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Single-tube Quantitative Rapid Detection for Coliform Bacteria Based on Enzyme Specific Technology

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Reagents and instruments

Coliform selective coloring medium formula: tryptone 15.0 g, lactose 4.5 g, sodium chloride 4.5 g, dipotassium hydrogen phosphate 2.1 g/L, potassium dihydrogen phosphate 1.0 g/L, sodium deoxycholate 200 mg. The volume of distilled water was adjusted to 1000 mL, pH 7.0. After 121℃, 0.11Mpa high-pressure steam sterilization, the filtered and sterilized ONPG solution was added to make the final concentration of ONPG 0.5 g/L.

The single-tube quantitative rapid detecting device was manufactured by Xiamen Sitandao scientific device company complemented with "microbes detecting system" software.

Coliform bacteria HX16.3 was isolated and identified in domestic sewage (including Escherichia coli, citric acid bacterium, Klebsiella spp., Enterobacter spp.), selected and preserved by college of environment and public health microorganism experiment of Xiamen Huaxia University. Different food samples, such as octopus, Brassica chinensis L, clams, oysters, salmon, chicken feet, chicken wings were purchased from the farmers market; milk tea, bread, bubble tea were purchased from the store; environmental water samples were collected from the sewage plant sewage outlet, Huaxia College Swan Lake, Jimei Dragon Boat Pond , Landscape Pool of Huaxia College, Yundang Lake on Hubin North Road in Xiamen city.

Sample preparation

Take 10 g (or mL) of the test sample into an Erlenmeyer flask containing 90 mL of sterile saline and glass beads, shake well before taking 10 mL of the sample suspension, and then it

is passed by 30 μm polypropylene primary filter to remove large solid particles. After filtration, the liquid passes through a 0.22 μm cellulose acetate secondary filter membrane to collect the bacteria which will be then put into 10 mL colorimetric coliform selective medium.

Specificity of coliform-selective coloring medium

The bacteria include *Escherichia coli* (1.90, 1.355, 1.506, 1.487, 1.96, 1.29, ATCC25922), E. coli mixed strain (HX16.3), *Bacillus subtilis* (CMCC (B) 63501), *Enterobacter cloacae* (1.2022) , *Enterobacter aerogenes* (1.2021), *Enterobacter sakazakii* (ATCC51329), *Vibrio parahaemolyticus* (1.1614), *Staphylococcus aureus* (ATCC25923), *Pseudomonas aeruginosa* (ATCC27853), *Micrococcus luteus* (1.193), *Shigella flexneri* (CMCC (B) 51573), *Shigella flexneri* (CMCC (B) 51592), *Klebsiella pneumoniae* (CMCC (B) 41002), *Salmonella paratyphi* A (CMCC (B) 50093), *Salmonella paratyphi* B (CMCC (B) 50094), *Micrococcus lyticus* (1.634), *Corynebacterium glutamicum* (1.1886). One mL bacterial suspensions were inoculated into 10 mL of coliform-selective coloring medium to incubate at 36±1℃ for 48 h. Observe the growth of microorganisms and the appearance of yellow products at 24h and 48h, respectively, to verify the specificity of the coliform-selective coloring medium.

Table S1. The specificity of the coliform-selective coloring medium. The symbol "+" indicates a positive result of growth; the symbol "-" indicates a negative result of growth.

Strain	Culture for 24h	Culture for 48h
Escherichia coli (1.90)	+	+
Escherichia coli (1.355)	+	+
Escherichia coli (1.506)	+	+
Escherichia coli (1.487)	+	+
Escherichia coli (1.96)	+	+
Escherichia coli (1.29)	+	+
Escherichia coli (ATCC25922)	+	+
mixed coliform bacteria (HX16.3)	+	+
Bacillus subtilis (CMCC(B)63501)	-	-
Enterobacter cloacae (1.2022)	+	+
Enterobacter aerogenes (1.2021)	+	+

