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EVALUATION OF LISTERIA RAPID TEST AND TRADITIONAL MTHOD FOR DETECTION OF LISTERIAS IN UNPASTEURIZED CREAM AND MARKET RAW MILK

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ABSTRACT

Thirty-five samples, 25 unpasteurized cream and 10 market raw milk, collected from street vendors and dairy shops in Damietta City for evaluation of Listeria Rapid test and FDA for qualitative detection of Listerias. The results obtained showed that superiority of Listeria Rapid Test which yielded 12% of Listeria positive samples, whereas FDA yielded 8%. Identification of isolated Listeria spp., confirmed the reliability of Listeria Rapid Test to detect L. monocytogenes(8%) two times more than that obtained by FDA (4%). L. seeligeri was found in only one unpasteurized cream sample by both methods.

One of ten(10%) market raw milk samples proved to be L. monocytogenes positive sample by both methods.

Availability of Listeria Rapid Test as a new method for rapid detection of Listerias in imported and exported dairy products.

INTRODUCTION

Listeria has attracted wide attention of recent Egyptian Standard because of that bacteria has gone through the stage of an emerging pathogen to a fully recognized food-borne pathogen of concern. Outbreaks of human Listeriosis involving many deaths linked with consumption of milk and dairy products. Indeed, the term Listeria can evoke a feeling of near panic within the dairy industry. Listeria monocytogenes survived more one year in Cheddar cheese (***Ryser and Marth, 1987***), tolerate famous preservatives and can grow at temperatures under zero (psychrophilic).

The genus Listeria is composed of seven species (L. monocytogenes, L. ivanovii, L. innocua, L. welshimeri, L. seeligeri, L. grayi and L. murrayi). Although L. monocytogenes is believed to be the most common Listerial causative agent of food poisoning, abortion and fetal meningitis, many investigators could isolate L. ivanovii from a woman who had aborted and announced the pathogenicity of L. welshimeri and L. seeligeri in men (***Seeliger and Jones, 1986***; ***Chakraborty and Goebel, 1988*** and ***Elischerova et al., 1990***).

Food-borne transmission through milk appear to be the major means of zoonotic transmission of Listeriosis. ***Farber et al.,*** (***1990***) discussed the danger of

shedding Listeria in apparently normal milk thus constituting a potential public health hazard.

There is no information in available literature about the presence of Listeria in cream.

This study was focused on evaluation of Listeria Rapid Test for detection of Listeria in food of animal origin under address of we need rapid answer beside study the role of cream in transmission of Listeria to human.

MATERIALS AND METHODS

Sampling :

Thirty-five samples, 25 unpasteurized cream (package, 125g) and 10 market raw milk (ca, 500ml), were collected from dairy shops and street vendors in Damietta City. The samples were kept in an insulated ice box ($4\pm1^{o}C$), transferred to the laboratory and subjected to qualitative detection of Listeria by using Listeria Rapid Test (***Oxoid, 2002***), (AOAC and AFNOR approved) and Food & Drug Administration (FDA) method (***Lovett, 1987***).

Preparation of cream samples:

Cream samples were thawed in a water bath adjusted at 40 ^{o}C /10 minutes (***Al-Ashmawy et al., 2002***).

I-Listeria Rapid Test:

a-Primary Enrichment:

Twenty-five ml/g of milk/cream samples were homogenized in a sterile wide mouth jar with 225ml of Fraser Broth (FB-CM895) supplemented with Half Fraser Selective Agent (SR 166A). The mixture was incubated at 30 ^{o}C/21 hours.

b-Secondary Enrichment:

0.1ml of FB culture was inoculated into 10ml volume of Buffered Listeria Enrichment Broth (BLEB-CM897)supplemented with BLSB Selective Agent (SR141E). The incubation was done at 30 ^{o}C/21 hours.

c-Antigen Extraction:

Two ml of upper region of BLEB culture were transferred to a small test tube incubated in a water bath adjusted at 80 ^{o}C/20 minuets, then tempered at room temperature.

d-Listeria Device:

the Device was left at room temperature shortly before use. 135 µl of BLEB extract were transferred (micropipette) onto a pad in the Sample Window. The antigen/latex complex was moved by capillary action to both Result Window and Control Window. After 20 minutes the Device was examined for appearance of a blue line in the Control Window and Result Window.

