A novel single tube method for biochemical identification of *Escherichia coli*

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Background: Identification of *E.coli* is an important task in both public health and clinical microbiology laboratories. *Escherichia coli* is responsible for a wide variety of diseases in human and animals, including urinary tract infections, 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 degree of specificity and sensitivity is desirable. We developed a single-tube method as a screening test for *E.coli* from various clinical specimens and also could be used for environmental samples . **Aim:** To evaluate a novel medium in a single tube, for screening isolates suspected to possibly represent *E.coli* and comparison of its results to results of the classical IMViC plus H_2S, Urease and Arabinose 7 tubes (FDA) procedure. **Materials and Methods:** To evaluate the method, (398) strains of Gram-negative isolates were tested. We tested this tube with, *(213)E.coli* isolates, and (185) Non *E.coli* Gram-negative isolates all were selected based on conventional biochemical reactions and FDA procedure. ATCC quality control organisms were evaluated as well to ensure accuracy. **Results:** 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 as *non-E.coli*. This tube correctly identified 100% of *E.coli* isolates compared to FDA procedure. **Conclusion:** This unique medium provides the most important biochemical reactions needed to screen for *E.coli* and other *Enterobacteriaceae* in a single-tube format, which decreases labor by 85% (i.e, 1 tube is inoculated vs 7).

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1. Introduction

Public health laboratories consider *E. coli* the most important indicator of fecal pollution of water and foods. They deal with a great work load and responsibility to examine all foods and drinking water; not only for *Salmonella* and *Shigella*, but also for- the equally important- *E coli*, and additionally to detect O157 (and other diarrheagenic *E. coli* serotypes).

Urine is virtually the commonest specimen received by the clinical microbiology laboratory for culture. More than 95% of urinary tract infections are caused by a single bacterial species. *E. coli* is the most frequent infecting organism in acute infections (1,2).

Considerable interest has been shown by public health officials regarding *Escherichia coli* in foods and water. The implications of *E. coli*, especially *E. coli* biotype I, as an indicator of fecal contamination vary with the food type and the handling that the food has received. Some workers have stated that the *Enterobacteriaceae* as whole, and not just *E. coli*, should be taken into account when considering the sanitary standards and hygiene of food handling (3).

Urine cultures are the most commonly performed tests in clinical laboratories, contributing significantly to laboratory expense and workload. In addition to *E. coli,* other common urine pathogens include *Klebsiella, Pseudomonas, Acinetobacter, Proteus*, *Enterobacter, Serratia*, *Citrobacter* and *Salmonella*. Many laboratories have attempted to decrease time and materials involved in UTI testing and diagnosis by using chromogenic media (4).

This study aimed to compare reliability of detecting *E. coli* from lactose fermenter colonies by the classical IMViC plus H2S, Urease and Arabinose (7 tubes) FDA procedure; against a single **Cellobiose tryptophan Iron medium** (CTIA tube). Colonies that were identified as *E.coli* by CTIA tube compared to FDA procedure were considered correctly identified.

2. Materials and Methods

This study evaluated (398) Gram negative clinical isolates. All clinical strains were isolated from clinical samples sent to our routine microbiology laboratory of Faculty of medicine, Cairo university hospitals. They were (n=213) *E. coli* and other non *E. coli* were (185) **(Table 1).** Quality control strains were (Supplied by Microbiologics and imported by EL-Magd company in Cairo, Egypt), they included, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 13883 strains. The quality control strains were tested each

time the CTIA tube reagents were prepared and each time the clinical isolates tested.

We selected a single colony of *E. coli* or other Gram negative isolates previously identified by conventional biochemical reactions in our laboratory and as previously described by Ewing 1968 they included: Triple Sugar Iron Agar (TSI), lysine iron agar, Simmons' citrate, Christensen's urea, ornithine decarboxylase and indole (5). Then each isolate was subjected to confirmation by FDA recommended biochemical tests including, TSI, Urease broth, Arabinose broth, tryptone broth, Methyl-red, Voges-Proskauer and Citrate (6,7).The same isolated *E. coli* or other Gram negative colony was inoculated into our CTIA-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.

Table (1): Total isolates selected by conventional biochemical reactions.

