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Research Article

Validation of Portable Culture Device for Enumerating Total Viable Count from Food Samples

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ABSTRACT

Bacterial contamination in food arises from various sources, including human contact and processing equipment, and often worsens during storage, affecting food quality. Traditional bacterial enumeration methods, though reliable, are time-consuming, while newer alternatives like biosensors provide faster results but are more expensive. The accurate identification of microorganisms is critical for food safety in the food industry. Portable culture devices (PCDs) offer a practical solution for on-site testing, particularly in resource-limited settings. This study aimed to validate the use of PCDs for semi-quantifying microorganisms in food samples by comparing their performance with conventional detection methods. PCDs were tested on both artificially contaminated (spiked) and naturally contaminated (non-spiked) food samples to evaluate their effectiveness and reliability. These attributes make PCDs valuable for use in settings with limited access to advanced laboratory equipment. The findings suggest that PCDs are suitable alternatives to traditional culture-based methods, providing similar results while offering advantages such as simplicity, cost-effectiveness, and suitability for on-site testing. This highlights the potential of PCDs to streamline the food testing process by reducing the time required for microbial detection, thus improving food safety practices. In conclusion, PCDs could play a significant role in enhancing food safety and quality monitoring.

Keywords: Portable culture device, Food analysis, Food quality, Reassured devices**.**

INTRODUCTION

Food serves as both nutrition and a breeding ground for microorganisms, carrying inherent and potentially harmful bacteria due to mishandling (Belina *et al*., 2021; N. Sharma *et al*., 2020). Its quality depends on chemical, physical, and biological factors (Mengistu *et al*., 2022; Yang *et al*., 2018). Bacterial contamination stems from human contact, equipment, and raw materials, increasing with storage time, even under cool conditions (Baltić *et al*., 2017; Madoroba *et al*., 2021). Modern bacterial detection and quantification methods include microscopy, culture-based, and culture-independent approaches (Hameed *et al*., 2018). Traditional microbial counting, through filtration, dilution, and plating, takes 48 hours for results (Abbasian *et al*., 2018; Apruzzese *et al*., 2019). Culture-independent methods use biosensors, fluorescence probes, assays, and flow cytometry, whereas antigen-antibody techniques like ELISA and PCR cannot differentiate between live and dead bacteria (Dada *et al*., 2021; Weng & Neethirajan, 2017)

Microbial levels indicate food safety and export quality, with considerable exports recalled for not meeting standards (Duan *et al*., 2017). Identifying viable bacteria is vital in food and water testing (Feizi *et al*., 2016). The plate count method, despite its common use, is laborious and may underestimate bacteria due to cell clumping or inhibition (Hasan *et al*., 2023; Santovito *et al*., 2021; Yang *et al*., 2018). Quick enumeration techniques, like direct microscopy, membrane filtration, and viable stains, allow fast viable bacteria counting by detecting cell dehydrogenase activity (Cadena-Herrera *et al*., 2015; Kim *et al*., 2016). Triphenyl tetrazolium chloride (TTC) reduction, turning red to indicate formazan, enables quantitative colorimetric analysis (Brown *et al*., 2013; Francisco *et al*., 2014).

Rapid, cost-efficient food testing and monitoring systems for field use are essential (Kerrouche *et al*., 2020; Mazur *et al*., 2023; Xu *et al*., 2021). Portable culture devices (PCDs) enable quick diagnosis and pathogen identification under resource constraints, meeting REASSURED criteria (Agustini *et al*., 2020; Nishat *et al*., 2021; Tang *et al*., 2016). PCDs can be used in point-of-control detection systems, are affordable for mass production, and can be operated by untrained users (Bordbar *et al*., 2021; Derda *et al*., 2015; Suntornsuk & Suntornsuk, 2020).

A standardized portable culture device, made of patterned paper, adhesive tape, PDMS, and cotton pads, was developed to semi-quantitatively count total viable

microorganisms in food samples by assessing their dehydrogenase activity with TTC as a color indicator (Tiwari *et al*., 2024). The efficacy of this detection method was evaluated by determining sensitivity, specificity, and predictive values (positive and negative) against gold standard tests to assess reliability and accuracy.

MATERIALS AND METHOD

Fabrication of the Device

The devices were constructed using Whatman filter paper no. 1 with specific patterns, a cotton pad serving as a reservoir for media, and masking tape for assembly (Tiwari *et al*., 2024c). The necessary stationery items were obtained from a nearby supply store, while the chemical components were sourced from SRL Chemicals in Mumbai.