Appearance of a blue line in the Control Window indicated the Device has worked correctly. Appearance of blue line in the Result Window indicated Listeria positive sample or result.

Which Listeria could isolated ?

A loopful of BLEB positive culture was streaked onto PALCAM Agar (CM877) and Oxoid Oxford Agar(CM856) (***Oxoid, 2002***) incubated at 30 °C /24 hours. Typical Listerial colonies were purified and identified basically on sugar fermentation (rhammnose, xylose and mannitol) according to ***Lovett*** (***1987***).

II-Traditional method(FDA), (*Lovett et al., 1987*):

a- Primary Enrichment:

Twenty-five ml/g of examined samples(milk/cream) were diluted with 225ml FDA broth supplemented with acriflavine -HCl (15mg/l), nalidixic acid(40mg/l) and cycloheximide (50mg/l), incubated at 30 °C / 24 hours.

b-Secondary Enrichment:

0.1ml of culture was inoculated into 10ml of FDA broth with one exception acriflavine-HCl concentration 25mg/l and incubated at 30 °C /24 hours.

A loopful from secondary enrichment was streaked onto Oxford and PALCAM media and incubated at 30 °C /24 hours.

Suspicious Listerial colonies:

Gray-black colonies with a black haloes (Oxford medium) and gray-green colonies with black sunken center and a black haloes (PALCAM medium).

Three to five colonies were purified and subjected to key of biochemical test {Gram's stain, umbrella like motility, catalase, oxidase, Kligler Iron

Agar(glucose-lactose fermentation, gases and H_2S)} and identified according to (***Lovett, 1987***).

RESULTS AND DISCUSSION

Table(**1**): Illustrated the evaluation of Listeria Rapid Test and FDA for qualitative detection of Listerias in unpasteurized cream. The results obtained showed Listeria Rapid Test was more productive for detection of pathogenic Listerias (12%) compared to FDA(8%).

Identification of isolated Listeria spp. confirmed superiority of Listeria Rapid Test in recovering the high rate of isolated L. monocytogenes in cream samples (8%). Whereas FDA was yielded 4% of L. monocytogenes positive samples. Only one sample of examined cream proved to be L. seeligeri positive by both methods (**Table**, **2**).

We have no information in available literature about the presence of Listeria spp. in cream. Only one recent study failed to detect Listeria monocytogenes in cream samples (***Pak et al., 2002***).

Table(**3**): showed the prevalence of Listeria monocytogenes in market raw milk by applying both methods. Our finding revealed that only one of ten (10%) examined samples was soiled with L. monocytogenes by using Listeria Rapid Test and FDA.

This observation was in agreement with that reported by ***Hayes et al.,*** (***1986***) and ***Azza-Deeb*** (***2000***). Higher prevalence percentages were reported by ***Dominguez-Rodriguez et al.,*** (***1985***) and ***Harvey and Gilomour*** (***1992***) while lower prevalence percentages were found by ***Rodler and Korbler*** (***1989***); ***Rea et al.,*** (***1992***) and ***Uraz and Yucel (1999)***.

Clearly Listeria Rapid Test gave a fast, reliable answer for detection of Listeria within 43 hours. On the other hand cold enrichment at 4 ^{o}C concumed very long time (2-3 months) (***Prentic and Neaves, 1988***) and the fast traditional method carried out through one week (***van Netten et al., 1991***).

RESULTS

Table (1): Evalution of Listeria Rapid Test and FDA for detection of Listeria in unpasteurized cream.