Bacterial isolates tested	Number of isolates
E. coli	213
Klebsiella spp.	86
Proteus mirabilis	8
Proteus vulgaris	2
Enterobacter spp.	3
Pseudomonas spp.	54
Acinetobacter spp.	32
Total	398

Cellobiose tryptophan Iron medium (CTIA-Tube):

CTIA tube is our novel single tube. It is a unique formula prepared and provided by a national laboratory in Cairo, Egypt, known as Microlab. It is the same formula as that of Triple Sugar Iron Agar described by Sulkin and Willett 1940 (**Table 2**), who recommended it for differentiation of enteric Gram-negative bacilli from clinical specimens, dairy samples, and food products. CTIA tube has a minor modification of replacement of lactose and sucrose sugars by cellobiose sugar **(8).**

The formula of CTIA tube includes glucose, cellobiose, tryptophan and components of H2S. It is known that cellobiose is fermented by *Klebsiella – Enterobacter* group but not by *E. coli* ***(9).***

We used this sugar in a tubed medium similar to KIA i.e. contains glucose and H2S, So that; in absence of cellobiose fermentation the tube identifies a non fermenter by its inability to ferment glucose. Black butt declares H2S production. We included tryptophan in the formula; so that TDA activity is observed by spontaneous dark brown slant. Indole production is inferred by the cherry red color when adding Kovac's reagent at the angle between the slant and butt **(Table 3, Figs 1 and 2).**

Table (2): TSI formula (8).

Ingredient	Concentration (g/L)
Enzymatic Digest of Casein	5
Enzymatic Digest of Animal Tissue	5
Yeast Enriched Peptone	10
Dextrose	1
Lactose	10
Sucrose	10
Ferric Ammonium Citrate	0.2
Sodium Chloride	5
Sodium Thiosulfate	0.3
Phenol Red	0.025
Agar	13.5

Adjust each medium to pH 7.3 ± 0.2 at 25°C

Table 3: formulation of CTIA tube.

Ingredient	Concentration (g/L)
Agar	17.0
Tryptone	20.0
L-Tryptophan	5.0
Yeast extract	3.0
Ferric ammonium citrate	0.3
Sodium thiosulphate	0.3
Phenol red	0.003
Glucose	1.0
Cellobiose	10
Sodium chloride	5.0

Adjust pH to 7.6 ± 0.2, Sterilization at 115 °C for 20 min.

The isolate will be considered *E coli* if orange butt (glucose fermentation), indole positive (rarely negative). *Klebsiella pneumoniae* will show yellow butt (glucose and cellobiose fermentation), gas is commonly produced (some *Klebsiella spp*. Do not produce gas), slant is pale red.

Proteus will show black butt (H_2S), brown slant (TDA), *Proteus mirabilis* is commoner (indole negative), *Proteus vulgaris* (indole positive).

Providencia spp. (occasional); donot produce H_2S (orange butt), brown slant and indole positive.

Citrobacter (or *Salmonella*) will show black butt (H_2S), red slant, occasionally; gas and pale slant (cellobiose fermentation) or positive indole rule out *Salmonella*. About non fermenters there will be no acid (no colour change in the butt), the colour is darker on the slant, *Pseudomonas aeruginosa* is oxidase positive and *Acinetobacter spp.* is oxidase negative **(Figures 1 and 2).**