Sample collection

Food samples, both solid and liquid, were obtained from various dining establishments across Mumbai. The samples were transported to the lab in plastic zip-lock bags that had been cleaned with alcohol and dried, accompanied by ice packs. Upon delivery, the samples were stored in a refrigerator until they were prepared for further examination and processed within 4 hours of receipt (Garrido-Maestu *et al*., 2017; Rahimi *et al*., 2013; Rosenquist *et al*., 2005; Tomás *et al*., 2009).

Culture preparation for spiking food samples

E. coli (MTCC 4040), were utilized to spike both solid and liquid food samples. The strain was obtained from the microbial type culture collection (MTCC) and Gene Bank located in Chandigarh. A saline suspension having an OD_{600} of 0.1 was used.

Conventional growth medium and substrate used

Dehydrated powder forms nutrient agar obtained from Himedia, and TTC from Sisco Research Laboratories located in Mumbai, India were used.

Processing of food samples

Food sample preparation adhered to protocols specified in the FDA Bacteriological Analytical Manual (BAM) and the Food Safety and Standards Authority of India (FSSAI) (Becker *et al*., 2006; Law *et al*., 2015). For solid food specimens, a homogenate was made by mixing 50 g of the sample with 450 ml of sterile physiological saline in a blender. Liquid samples were used as it is without additional processing.

Preparation of artificially contaminated food samples

10g or 10 ml of the autoclaved food homogenate/ sample was inoculated with 1-mL of bacterial suspension of *E. coli* having an OD_{600} of 0.1. These spiked samples were then diluted serially.

Determination of TVC of food samples using PCD

The experimental setup involved inoculating the devices with a growth medium and 20 µL of food samples. These samples were either naturally contaminated (liquid or homogenized) or artificially contaminated (selected dilutions from spiked liquid or homogenized food). The sealed devices underwent incubation for 24 hours at 28°C. Subsequently, they were examined for color changes, with a pinkish-red color indicating the presence of viable organisms (Tiwari *et al*., 2024). The total viable counts of organisms present in the samples were determined using the user interpretation chart (Fig. 2).

Quantifying the number of target cells in the diluted spiked sample preparations and non-spiked samples

Using the Miles and Misra method (Miles *et al*., 1938), the number of viable cells in the diluted spiked sample dilutions chosen for device inoculation and the non-spiked samples were enumerated.

Validation of PCD

The efficacy of PCD in detecting target organisms was assessed using Hübner's method (A. Anderson *et al*., 2011; Garrido-Maestu *et al*., 2017; Tomás *et al*., 2009; Yoshitomi *et al*., 2015). Results were classified as true positives, true negatives, false positives, or false negatives. Various metrics, including relative accuracy, sensitivity, specificity, false-positive and false-negative rates, positive

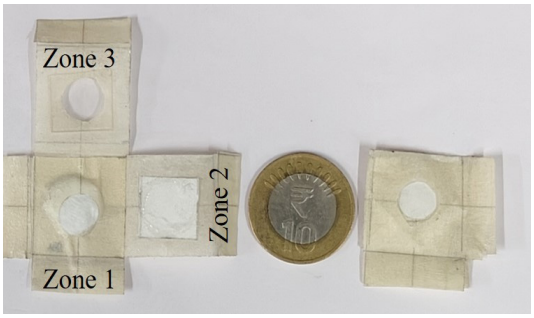

Fig 1: Fabricated device (₹10 coin is placed to display the dimension of the device)

Time (h) / Cell number (CFU/ml)	0	8	10	12	14	16	18	20	22	24
10^8	0	1.00	2.00	3.00	4.00	5.00	5.00	5.00	5.00	5.00
10^7	0	0.00	1.00	2.00	3.00	4.00	5.00	5.00	5.00	5.00
10^6	0	0.00	0.00	1.00	2.00	3.00	4.00	5.00	5.00	5.00
10^5	0	0.00	0.00	0.00	1.00	2.00	3.00	4.00	5.00	5.00
10^4	0	0.00	0.00	0.00	0.00	1.00	2.00	3.00	4.00	4.00
10^3	0	0.00	0.00	0.00	0.00	0.00	1.00	2.00	3.00	3.00
10^2	0	0.00	0.00	0.00	0.00	0.00	0.00	1.00	2.00	2.00
10^1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00