Enterobacter sakazakii (ATCC51329)	+	+
Vibrio parahaemolyticus (1.1614)	-	-
Staphylococcus aureus (ATCC25923)	-	-
Pseudomonas aeruginosa (ATCC27853)	-	-
Micrococcus luteus (1.193)	-	-
Shigella flexneri (CMCC(B)51573)	-	-
Shigella sonnei (CMCC(B)51592)	-	-
Klebsiella pneumoniae (CMCC(B)41002)	+	+
Salmonella paratyphi A (CMCC(B)50093)	-	-
Salmonella paratyphi B (CMCC(B)50094)	-	-
Micrococcus lyticus (1.634)	-	-
Corynebacterium glutamicum (1.1886)	-	-

The optimization of the detection wavelength

To determine the wavelength best suited for the detection, a comparison between different four samples were carried out. The four types of samples were respectively: coliform HX16.3 bacteria cultured for 10 hours at 37℃ in coliform-selective coloring medium, coliform-selective color development medium itself, coliform HX16.3 bacterial cultured for 10 hours at 37℃ in normal medium and ddH_2O. The absorption curves at wavelengths of 200 to 800 nm were measured.

Temperature and pH influence on coliform growth

HX16.3 was inoculated into the coliform-selective coloring medium, and cultured in a thermostatic incubator at 35 ℃, 37 ℃, 40 ℃, 42 ℃ and 44 ℃, respectively. The numbers of coliform bacteria were determined at 0, 2, 4, 6, 8, 10, 12, 24, 36, 48, 60 and 72 hours by traditional plate counting method. Meanwhile, take a measurement of the absorbance at the optimal wavelength (410 nm) for the coliform-selective coloring medium itself without bacteria under the same culture temperature to observe the influence.

HX16.3 was inoculated in the coliform-selective coloring medium with different pH values (5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0). The bacterial concentrations were determined by the traditional plate counting method and the absorbance value at the optimal wavelength were

monitored to verify the influence of pH. The experiments were done in three parallel replicates.

Single-tube quantitative rapid detecting process

The original coliform HX16.3 was diluted in a series of concentrations for separately 10^3, 10^4, 10^5, 10^6, 10^7 folds. Different concentrations of HX16.3 were incubated with the sterilized steamed bun to mimic the real food sample and pretreated as abovementioned. Subsequently HX16.3 was quantified by the plate counting method as a standard reference. Meanwhile, the same pretreated HX16.3 were put into the single-tube quantitative rapid detector for the absorbance measurement every 10 seconds during the cultivation at 37 °C for 16 hours. The relationship between the concentration and absorbance value was then established according to the results.

The precision of the method

Take the coliform solution of the same concentration (10^3 folds dilution) in the mimic white steamed bun sample into eight paralleled channels separately after sample pretreatment, and make a quantitation of each channel to examine the precision among different channels.

Measure the concentration of coliform bacteria (580 cfu/mL and 34 000 cfu/mL) in one channel for 10 times by the device. The precision of the single-tube rapid detection method can be validated from the reproducible results.

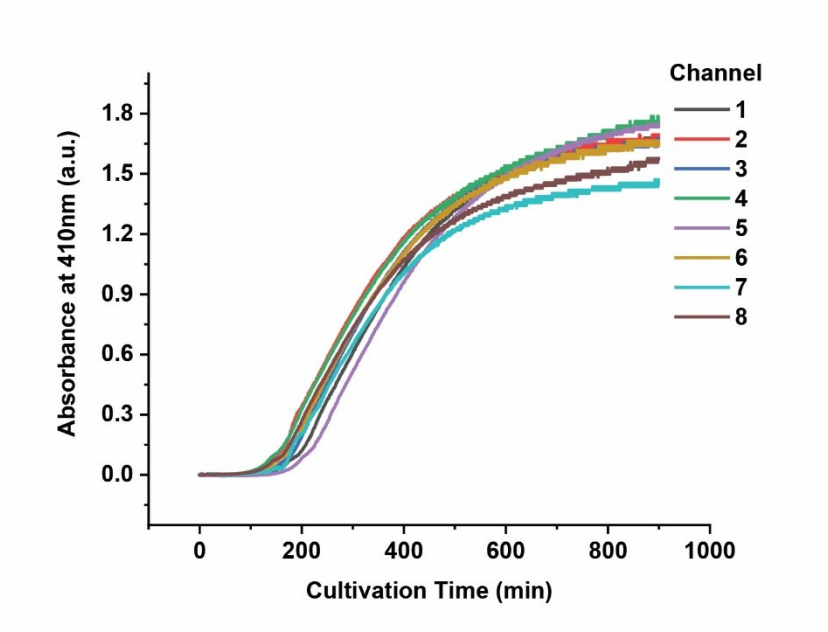

Fig S1. The absorbance changes over cultivation time among eight channels.