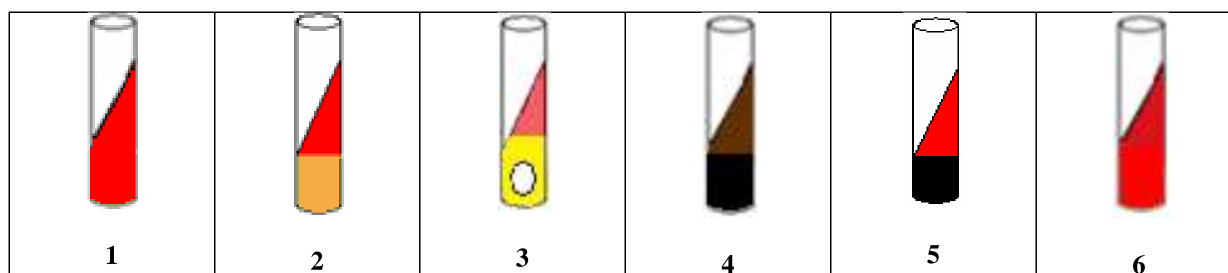
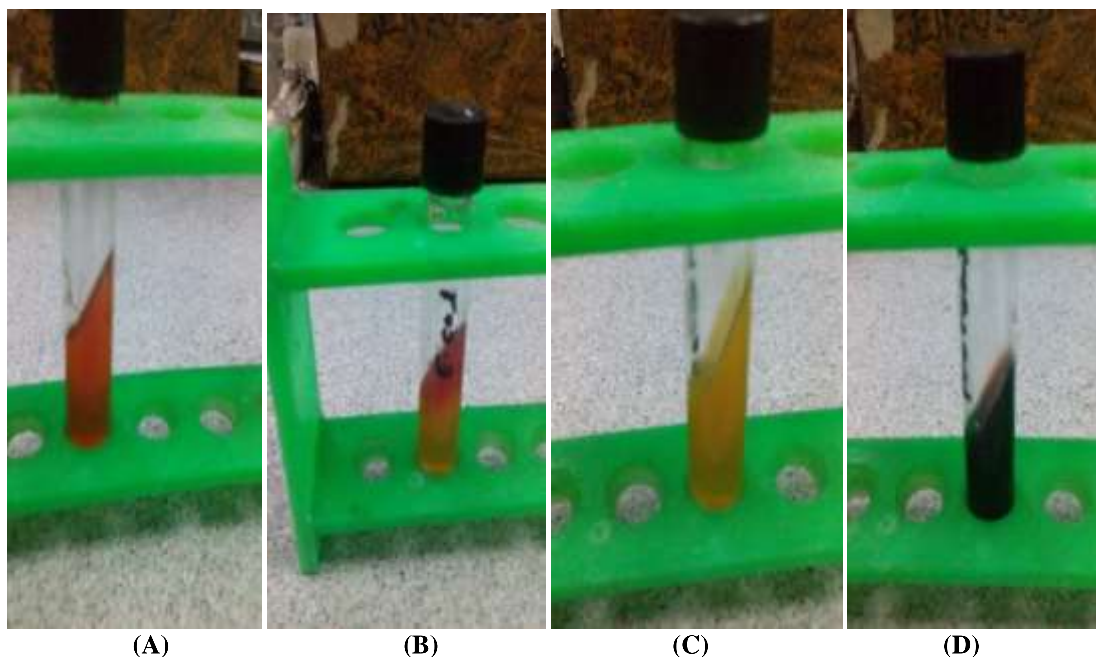


Figure 1: 1) Uninoculated tube ***2)*** *E. coli* ***3)*** *Klebsiellapneumoniae* ***4)*** *Proteus* ***5)*** Citrobacter (or Salmonella) **6)** *Pseudomonas or Acinetobacter spp.*



(A) **(B)** **(C)** **(D)**

Figure 2: The CTIA tube. (**A**) An uninoculated tube. **B**) Inoculated with *E. coli.* (**C**) Inoculated with *Klebsiella* (**D**) tube inoculated with *Proteus.*

3. Results

The CTIA tube correctly screened all 213*E. coli* isolates, with 100% sensitivity and 100% specificity as compared to FDA procedure. Also other (185) isolates were correctly identified as non *E. coli.*

4. Discussion

Biochemical identification of *E coli* is not a simple test. FDA in bacteriological analytical manual; identified lactose fermenting colonies as *E.coli* by: H2S negative, urease negative, arabinose positive, indole positive, methyl red positive, Vogues Proskauer negative and citrate negative reactions. The authors described primary 20 hours screening with TSI, urease, arabinose, and indole tests to be followed by secondary 48 hours screening including the IMViC tests (10).

According to FDA instructions; biochemical identification of *E coli* needs-at least- 7 conventional test tubes (TSI, Urease broth, Arabinose broth, tryptone broth, Methyl-red, Voges-Proskauer and Citrate), 3 days (or 2 days if both primary and secondary screening tests were done simultaneously). 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 (10).

We noticed that most lactose fermenter species of *Enterobacteriaceae*, do also ferment cellobiose, or

produce H_2S or donot produce indole. *E. coli* is the unique exception among lactose fermenters in that it does not ferment cellobiose, nor produce H2Sand 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 **(11).** 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. of *Enterobacteriaceae* including *Shigella* share (inactive) *E coli* this profile (9). To gather testing glucose and cellobiose fermentation, H_2S production, tryptophan deaminase activity and indole production in a single tube; we formulated a new medium similar in principles and colour changes to KIA. This formula is nearly identical except for cellobiose that replaced lactose, and 5 grams of L- tryptophan were added.

We suggested the name Cellobiose Tryptophan Iron Agar (CTIA) for this formula; a new differential medium to be used in a tube (slant and butt); mainly directed to identify *E. coli* (rather than *Salmonella* and *Shigella*, the main targets of KIA and TSI).

