The rapid detection of *E. coli* 0157 Antigen in meat products by using ELISA Test Kit

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Summary

This study was conducted for rapid detection of contamination of meat products with *E. coli* 0157 by usage of ELISA test kit as one of the most rapid and newest test. The study showed the efficiency of ELISA test kit used in this study in detection of bacteria antigen in meat products samples of raw beef meat , kabab and beef burger which subjected to improper storage conditions or undercooked and hence when some peoples uptake some of these products they suffered from clinical intoxication signs like diarrhea ,vomiting and hyperthermia. The kit showed the presence of (9) samples positive among (90) samples ; (2) positive out of (30) kabab samples,(3) positive out of (30) beef burger and (4) positive out of (30) raw beef meat after 16 hrs of enrichment of all samples in EC Modified broth including Novobiocin supplement as inhibitor of other bacteria and then the liquid supernatants from all prepared samples got tested by ELISA kit used in this study and the data recovered in less than 1 hr.The study indicated the ability of using ELISA kit for detection of *E. coli* 0157 antigens in food stuffs and reduce the time for releasing the results in less than 24 hrs when compared with conventional culturing procedure which reuiqred more than 3 days and launch the food products for consumption with focusing on the main point here which is the protection of our consumer safety.

Key words: E.coli 0157 , antigen , Elisa .

التشخيص السريع لمستضدات الايشيريشيا القولونية نوع O157 في منتجات اللحوم باستخدام عُدة فحص الاليزا

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الخلاصة

اجريت هذه الدراسة لتشخيص تلوث منتجات اللحوم بجراثيم *E.coli* نوع O157 باستخدام عُدة فحص الاليزا كاحد الفحوصات السريعة الحديثة. اذ اظهرت الدراسة كفاءة فحص الاليزا بتشخيص وجود مستضدات الجرثومة في نماذج منتجات لحوم الابقارالغير المطبوخة والكباب والبركر البقري المستورد والتي لم يتم خزنها في ظروف مثالية اوتعرضت الى الطبخ غير السليم وحدث نتيجة تناولها من بعض الاشخاص الى ظهور اعراض تسمم سريرية منها الاسهال الحاد والقئ وارتفاع درجات الحرارة.اظهر الاختبار وجود (9) نماذج موجبة من اصل (90) وبواقع (2) انموذج من

اصل (30) نموذج كباب و (3) انموذج من اصل (30) انموذج بركر بقري و (4) انموذج من اصل (30) انموذج لحم بقري بعد تنمية جميع النماذج لمدة 16 ساعة في وسط الـ EC Modified broth المضاف له مضاد الـ Novobiocin كمثبط لنمو الجراثيم الاخرى ومن ثم فحص السائل الطافي من تحضير النماذج جميعها بعُدة فحص الاليزا المستخدمة في هذه الدراسة خلال مدة اقل من ساعة والحصول على النتائج مقارنة بطول المدة اللازمة لطريقة العزل الجرثومي التقليدية.وبذلك يمكن الاعتماد على استخدام عدة فحص الاليزا للتشخيص السريع لوجود مستضدات هذه الجراثيم في نماذج الغذاء واعطاء النتائج بوقت اسرع وباقل من 24 ساعة واطلاق المنتجات للاستهلاك مع توفير النقطة الرئيسة وهي حماية المستهلك.

Introduction

Strains of *Escherichia coli* that produce Shiga-like toxins (SLTs), also known as Verocytotoxins (VTs), are an important cause of human disease. Clinical manifestations of infection include diarrhea, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). Although Shiga-like toxin producing *E. coli* (SLTEC) belong to many O serogroups, serotypes, 0157:H7 and 0157: H ; are the predominant SLTEC associated with HC and HUS (1 and 2). Outbreaks of HC and HUS due to E. coli 0157 have been linked to consumption of undercooked ground beef (1 , 3 and 4). Prevalence studies indicate that up to 3.7% of retail meats may contain *E.coli* 0157 (5 and 6).And cattle also known as a source of Verocytotoxin *E. coli* O157 infections in man when samples from meat and surface swabs cultured from bovine carcasses indicated isolation of *E. coli* O157 (7). Although this may seem to be low compared to some other food borne pathogens, the seriousness of disease caused by *E. coli* 0157 has made this pathogen a major food safety concern (5 and 7).Detection of *E. coli* 0157:H7 in meats by cultural methods is time consuming, requiring several days to obtain presumptive positive results (8). ELISA is a rapid and reliable test which significantly reduces the time required to screen meats for the presence of *E. coli* 0157. Primary enrichment cultures grown for 8-16 hours can be tested in less than one hour, allowing ELISA-negative product to be released within 24 hours. Enrichment broths presumptively positive for *E. coli* 0157on the basis of positive ELISA tests can be cultured further for confirmation by standard methods (9 and 10).