Total No. of Samples	Positive Samples		Applied Method
	No.	%	
25	3	12	Listeria Rapid Test
	2	8	FDA

Table (2): Evaluation of Listeria Rapid Test and FDA for detection of L. monocytogenes and L. seeligeri in unpasteurized cream samples.

Listeria spp.	Positive Samples		Applied Method
	No.	%	
L.monocytogenes	2	8	Listeria Rapid Test
	1	4	FDA
L.seeligeri	1	4	Both

Table (3): Qualitative detection of L. monocytogenes in market raw milk by using Listeria Rapid Test and FDA.

Total No. of Samples	Positive Samples		Applied Method
	No.	%	
10	1	10	Both

CONCLUSION

How dose Listeria Rapid Test work?

1. primary (Fraser Broth) and secondary (Buffered Listeria Selective Broth/BLSB) enrichment stimulate the productivity of B-flagella antigen.
2. Heating cultures of BLSB at 80 °C /20 minutes facilitate the extraction of B-flagella antigen (***Seeliger and Jones, 1986***; ***Holbrook et al., 1993***; ***Parry et al., 1993***and ***Holbrook et al., 1994***).
3. Listeria except L. grayi possess a common B-flagella antigen which is not shared by other bacteria.
4. The specific monoclonal Listerial antibodies in the Device (coated latex) have been tested against B-flagella antigen giving a blue line in a Result Window in case of positive result.

Superiority or necessity to Listeria Rapid Test:

It is simple system, unlike Elisa no reagents, mixing or washing steps are required.

It is a sensitive test which can be detected the low level of Listerial cells (two cells/ml or gram) in foods.

It gives a clear visual rapid result, the time consumed (43 hours) less than one third (1/3) the time taken by traditional methods (about one week) and cold enrichment (2-3 months).

No rapid judgment without rapid answer.

It is designed for the detection of Listerias in foods including milk, dairy products, meats, poultry, vegetables and fishes. Besides environmental samples (stainless steel, plastic, rubber, glasses, wood, air filter).

It is recommended by AOAC Research Institute (USA).

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تقييم اختبار الليستيريا السريع و الاختبار التقليدي للكشف عن ميكروبات الليستيريا في القشدة غير المبسترة و اللبن الخام

*ا.د. محمد الشربينى السيد** – د. مدحت إبراهيم محمد عبدالله***

أستاذ الرقابة الصحية على الألبان و منتجاتها و رئيس قسم الراقبة الصحية على الأغذية –كلية الطب البيطرى –جامعة المنصورة - مصر*

باحث بمعمل فحوص الأغذية بميناء دمياط البحرى - معهد بحوث صحة الحيوان – الدقى - القاهرة**

أجريت الدراسة على عدد ٣٥ عينة (٢٥ عينة قشدة غير مبسترة و ١٠ عينة من اللبن الخام المباع بالأسواق) تم تجميعها من مدينة دمياط وذلك لتقييم اختبار الليستيريا السريع Listeria Rapid Test بالمقارنة مع الاختبار التقليدي FDA في الكشف عن الليستيريا Listerias .

أسفرت النتائج عن تفوق استخدام الطريقة الحديثة فكانت أسرع و أدق و اسهل و اكثر كفاءة في عزل الليستيريا حيث دلت على نسبة أعلى (١٢%) عنها باستخدام FDA (٨%) في القشدة غير المبسترة . بالتعرف على أجناس الليستيريا أظهرت النتائج عزل عترة L. monocytogenes باستخدام Listeria Rapid Test بنسبة (٨%) بينما كانت النسبة التي توصلت لها الطريقة التقليدية FDA (٤%) . تواجدت عترة L. seeligeri في عينة واحدة(٤%) من القشط غير المبسترة باستخدام الطريقتين . بالنسبة للبن الخام المباع بالأسواق تواجدت عترة L. monocytogenes في عينة واحدة بنسبة (١٠%) باستخدام الطريقتين.