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. *Yersinia enterocolitica*); that show the IMViC profile ++--, H2S negative, Urease negative, Arabinose positive and consequently misidentified as *Ecoli*; yield a sum of 122.3 (that may be imagined as 1.2 spp.) falsely identified as *E. coli* (9,12).

Calculation of the same spp. That show the profile: Cellobiose negative, H2S negative, TDA negative, Indole positive – according to our proposal- to be misidentified as *E.coli*; yield a sum of only 15.1 (i.e. 0.15 spp.) (9,12). 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 IMViC plus H2S, Urease and Arabinose group of tests (in 7 tubes and 2-3 days). This theoretical prediction; proved to be practically correct and made biochemical identification of *E.coli* in a single tube much easier; saves time, effort, cost and denies the need for computer assisted system (**9,12**).

The detection of all 213 *E. coli* isolates tested demonstrates the high sensitivity (100%) of the CTIA tube.

This tube identification of previously known lactose or non lactose fermenter provides five biochemical reactions including glucose fermentation, cellobiose fermentation , H_2S production , indole production and tryptophan deamination. For interpretation of A/A reaction including *Klebsiella* (the commoner) and less commonly *Enterobacter*. In our own practice ; we observe motility on microscope as described by Reynolds 2011, using 5-10 µl of bacterial suspension in saline at the angle of a coverslip on ordinary glass slide . In this way we simply differentiate between *Klebsiella* (non motile and *Enterobacter* (motile) (13). We suppose that this practice is easy and more reliable than observing motility in semisolid agar tube e.g. MIO.

In conclusion this medium decreases labor in preparation and autoclaving of 7 tubes versus one tube and provides an ease of interpretation , also it reduces cost and saves time.

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References

1. Jellheden B, Norrby RS, Sandberg T. Symptomatic urinary tract infection in women in primary health care: Bacteriological, clinical and diagnostic aspects in relation to host response to infection. *Scand J Prim Health Care.*1996; 14: 122-8.
2. Ronald A. The etiology of urinary tract infection: Traditional and emerging pathogens. *Am J Med.* 2002; 113: Suppl 1A:*14S-9S.*
3. Michael E. Stiles and Lai-King. Biochemical Characteristics and Identification of Enterobacteriaceae Isolated from Meats. Applied and environmental microbiology, 1981, 41(3):639-645.
4. Olle Aspevall, Björn Osterman, Rakel Dittmer, Lena Ste´n, Emma Lindbäck, and Urban Forsum. Performance of four chromogenic urine culture media after one or two days of incubation compared with reference media. J. Clin. Microbiol. 2002;40:1500-1503.
5. Ewing, W. H. Differentiation of Enterobacteriaceae by biochemical reactions. National Communicable Disease Center, Atlanta, Ga.1968.

6. Mac Faddin JF. Biochemical tests for identification of medical bacteria. 3rd ed. Philadelphia: Lippincott Williams and Wilkins, 2000.
7. Forbes BA. Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic microbiology, 12th edition, Mosby Elsevier, 2007; 842-55.
8. Sulkin, S. E., and J. C. Willett, A triple sugar-ferrous sulfate medium for use in identification of enteric organisms. J. Lab. Clin. Med.1940; 25:649-653.
9. Farmer, J. J., III. Enterobacteriaceae: introduction and identification, p. 442–458. In P. Murray *et al.* (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.1999.
10. Peter Feng, Stephen D. Weagant, Michael A. Grant, William Burkhardt, FDA, Bacteriological Analytical Manual, Enumeration of *Escherichia coli* and the Coliform Bacteria. 2002. Chapter 4.
11. Vinuselvi P.: S. K. Lee. Engineering Escherichia coli for efficient cellobiose utilization. Appl Microbiol Biotechnol. 2011; 92:125–132.
12. FarmerJ, Betty R. Davis, F. W. Hickman-Brenner, Alma McWhorter, G.P. Huntleycarter, M. A. Asbury, Conradine Riddle, H. G. Wathen-Grady, Elias, G. R. Fanning, A. G. Steigerwalt, Caroline A, M. O'hara, G. K. Morris,' P. B. Smith, and Don, J. Brenner. Biochemical Identification of New Species and Biogroups of *Enterobacteriaceae* Isolated from Clinical Specimens. Journal of clinical microbiology. 1985; 21(1): 46-76.
13. Jackie Reynolds, Richland College, Biology 2420 Lab manual, 2011. http://delrio.dcccd.edu/jreynolds/microbiology/RLCmicroindex.html.

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