Materials and Methods

Fisrt of all, the samples cultivated into modified EC broth (Accumedia-NeogenEurope,USA) with addition of Novobiocin supplement (Oxoid Limited) and then the sample supernatants used to be checked by ELISA(SafePath® *E. coli* 0157 ELISA kit from Safepath labs,USA).

Twenty five (25) gram of 90 of each meat products (30 samples of beef burger , 30 samples of Kabab which were stored and cooked improperly and

30 samples of raw meat which were stored improperly) added to 225ml of Modified EC broth + Novobiocin Supplement (final concentration 20ug/ml) together in a sterile stomacher bag. The samples stomached for 2 minutes
The samples content transferred to s sterile flask and shaked at 37°C for 16 hrs. One (1) ml aliquot from each sample placed in a separate clean screw top test tube.

The plate pouch opened and 12 strips used to run 96 wells (90 samples in single wells as well as to positive and negative controls in triplicate) 100ul of positive control added to well A1 and B1 and 100ul of negative control added to C1 and D1and then 100ul of the samples supernatants from the samples preparation added subsequently to the rest of wells.
The plate incubated at room temperature for 15 minutes, and then washed 3 times by the washing buffer (prepared by addition of the wash buffer concentrate to 475ml of distilled water). 100ul of enzyme conjugate added to each well. The plate incubated for 10 minutes and washed as above, then rinsed with distilled water 1 time.

Fifty (50)ul of the Chromogen added to each well and the wells mixed thoroughly by gentle tapping the side of the strip holder with index finger. The plate incubated for 5 minutes. Hundred ul of stop solution added and wells mixed.

The plate read with ELISA reader (STATFAX, Neogen Europe, USA) at 450/620-650 nm within 4 hrs from the stopping of reaction.

Kit Validity:

The Positive and Negative Controls run each time as the assay is performed. The Negative Control below 0.12 ODs and the Positive Control greater than 1.0 OD units.

Visual Result:

Positive: Any sample well that has significant and obvious yellow color.
Negative: Any sample well that does not have significant and obvious yellow color.

ODs – Results:

Positive Samples: OD readings of 0.20 and above.
Negative Samples: OD readings of less than 0.20.

Results

Kit validity evaluated through the OD got from ELISA reader which showed the following values at 450nm:
-The Mean Negative Control OD was 0.097
-The Mean Positive Control OD was 1.257
So, the above mentioned values proves that the kit is valid.

Table 1: Shows the results of ELISA testing samples

Meat Products	No. of Samples	Positive Elisa result at 450nm (Mean samples OD ≥ 0.20)	Negative Elisa result at 450nm (Mean samples OD < 0.20)
Kabab	30	2	28
Beef Burger	30	3	27
Raw Meat	30	4	26

Meat samples tested by ELISA technique (table1) showed 2 positive kabab samples out of 30 (6.6%), 3 positive beef burger out of 30 (10%) and 4 positive meat trim samples out of 30 (13.3%) .The value of positive samples considered positive when their OD value at 450nm were ≥ 0.20 ,while the negative ones considered negative when their OD values were < 0.20 according to kit leaflet instruction which followed strictly. Meats results obtained after 16 hrs enrichments of each samples with modified EC broth plus Novobiocin supplement.

Discussion

Elisa technique could be considered as a useful aid for rapid detection of contaminated meat products with *E. coli* O157 whether food improperly stored or undercooked and this study showed that the percent of most of contaminated samples exceed 3.6% (1 and 3). The kit has a limit of detection of approximately 1,000 CFU/ml of meat samples and this depend on the strain of *E. coli* O157 tested (11,12 and 13) and this indicated that most of positive samples tested in this study have a limit of 1,000 CFU/ml and more and the negative one less .The enrichment of samples mandatory required 8-18 hrs for optimum growth of *E. coli* O157 to get a significant number of the bacteria to enable ELISA kit of capturing them.The kit is highly specific and sensitive to give a positive reaction if 1 CFU/g of *E. coli* O157 inoculated in a meat sample after 16 hrs of enrichment.Thus conclude,that using ELISA technique in parallel with conventional methods, as ELISA reduce the time required to screen meats for the presence of *E. coli* O157 (11,13,12,5 and 10).

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