Fig. 2: Results interpretation chart

and negative predictive values, index of concordance, and chi-squared value, were calculated to evaluate PCD's performance (Eijkelkamp *et al*., 2009; Jamali *et al*., 2013; Mata & Vanetti, 2012). The research defines nine parameters to compare the performance of PCD and conventional methods: relative accuracy, sensitivity, specificity, false positive and negative rates, positive and negative predictive values, index of concordance (kappa, κ), and chi-square value (A. Anderson *et al*., 2011; Eijkelkamp *et al*., 2009; Garrido-Maestu *et al*., 2017; Godard *et al*., 2013; Mata & Vanetti, 2012; Olstein *et al*., 2013; Tomás *et al*., 2009; Yoshitomi *et al*., 2015).

RESULTS AND DISCUSSION

Sample collection

The study examined 35 diverse food samples, including thirteen liquids and 22 solids, with twenty colored and fifteen neutral-hued items (Fig. 3). Various foods can harbor foodborne pathogens, necessitating their detection due to minimal processing (Law *et al*., 2014). The samples

i. Milk 2 ii. Mosambi Juice iii. Chaas iv. Rice 1

Fig. 3: Samples collected from various locations

Fig. 4: PCD inoculated with spiked and non-spiked fruit juice 1 incubated for 24 h (from top to bottom)

Table 1: Categorization of spiked food samples for inoculation on PCD

Sample	*Observed CFU/mL of the dilution selected*	*Inoculum Level*
Chicken, Shrikhand, Vegetable Salad, Sprouts 1 and 2, Green Chutney 1, Rice 2, Icecream 1, Falooda, Mayonnaise, Cake and Prawns	10^5–10^7	High
Dahi, Egg yolk, Rice 1, Icecream 3, Green Chutney 2, Sandwich 2, Fruit Salad, Bhel and Fish	10^3–10^4	Medium
Milk 1 and 2, Fruit Juice 1 and 2, Lassi, Popsicle, Milkshake, Pani Puri water, Lime Juice, Mosambi Juice and Chaas, Kharvaas, Sandwich 1 and Icecream 2	10^1–10^2	Low

Table 2: Results for enumeration of total viable count of spiked and non-spiked food samples using the conventional method (Miles and Misra)

S. No	*Name of the sample*	*Miles and Misra ($CFUmL^{-1}$)*	
	Liquid sample	*Results for artificially contaminated samples*	*Results for naturally contaminated samples*
i	Milk 1	4×10^2	2.13×10^2
ii	Dahi	4.2×10^3	5.11×10^2
iii	Egg Yolk	5.0×10^3	1.45×10^5
iv	Fruit Juice 1	3.0×10^2	1.23×10^1
v	Lassi	3.7×10^2	1.56×10^2
vi	Pepsi	3.9×10^1	2.45×10^1
vii	Milkshake	4.6×10^2	1.87×10^2
viii	Fruit Juice 2	4.2×10^1	2.54×10^2
ix	Lime Juice	4.7×10^1	8.23×10^2
x	Pani Puri Water	2.3×10^1	1.37×10^1
xi	Milk 2	6.1×10^2	1.38×10^2
xii	Mosambi Juice	5.9×10^2	1.29×10^3
xiii	Chaas	1.1×10^2	1.83×10^2
xiv	Rice 1	1.3×10^3	1.92×10^1
xv	Chicken	5.1×10^6	2.58×10^4
xvi	Shrikhand	4.2×10^5	1.29×10^3
xvii	Vegetable Salad	1.7×10^5	1.20×10^2
xviii	Sprouts	1.3×10^5	1.92×10^4
xix	Malai	3.3×10^2	2.38×10^6
xx	Sandwich 1	5.11×10^2	2.93×10^1
xxi	Green Chutney 1	1.14×10^6	8.37×10^2
xxii	Rice 2	1.62×10^7	3.28×10^3
xxiii	Sprouts 2	4.16×10^6	2.83×10^5
xxiv	Icecream 1	7.31×10^5	1.48×10^3
xxv	Icecream 2	4.36×10^2	1.37×10^3
xxvi	Falooda	2.52×10^7	2.0×10^4
xxvii	Icecream 3	1.38×10^3	1.83×10^4
xxviii	Mayonnaise	1.24×10^7	1.96×10^2
xxix	Cake	9.13×10^6	3.0×10^1
xxx	Prawns	4.44×10^7	1.63×10^3
xxxi	Green Chutney 2	3.2×10^4	1.72×10^4
xxxii	Sandwich 2	1.53×10^4	6.0×10^3
xxxiii	Fruit Salad	1.37×10^4	5.0×10^4
xxxiv	Bhel	3.2×10^4	7.9×10^3
xxxv	Fish	3.73×10^5	5.93×10^7

