j.Egypt.soc.med.Assoc 77, no 3. 435 – 442 (2017)

NOVEL MEDIA TO IDENTIFY SORBITOL NEGATIVE *E. COLI* TO EASE DETECTION *E. COLI* O157:H7. CANDIDATE NAME: AMANY EL-DESOKY RAMADAN BADAWY

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ABSTRACT

Identification of *Escherichia coli* is an important task in both public health and Clinical microbiology laboratories. *E.coli* is responsible for a wide variety of diseases in human and animals, including diarrhea, septicemia, hemorrhagic enteritis, respiratory diseases and ear infections. Pathogenic isolates of *E.coli* are of special significance, therefore, a rapid, inexpensive method to presumptively identify *E.coli* isolates with a high specificity and sensitivity is desirable. We developed a single-tube method as a screening test for *E.coli* sorbitol-negative from various clinical human stool, bovine feces and food specimens also could be used for environmental samples.

In the present study novel: cellobiose-inositol-sorbitol- Iron-indole agar used as a new medium (in single tube) as a selective/differntional to identify sorbitol negative *E. coli* to ease detection *E. coli* O157:H7 versus conventional identification.

The aim of study: To evaluate a novel medium in a single tube, for screening isolates suspected to possibly represent sorbitol negative *E.coli* and comparison of its results to results of the classical biochemical reactions (FDA) procedure includes: TSI, LIA, MIO, Urea, Simmons Citrate and sorbitol tube.

The novel medium that screens sorbitol negative *E. coli* in single tube will be cost effective and time saving procedure to ease detection of serotype O157 that is why we selected this new medium as a subject of study.

Keywords:

E.coli, Screening; Gram-negative bacilli; Culture media.

INTRODUCTION

Despite the fact that *E. coli* as commensal bacteria can be found in the intestinal microflora of human and animals, most strains are harmless, and some pathogenic cause disease as well as human, mammals and birds **(Riley LW *et al.,* 1983)**.The first recognized *E. coli* O157 outbreak occurred in 1982 in Oregon and Michigan and was associate with eating hamburger form a particular fast food chain **(Riley LW *et al.,*1983**).Evidence indicating rare sporadic infection occurred prior to 1982 comes from retrospective review by the centers for disease control and prevention (CDC) of over 3,000 *E. coli* serotype identified from 1973-1983, in which O157:H7 was detected only once a 1975 isolate from a 50 years old California woman. The subsequent occurrence of large outbreaks and the wide spread distribution of cases has led to the designation of *E. coli* O157:H7as a new, emerging pathogen. The disease caused by *E. coli* O157:H7 is hemorrhagic colitis; and is characterized by severe cramping (abdominal pain) and diarrhea "watery and/or bloody". Other symptoms may include vomiting and/or low grade fever. The illness lasts an average of 8 days. Treatment for *E. Coli* O157:H7 infection is primarily supportive including management of dehydration and complications such as “anemia and renal failure” The proportion of all cases of diarrhea estimated to be associated with *E. coli* O157:H7 is 0.6% to 2.4% of all cases of bloody diarrhea or hemorrhagic colitis, 15% to 36% are estimated to be caused by *E. coli* O157:H7. Serious complications of *E. coli* O157 disease occur in 0 to 15% of cases and are experienced more frequently by the very young and the elderly. Our study described using of a new medium that’s identified *E. coli* O157:H7, which formulated a tube medium based on identify sorbitol negative *E. coli* among lactose fermenters growing on MacConkey agar.

The principle:

Is that a lactose positive colony; that fails to ferment cellobiose, inositol nor sorbitol, but produces indole is exclusively a sorbitol negative *E. coli* that should be serologically tested for O157.The medium has been evaluation by Egyptian Academy of Scientific Research.

MATERIAL AND METHODS

To evaluate the method, (250) strains of Gram-negative isolates were tested. We tested this tube with all isolates all were selected based on conventional biochemical reactions and FDA procedure.

Samples:

Samples were collected from three sources; human stool and bovine feces specimens and food. A total of two hundred and fifty were collected:100 human, 100 bovine and50 samples of food "ground meat and hamburger", were presenting with enteric symptoms suspected to be *E. coli*.

Isolation and identification of the organisms by culture methods.

Human fecal specimens, bovine fecal specimens and food stuffs specimens; were collected according to standard method for diagnosis of *E. coli* O157 (BAM/FDA) chapter 4.

Human fecal specimens:

Specimens were streaked on MacConkey agar, then incubated aerobically at 34°C for 24 hours for enrichment and then suspected *E. coli "*pink colonies*"* colonies were picked up for conventional biochemical reaction then serological identification and compared with the new tested medium.

