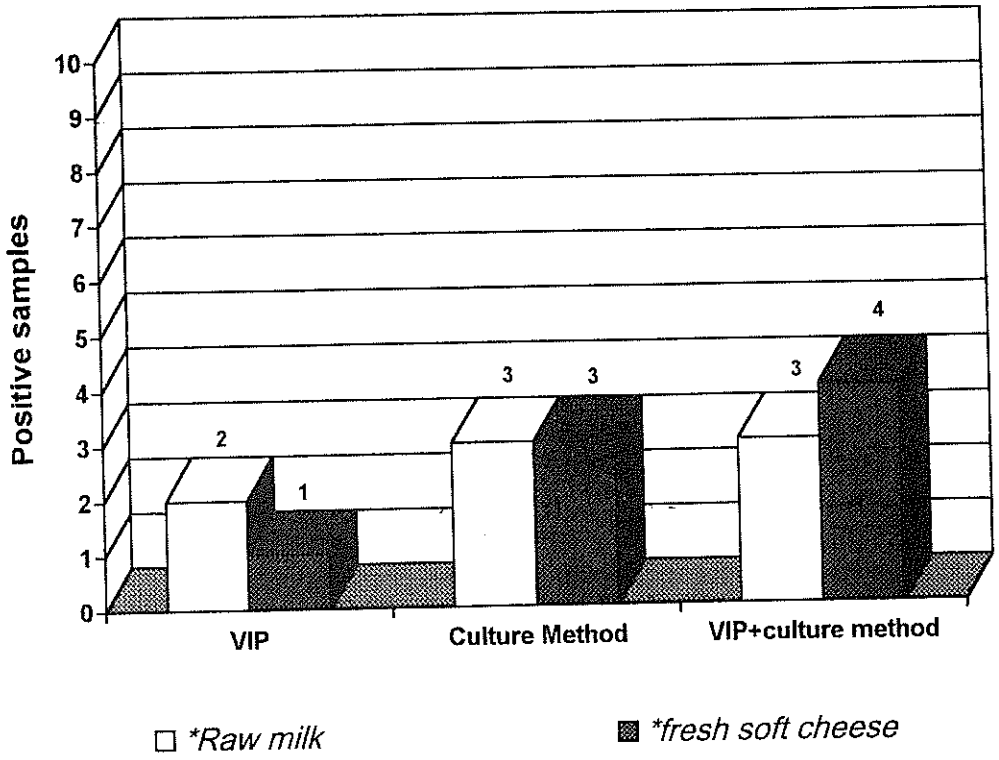


Figure (2)
Comparison between VIP™ assay and culture method for detection of *E.coli* O157:H7 in raw milk and raw milk cheeses



* No. of examined samples = 100

Figure (3)
Evaluation of the VIP™ assay for detection of *E.coli* O157:H7 in raw milk and raw milk cheeses

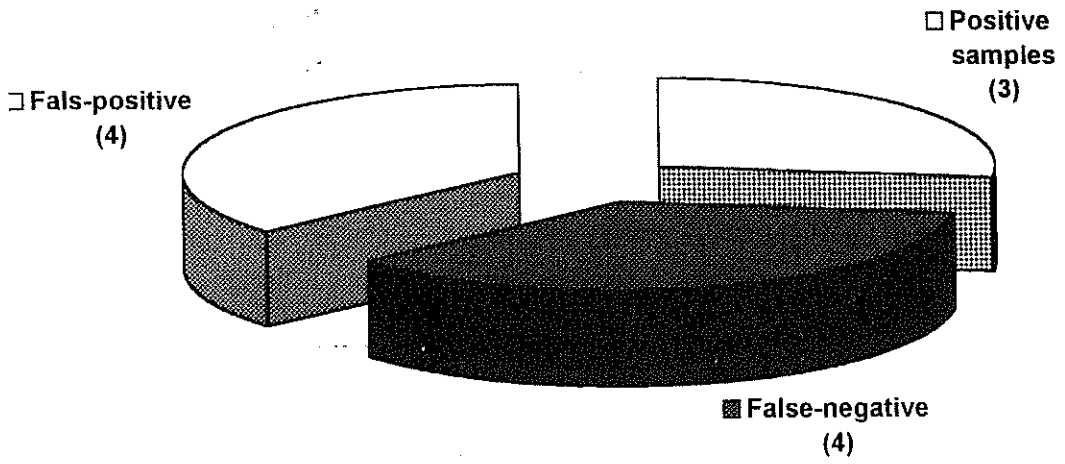
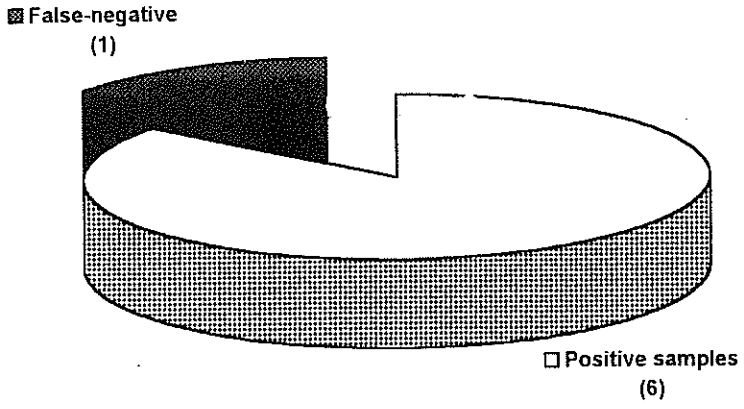


Figure (4)
Evaluation of the culture method for detection of *E.coli* O157:H7 in raw milk and raw milk cheeses



تقييم اختبار ال VIP™ للكشف عن ميكروب *الاشيرشيا كولاى* O157:H7 في اللبن الخام والجبنالمصنوع من اللبن الخام

عبد العزيز محمود أبو العينين

أجريت الدراسة على مائتين عينة من اللبن الخام و الجبن الطازج المصنوع من اللبن الخام (١٠٠ عينة لكل نوع) جمعت من أماكن مختلفة من محافظة القليوبية. تم تقييم اختبار ال VIP™ للكشف السريع على ميكروب الاشيرشيا كولاىO157:H7 وذلك بمقارنته بطريقة العزل الموصى بها من قبل منظمه الأغذية والأدوية الأمريكية (BAM Cultural Method) . تم عزل ميكروب الاشيرشيا كولاى O157:H7 بنسبة ٣،٥ %من إجمالي عدد العينات. كذلك تم عزل الميكروب من اللبن الخام والجبن الطازج الطري بنسبة ٣% و ٤% على التوالي. أثبتت الدراسة عدم كفاءة الVIP™ كاختبار سريع للكشف عن ميكروب الاشيرشيا كولاىO157:H7 حيث لم يكتشف الميكروب في ٥٧،١% من العينات التي تم عزل الميكروب منها بالطريقة التقليدية ، في المقابل أظهر نظام ال B AM Cultural Method تفوقا على اختبار ال VIP™ حيث لم يكتشف الميكروب في عينة واحدة فقط من العينات التي تم عزل الميكروب منها بطريقة ال VIP™ . وقد تم مناقشة النتائج والاحتياطات الواجب اتخاذها لتعظيم طرق الكشف عن هذا الميكروب وكذلك الإجراءات الوقائية التي يجب اتخاذها لتقليل تواجد هذا الميكروب في الألبان و منتجاتها.