Table 3: Results for enumeration of total viable count of spiked and non-spiked food samples using PCD

Sr. No.	*Name of the liquid sample*	*Results for artificially contaminated samples*		*Results for naturally contaminated samples*	
		Time of appearance of color (hours)	*≈Viable Count as per score card ($CFUmL^{-1}$)*	*Time of appearance of color (h)*	*≈Viable Count as per score card ($CFUmL^{-1}$)*
i	Milk 1	20	10^2	20	10^2
ii	Dahi	18	10^3	20	10^2
iii	Egg Yolk	18	10^3	14	10^5
iv	Fruit Juice 1	20	10^2	22	10^1
v	Lassi	20	10^2	20	10^2
vi	Pepsi	22	10^1	22	10^1
vii	Milkshake	20	10^2	20	10^2
viii	Fruit Juice 2	22	10^1	20	10^2
ix	Lime Juice	22	10^1	20	10^2
x	Pani Puri Water	22	10^1	22	10^1
xi	Milk 2	20	10^2	20	10^2
xii	Mosambi Juice	20	10^2	18	10^3
xiii	Chaas	20	10^2	20	10^2
xiv	Rice 1	18	10^3	22	10^1
xv	Chicken	12	10^6	16	10^4
xvi	Shrikhand	14	10^5	18	10^3
xvii	Vegetable Salad	14	10^5	20	10^2
xviii	Sprouts	14	10^5	16	10^4
xix	Malai	20	10^2	12	10^6
xx	Sandwich 1	20	10^2	22	10^1
xxi	Green Chutney 1	12	10^6	20	10^2
xxii	Rice 2	10	10^7	18	10^3
xxiii	Sprouts 2	12	10^6	14	10^5
xxiv	Icecream 1	14	10^5	18	10^3
xxv	Icecream 2	20	10^2	18	10^3
xxvi	Falooda	10	10^7	16	10^4
xxvii	Icecream 3	18	10^3	16	10^4
xxviii	Mayonnaise	10	10^7	20	10^2
xxix	Cake	12	10^6	22	10^1
xxx	Prawns	10	10^7	18	10^3
xxxi	Green Chutney 2	16	10^4	16	10^4
xxxii	Sandwich 2	16	10^4	18	10^3
xxxiii	Fruit Salad	16	10^4	16	10^4
xxxiv	Bhel	16	10^4	18	10^3
xxxv	Fish	14	10^5	10	10^7

Table 4: Comparison of the conventional method with PCD for enumeration of total viable count from food samples