Table S2. The precision of the single-tube quantitative rapid method for coliform.

Measurement times	coliform conc. (580 cfu/mL)	coliform conc. (34000 cfu/mL)
1	538	39002
2	620	42501
3	550	38899
4	578	36823
5	559	32808
6	553	35086
7	498	40012
8	563	30899
9	585	33566
10	593	35866
Average (cfu/mL)	564	36546
RSD (%)	5.91	9.84

Real sample detection for coliform bacteria

Select representative real samples, including solid samples, liquid samples, and water samples in various environments, make quantitation of each sample with single-tube quantitative rapid detector for coliform bacteria in 3 parallel replicates, and compared the results with that of Chinese national standard method (GB4789.3-2016) by Student's t test to determine the

significance difference.

Table S3. The detection results for coliform of single-tube quantitative rapid detection method and national standard method in environmental water

Samples	Rapid detection method		Multi-tube fermentation method	
	Coliform cfu/mL	RSD (%)	Coliform cfu/mL	RSD (%)
Sewage plant drain 1	531±31	5.79	811±231	28.55
Sewage plant drain 2	162±32	19.54	213±69	32.58
Huaxia College Swan Lake 1	26574±4733	17.81	27245±11695	42.93
Huaxia College Swan Lake 2	15132±1570	10.38	17425±3759	19.85
Jimei Dragon Boat Pond 1	112±10	8.61	164±27	16.59
Jimei Dragon Boat Pond 2	46±13	28.50	73±35	47.88
Huaxia College Landscape Pool 1	1902±192	10.08	1706±674	39.49
Huaxia College Landscape Pool 2	922±125	13.55	1224±773	63.11
Yundang Lake from Hubin North Road 1	136±21	15.02	174±80	45.91
Yundang Lake from Hubin North Road 2	244±32	13.01	329±201	61.14

Table S4. A comparison of different methods for coliform detection.

Methodology	Analysis Time	LOD	Refence
Voltammetry for pH Sensing	4 hours	10^3 cfu/mL	[1]
Voltammetry with enzyme-phage	3-7 hours	10^2 cfu/mL	[2]
Electrochemical magneto immunosensor	2 hours	33 cfu/mL	[3]
Lateral flow test strip	8 hours	49 cfu/g	[4]
Impedimetric cytosensor	4-5 hours	14 cfu/mL	[5]
DNAzyme biosensor	more than 2 hours	400 cfu/mL	[6]
Single-tube quantitative method	275-558 minutes	7 cfu/mL	Our method

References

1. T. Bigham, C. Casimero, J. S. Dooley, N. G. Ternan, W. J. Snelling and J. Davis, *Electrochem. Commun.*, 2019, **101**, 99-103.
2. D. Wang, J. Chen and S. R. Nugen, *Anal. Chem.*, 2017, **89**, 1650-1657.
3. L. V. Tarditto, M. A. Zon, H. G. Ovando, N. R. Vettorazzi, F. J. Arévalo and H. Fernández, *Talanta*, 2017, **174**, 507-513.
4. T. Tominaga, *J. Microbiol. Methods*, 2019, **160**, 29-35.
5. M. Sedki, X. Chen, C. Chen, X. Ge and A. Mulchandani, *Biosens. Bioelectron.*, 2020, **148**, 111794.
6. N. Zaouri, Z. F. Cui, A. S. Peinetti, Y. Lu and P. Y. Hong, *Environ. Sci.-Wat. Res.*, 2019, **5**, 2260-2268.