Bovine fecal specimens:

Specimens were streaked on Sorbitol MacConkey **(March SB *et al.*, 1986)** with then incubated at 35 °C for 24 hours for enrichment and the suspected *E. coli* pale colonies were picked for further conventional biochemical reaction then serological and compared with the new tested medium.

Food stuffs specimens:

Add 25gm of food stuffs specimens to 225 ml buffer field's phosphate buffered water, incubated at 37 ± 0.5 ^{0}C for 24 hours shaking. Streak one loop-full of the enrichment on Tellurite-Cefixime-Sorbitol MacConkey agar "TCSMAC". Then incubated at 35-37^{0}C for 18- 24 hours with for enrichment. Typical suspected *E. coli* O157:H7 are colorless or neutral gray with a smoky center and 1-2 mm in diameter, were picked-up for further conventional biochemical reactions then serological and compared with the new tested medium. Each suspected *E. coli* isolates were subjected to confirmation by FDA recommended conventional biochemical **(Ewing *et al.*, 1986**) tests includes: Triple Sugar Iron Agar (TSI), Lysine iron agar (LIA), Simmons' citrate, Motility Indole Ornithine Medium (MIO), Urea Agar, sorbitol tube, then the same isolate was inoculated into our new tube, and incubated for 24 hours at 35°C. All of the above media and diagnostic reagents were obtained from (Oxoid limited Basingstoke), and were prepared and quality controlled with each time.

Celloboise, Sorbitol, Inositol, Iron, Indole agar in one tube "the novel medium".

The novel medium (CSTIA tube) is dispensed into tubes, autoclaved at 121 ^{0}C for 20 min., allowed to cool forming 1 inch butt and slant. In contrast to other sugar fermentation broths or semi-solid formula e.g.M116 of BAM/FDA; the inconvenience of tantalization or filtration doesn't exist. A sterile wire is used to stab the butt and streak the slant with a single colony.

Formula of the novel medium (CSTIA tube):

Peptone 10.0, tryptone 10.0, yeast extract 3.0, beef extract 1.0, L-tryptophan 2.0, Na chloride 5.0, D-Sorbitol 3.0, cellobiose 3.0, Myo-inositol 3.0, ferric citrate 0.33, Na thiosulphate 0.08, bromothymol blue 0.06 and Agar 15.0.

PH 7.4 ± 0.2 at 25^{0}C.

-The un-inoculated medium is green.

-The un-change in color with formation of red ring by adding few drops of Kovac's reagent indicates sorbitol negative *E. coli*.

In this way the tested medium in this single tube proves that:

-Sorbitol not fermented.

-Cellobiose not fermented.

-Inositol not fermented.

-Oxidase negative.

-H2S not produced.

-Indole positive.

Sorbitol negative *E. coli* more commonly *E. coli* O157:H7, however other serotypes may be detected.

RESULT

All (100%) of *E. coli* isolates tested were appropriately characterized by using this single tube with this medium. Similarly, (100%) of other Gram-negative bacilli were appropriately screened a sorbitol negative *E. coli.* This tube correctly identified 100% of *E.coli sorbitol negative* isolates compared to FDA procedure. The new tube correctly screened all sorbitol negative *E. coli* with 100% sensitivity and 100% specificity as compared with classical biochemical identification as shown in (Tables 1, 2, 3 and 4).

Food samples were empty from sorbitol negative *E. coli*.

Table (1): Showing result of biochemical reaction of human specimens.

Source	Biochemical reactions
Human specimens	**53 sorbitol positive *E. coli***
Lactose fermenter colonies	**8 sorbitol –negative *E. coli***
	39 other *Enterobacteriaceae*

Table (2): showing result of new one tubed medium**.**

Source	Sorbitol, cellobiose, inositol, iron, indole tube
Human specimens Lactose fermenter colonies	**91 specimens sorbitol positive**
	8 specimens sorbitol negative
	1 sorbitol negative, cellobiose positive

Non-sorbitol fermenter "NSF" colonies were examined in two pass:

1- Conventional biochemical reactions.

2- New one tubed medium "cellobiose, sorbitol, inositol, iron, indole tube".

Table (3): Showing result of culture bovine specimens on SMAC.

Source	Sorbitol- MacConkey "SMAC"
Bovine specimens	**22 sorbitol negative colonies**
	78 sorbitol positive colonies

Table (4): showing result of conventional biochemical reaction on Non-sorbitol fermenter colonies.