Sr. No	*Name of the liquid sample*	*Results for spiked samples*			*Results for non-spiked samples*		
		Time of appearance of color (hours)	*PCD as per the score card (≈CFUmL^{-1})*	*Miles and Misra (CFUmL^{-1})*	*Time of appearance of color (hours)*	*PCD as per score card (≈CFUmL^{-1})*	*Miles and Misra (CFUmL^{-1})*
i	Milk 1	20	10^2	4×10^2	20	10^2	2.13×10^2
ii	Dahi	18	10^3	4.2×10^3	20	10^2	5.11×10^2
iii	Egg Yolk	18	10^3	5×10^3	14	10^5	1.45×10^5
iv	Fruit Juice 1	20	10^2	3×10^2	22	10^1	1.23×10^1
v	Lassi	20	10^2	3.7×10^2	20	10^2	1.56×10^2
vi	Pepsi	22	10^1	3.9×10^1	22	10^1	2.45×10^1
vii	Milkshake	20	10^2	4.6×10^2	20	10^2	1.87×10^2
viii	Fruit Juice 2	22	10^1	4.2×10^1	20	10^2	2.54×10^2
ix	Lime Juice	22	10^1	4.7×10^1	20	10^2	8.23×10^2
x	Pani Puri Water	22	10^1	2.3×10^1	22	10^1	1.37×10^1
xi	Milk 2	20	10^2	6.1×10^2	20	10^2	1.38×10^2
xii	Mosambi Juice	20	10^2	5.9×10^2	18	10^3	1.29×10^3
xiii	Chaas	20	10^2	1.1×10^2	20	10^2	1.83×10^2
xiv	Rice 1	18	10^3	1.3×10^3	22	10^1	1.92×10^1
xv	Chicken	12	10^6	5.1×10^6	16	10^4	2.58×10^4
xvi	Shrikhand	14	10^5	4.2×10^5	18	10^3	1.29×10^3
xvii	Vegetable Salad	14	10^5	1.7×10^5	20	10^2	1.20×10^2
xviii	Sprouts	14	10^5	1.3×10^5	16	10^4	1.92×10^4
xix	Malai	20	10^2	3.3×10^2	12	10^6	2.38×10^6
xx	Sandwich 1	20	10^2	5.11×10^2	22	10^1	2.93×10^1
xxi	Green Chutney 1	12	10^6	1.14×10^6	20	10^2	8.37×10^2
xxii	Rice 2	10	10^7	1.62×10^7	18	10^3	3.28×10^3
xxiii	Sprouts 2	12	10^6	4.16×10^6	14	10^5	2.83×10^5
xxiv	Icecream 1	14	10^5	7.31×10^5	18	10^3	1.48×10^3
xxv	Icecream 2	20	10^2	4.36×10^2	18	10^3	1.37×10^3
xxvi	Falooda	10	10^7	2.52×10^7	16	10^4	2×10^4
xxvii	Icecream 3	18	10^3	1.38×10^3	16	10^4	1.83×10^4
xxviii	Mayonnaise	10	10^7	1.24×10^7	20	10^2	1.96×10^2
xxix	Cake	12	10^6	9.13×10^6	22	10^1	3×10^1
xxx	Prawns	10	10^7	4.44×10^7	18	10^3	1.63×10^3
xxxi	Green Chutney 2	16	10^4	3.2×10^4	16	10^4	1.72×10^4
xxxii	Sandwich 2	16	10^4	1.53×10^4	18	10^3	6×10^3
xxxiii	Fruit Salad	16	10^4	1.37×10^4	16	10^4	5×10^4
xxxiv	Bhel	16	10^4	3.2×10^4	18	10^3	7.9×10^3
xxxv	Fish	14	10^5	3.73×10^5	10	10^7	5.93×10^7

Table 5: Results for enumeration of total viable count from food samples

Category	*Numbers*
Total number of liquid samples	13
No. of negative liquid samples	00
No. of positive liquid samples	13
Total number of solid samples	22
No. of negative solid samples	00
No. of positive solid samples	22
Total number of samples	35
No. of negative samples	00
No. of positive samples	35

Table 6: Parameters of reliability of PCDs for enumerating TVC

Parameter	*Values*
True positives (a)	35
True negatives (b)	0
False negatives (c)	0
False positives (d)	0
Relative accuracy (%)	100
Relative sensitivity (%)	100
Relative specificity (%)	100
False positive rate (%)	0
False negative rate (%)	0
Positive predictive value (PPV) (%)	100
Negative predictive value (NPV) (%)	100
Kappa (κ)	1
Chi-squared value (χ^2)	0

were suspected to be contaminated potentially from natural environments or human contact (Eijkelkamp *et al*., 2009; Godard *et al*., 2013; Mata & Vanetti, 2012).

Determination of concentration of target organisms in artificially contaminated food samples

To assess the concentration of spiked food inoculum used for PCD inoculation, the Miles and Misra method was used. Several samples, including various fruit juices, lime juice, pani puri water, and fruit salad, exhibited acidic pH levels, resulting in lower observed counts than anticipated. A similar reduction in observed counts was noted in Shrikhand due to its sugar content, egg yolk because of inherent inhibitors, and sandwiches 1 and 2 owing to their dressing, salt, and other components. The *E. coli* concentration in the spiked food samples was used to categorize the inoculum as high, medium, or low. Inoculum levels were classified as follows: high (10^8–10^5 $CFUmL^{-1)}$, medium (10^4–10^3 $CFUmL^{-1}$), and low (10^2–10^1 $CFUmL^{-1}$) (Table 1).

Results for enumeration of total viable count from samples using conventional method and PCD

The traditional Miles and Misra method was used to detect the number of viable organisms present in the spiked and non-spiked food samples. Media's capacity to support the growth of *E. coli* was confirmed by its growth on nutrient media, validating the purity and nature of the culture used for spiking. The results are tabulated in Table 2. Selected dilutions of spiked food sample homogenates and non-spiked food homogenates were inoculated on PCDs (Fig. 4). Viable counts of the spiked and non-spiked samples enumerated using PCDs are represented in Table 3. The pink coloration on PCDs (Fig. 4) and colony growth on conventional media were observed in all 35 samples inoculated with various concentrations (high, medium, and low) of microorganisms. Naturally, contaminated samples also contained microorganisms, as evidenced by red coloration on all test devices (Fig. 4). Earlier research has indicated the possibility of post-production contamination in food products (Law *et al*., 2015; Mazur *et al*., 2023; Meldrum *et al*., 2010; S. Sharma *et al*., 2017).

Comparison the conventional method with PCD for enumeration of total viable count from food samples

PCDs effectively identified viable microorganisms in 35 food samples, including those containing natural and artificial colorants, yielding results consistent with conventional techniques (Table 4). Despite using a minimal 20 µL sample volume, color development was not impeded, and all devices with inoculated samples showed coloration, indicating that the complexity of food matrices did not interfere with color formation or target organism growth (Table 4). The number of samples showing similar results on PCD and the conventional method for 35 samples tested for enumeration of total viable count is tabled in Table 5.

Parameters for reliability of PCD for enumerating total viable counts

The effectiveness of a testing method, also referred to as its suitability for intended use, is characterized by its accuracy, sensitivity, specificity, and both positive and negative predictive values (A. Anderson *et al*., 2011; Olstein *et al*., 2013). Table 6 displays these metrics for PCD for enumeration of TVC. These values reached 100%, surpassing the 90% threshold considered acceptable (M. Anderson *et al*., 2011; Eijkelkamp *et al*., 2009; Olstein *et al*., 2013). When using PCD to enumerate TVC, both false-negative and false-positive rates were 0%, falling below the permissible limit (Garrido-Maestu *et al*., 2017). The kappa value (κ) was 1, indicating excellent agreement

between PCD as an enumeration method and the conventional method (κ value of 0.81-1 is acceptable) (A. Anderson *et al*., 2011; Garrido-Maestu *et al*., 2017; Gelinski *et al*., 2002; Huang *et al*., 2017; Yoshitomi *et al*., 2015). Chi-squared values was 0 across the 35 samples tested, suggesting no statistically significant difference between PCD enumerated numbers and those enumerated by conventional medium (reference) at a 5% significance level. (Garrido-Maestu *et al*., 2017; Olstein *et al*., 2013; Yoshitomi *et al*., 2015).

CONCLUSION

Evaluating a test's effectiveness requires examining its sensitivity, specificity, and predictive values (both positive and negative). These metrics are frequently used to assess the accuracy of detection methods by comparing them to established gold standard tests. Experimental comparisons between PCD and traditional methods revealed comparable results. The feasibility of integrating PCDs into routine food safety testing is explored, and the results indicate that they can serve as reliable substitutes for conventional methods, particularly in situations where rapid, on-the-spot testing is needed. Consequently, PCD could be considered a viable alternative to standard microbiological analysis for enumerating total viable counts in food samples, particularly in remote locations.

STATEMENTS AND DECLARATIONS

Conflicts of Interest

The authors have no relevant financial or non-financial interests to disclose.

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Ethics Compliance Statement

This article does not contain any studies involving human participants performed by any of the authors.

Data Access Statement

All data supporting the findings of this study are available within the manuscript

Author's Contributions

All authors contributed equally.