Source	Conventional biochemical reactions
Non-sorbitol fermenter colonies	**10 sorbitol negative *Escherichia coli***
	3 non-fermenter sp.
	9 other *Enterobacteriaceae*

DISCUSSION

Biochemical identification of *E coli* is not a simple test. FDA in bacteriological analytical manual identified lactose fermenting colonies as *E.coli* by: H_2S negative, urease negative, indole positive, citrate negative reactions. The authors described primary 24 hours screening with TSI, LIA, MIO, Urea, Simmons citrate and Sorbitol Tube; biochemical identification of *E coli* needs-at least- 6 conventional test tubes. The authors in both chapters suggested alternative use of API 20E or the automated VITEK biochemical assay to identify the organism as *E coli.* That means too many tests that could be difficult to interpret, so that a computer assisted system- manual or automated– may be preferably used **(Peter Feng *et al.*, 2002**).We noticed that most lactose fermenter species of *Enterobacteriaceae*, do also ferment cellobiose or produce H_2S or do not produce indole. *E. coli* is the unique exception among lactose fermenters in that it does not ferment cellobiose, nor produce H_2S and produces indole. It is known that *E. coli* cannot utilize the β- glucoside sugar cellobiose as a carbon and energy source unless a stringent selection pressure for survival is present. **(Vinuselvi and Lee 2011)** assumed that Engineering *E. coli* is required for efficient cellobiose utilization. This required mutations in the two cryptic operons to give the property of cellobiose fermentation **(Vinuselvi *et al,* 2011).** We carefully studied Farmers tables 1999 for biochemical identification of *Enterobacteriaceae*, and we concluded that: an oxidase negative Gram negative bacillus that is: Glucose fermenter, Non cellobiose fermenter, H_2S negative, Tryptophan deaminase negative, and Indole positive can be identified as *E. coli*. This identification is certain if the test colony is a lactose fermenter, while a few non-lactose fermenter spp. if *Enterobacteriaceae* including *Shigella* share (inactive) *E coli* this profile **(Farmer *et al*, 1999**). To gather testing glucose and cellobiose fermentation, sorbitol fermentation and H2S production, tryptophan deaminase activity and indole production in a single tube; we formulated a new medium similar in principles and color changes to KIA. This formula is nearly identical except for cellobiose that replaced lactose, sorbitol, inositol, and 2 grams of L- tryptophan were added. We suggested the name Cellobiose Sorbitol Tryptophan Iron Agar **(CSTIA**) for this formula; a new differential medium to be used in a tube (slant and butt); mainly directed to identify **sorbitol negative *E. coli.*** According to **(Farmer 1999 and Farmer *et al.*, 1985)**, calculation of the percent probability of lactose fermenter spp. other than *E. coli* (including that rarely ferment lactose e.g. *Pantoea agglomerans* and Yersinia intermedia) that show the biochemical identification lactose

fermenter, indole positive, H_2S negative, urease negative and sorbitol negative. And consequently misidentified as *E. coli*; yield a sum of 12 (that may be imagined as 1.2 spp.) falsely identified as *E. coli* **(Farmer *et al.,* 1999 and Farmer J *et al.,* 1985).** Calculation of the same spp. That show the profile: Cellobiose negative, H_2S negative, sorbitol negative, oxidase negative and indole positive – according to our proposal to be misidentified as *E.coli*; yield a sum of only 15.1 (i.e. 0.15 spp.) **(Farmer *et al.,* 1999 and Farmer J *et al.,* 1985**). These calculations theoretically predict a higher specificity of our group of reactions (in a single tube, and 23 hours) for identification of *E.coli,* than the classical biochemical identifications (in 6 tubes) This theoretical prediction; proved to be practically correct and made biochemical in a single tube much easier; saves time, effort, cost identification of **sorbitol negative *E.coli.*** And denies the need for computer assisted system **(Farmer *et al.,* 1999 and Farmer J *et al.,* 1985**). The detection of all sorbitol negative *E. coli* isolates tested demonstrates the high sensitivity (100%) of the CSTIA tube. We observe motility on microscope as described by Reynolds 2011, using 5-10µl of bacterial suspension in saline at the angle of a coverslip on ordinary glass slide. In this way we suppose that this practice is easy and more reliable than observing motility in semisolid agar tube e.g. MIO. In conclusion this medium unique decreases labor in preparation and autoclaving of 6 tubes versus one tube and provides an ease of interpretation, showing it is capable to identify sorbitol negative *E. Coli* strains and it will be a selective medium for sorbitol negative *E. Coli* strains also it reduces cost and time saving procedure to detect serotype O157:H7. This medium provides the most important biochemical reactions needed to screen for *E.coli* sorbitol negative in a single-tube format, which decreases labor by 85% (i.e., 1 tube is inoculated vs 6).

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