REFERENCES

1. Abbasian, F., Ghafar-Zadeh, E., & Magierowski, S. (2018). Microbiological sensing technologies: A review. *Bioengineering*, *5*(1), 1–33. https://doi.org/10.3390/bioengineering5010020
2. Agustini, D., Fedalto, L., Agustini, D., de Matos dos Santos, L. G., Banks, C. E., Bergamini, M. F., & Marcolino-Junior, L. H. (2020). A low cost, versatile and chromatographic device for microfluidic amperometric analyses. *Sensors and Actuators, B: Chemical*, *304*. https://doi.org/10.1016/j.snb.2019.127117
3. Anderson, A., Pietsch, K., Zucker, R., Mayr, A., Müller-Hohe, E., Messelhäusser, U., Sing, A., Busch, U., & Huber, I. (2011). Validation of a Duplex Real-Time PCR for the Detection of Salmonella spp. in Different Food Products. *Food Analytical Methods*, *4*(3), 259–267. https://doi.org/10.1007/s12161-010-9142-8
4. Anderson, M., Hinds, P., Hurditt, S., Miller, P., McGrowder, D., & Alexander-Lindo, R. (2011). The microbial content of unexpired pasteurized milk from selected supermarkets in a developing country. *Asian Pacific Journal of Tropical Biomedicine*, *1*(3), 205–211. https://doi.org/10.1016/S2221-1691(11)60028-2
5. Apruzzese, I., Song, E., Bonah, E., Sanidad, V. S., Leekitcharoenphon, P., Medardus, J. J., Abdalla, N., Hosseini, H., & Takeuchi, M. (2019). Investing in Food Safety for Developing Countries: Opportunities and Challenges in Applying Whole-Genome Sequencing for Food Safety Management. *Foodborne Pathogens and Disease*, *16*(7), 463–473. https://doi.org/10.1089/fpd.2018.2599
6. Baltić, T., Ćirić, J., Velebit, B., Petronijević, R., Lakićević, B., Dordević, V., & Janković, V. (2017). Changes in total viable count and TVB-N content in marinated chicken breast fillets during storage. *IOP Conference Series: Earth and Environmental Science*, *85*(1). https://doi.org/10.1088/1755-1315/85/1/012073
7. Becker, B., Schuler, S., Lohneis, M., Sabrowski, A., Curtis, G. D. W., & Holzapfel, W. H. (2006). Comparison of two chromogenic media for the detection of Listeria monocytogenes with the plating media recommended by EN/DIN 11290-1. *International Journal of Food Microbiology*, *109*(1–2), 127–131. https://doi.org/10.1016/j.ijfoodmicro.2006.01.030
8. Belina, D., Hailu, Y., Gobena, T., Hald, T., & Njage, P. M. K. (2021). Prevalence and epidemiological distribution of selected foodborne pathogens in human and different environmental samples in Ethiopia: a systematic review and meta-analysis. *One Health Outlook*, *3*(1). https://doi.org/10.1186/s42522-021-00048-5
9. Bordbar, M. M., Sheini, A., Hashemi, P., Hajian, A., & Bagheri, H. (2021). Disposable paper-based biosensors for the point-of-care detection of hazardous contaminations—a review. *Biosensors*, *11*(9), 1–51. https://doi.org/10.3390/bios11090316
10. Brown, H. L., van Vliet, A. H. M., Betts, R. P., & Reuter, M. (2013). Tetrazolium reduction allows assessment of biofilm formation by Campylobacter jejuni in a food matrix model. *Journal of Applied Microbiology*, *115*(5), 1212–1221. https://doi.org/10.1111/jam.12316
11. Cadena-Herrera, D., Esparza-De Lara, J. E., Ramírez-Ibañez, N. D., López-Morales, C. A., Pérez, N. O., Flores-Ortiz, L. F., & Medina-Rivero, E. (2015). Validation of three viable-cell counting methods: Manual, semi-automated, and automated. *Biotechnology Reports*, *7*, 9–16. https://doi.org/10.1016/j.btre.2015.04.004
12. Dada, A. C., Somorin, Y. M., Ateba, C. N., Onyeaka, H., Anyogu, A., Kasan, N. A., & Odeyemi, O. A. (2021). Microbiological hazards associated with food products imported from the Asia-Pacific region based on analysis of the rapid alert system for food and feed (RASFF) notifications. *Food Control*, *129*(May), 108243. https://doi.

org/10.1016/j.foodcont.2021.108243
13. Derda, R., Gitaka, J., Klapperich, C. M., Mace, C. R., Kumar, A. A., Lieberman, M., Linnes, J. C., Jores, J., Nasimolo, J., Ndung'u, J., Taracha, E., Weaver, A., Weibel, D. B., Kariuki, T. M., & Yager, P. (2015). Enabling the Development and Deployment of Next Generation Point-of-Care Diagnostics. *PLoS Neglected Tropical Diseases*, *9*(5), 1–17. https://doi.org/10.1371/journal.pntd.0003676
14. Duan, H. W., Zhu, R. G., Yao, X. D., & Lewis, E. (2017). Sensitive variables extraction, non-destructive detection and visualization of total viable count (TVC) and pH in vacuum packaged lamb using hyperspectral imaging. *Analytical Methods*, *9*(21), 3172–3183. https://doi.org/10.1039/c6ay03321k
15. Eijkelkamp, J. M., Aarts, H. J. M., & Van Der Fels-Klerx, H. J. (2009). Suitability of rapid detection methods for salmonella in poultry slaughterhouses. *Food Analytical Methods*, *2*(1), 1–13. https://doi.org/10.1007/s12161-008-9040-5
16. Feizi, A., Zhang, Y., Greenbaum, A., Guziak, A., Luong, M., Chan, R. Y. L., Berg, B., Ozkan, H., Luo, W., Wu, M., Wu, Y., & Ozcan, A. (2016). Rapid, portable and cost-effective yeast cell viability and concentration analysis using lensfree on-chip microscopy and machine learning. *Lab on a Chip*, *16*(22), 4350–4358. https://doi.org/10.1039/c6lc00976j
17. Francisco, F. L., Saviano, A. M., Pinto, T. de J. A., & Lourenço, F. R. (2014). Development, optimization and validation of a rapid colorimetric microplate bioassay for neomycin sulfate in pharmaceutical drug products. *Journal of Microbiological Methods*, *103*, 104–111. https://doi.org/10.1016/j.mimet.2014.05.023
18. Garrido-Maestu, A., Azinheiro, S., Carvalho, J., Abalde-Cela, S., Carbó-Argibay, E., Diéguez, L., Piotrowski, M., Kolen'ko, Y. V., & Prado, M. (2017). Combination of microfluidic loop-mediated isothermal amplification with gold nanoparticles for rapid detection of Salmonella spp. in food samples. *Frontiers in Microbiology*, *8*(NOV), 1–8. https://doi.org/10.3389/fmicb.2017.02159
19. Gelinski, J. M. L. N., Martin, G., Destro, M. T., Landgraf, M., & De Melo Franco, B. D. G. (2002). Rapid detection of Salmonella in foods using a combination of SPRINT™, MSRV™ and Salmonella Latex Test™. *Revista Brasileira de Ciencias Farmaceuticas/Brazilian Journal of Pharmaceutical Sciences*, *38*(3), 315–322. https://doi.org/10.1590/S1516-93322002000300007
20. Godard, R. D., Sharma, P., Wagner, C. J., Wilson, C. M., Fleming-Haddock, A., & Beach, R. L. (2013). Repeated detection of microbes in beverages dispensed from soda fountain machines and the effect of flushing on microbial density. *International Journal of Food Microbiology*, *163*(2–3), 218–222. https://doi.org/10.1016/j.ijfoodmicro.2013.03.007
21. Hameed, S., Xie, L., & Ying, Y. (2018). Conventional and emerging detection techniques for pathogenic bacteria in food science: A review. *Trends in Food Science and Technology*, *81*, 61–73. https://doi.org/10.1016/j.tifs.2018.05.020
22. Hasan, M. R., Hossain, M. M., Islam, M. S., Sunny, A. R., Ferdous, J., Chowdhury, M. Z. A., Al Mazed, M., Al Shiam, S. A., Mojumder, M. A. N., Rahman, M. A., Hamid, S. M. A., & Sultana, A. (2023). Seasonal Variation of Quality and the Total Viable Count of Lean and Fatty Fish. *Egyptian Journal of Aquatic Biology and Fisheries*, *27*(5), 1337–1356. https://doi.org/10.21608/ejabf.2023.324732
23. Huang, G., Huang, Q., Xie, L., Xiang, G., Wang, L., Xu, H., Ma, L., Luo, X., Xin, J., Zhou, X., Jin, X., & Zhang, L. (2017). A rapid, low-cost, and microfluidic chip-based system for parallel identification of multiple pathogens related to clinical pneumonia. *Scientific Reports*, *7*(1), 1–10. https://doi.org/10.1038/s41598-017-06739-2
24. Jamali, H., Chai, L. C., & Thong, K. L. (2013). Detection and isolation of Listeria spp. and Listeria monocytogenes in ready-to-eat foods with various selective culture media. *Food Control*, *32*(1), 19–24. https://doi.org/10.1016/j.foodcont.2012.11.033
25. Kerrouche, A., Lithgow, J., Muhammad, I., & Romdhani, I. (2020). Towards the development of rapid and low-cost pathogen detection systems using microfluidic technology and optical image processing. *Applied Sciences (Switzerland)*, *10*(7). https://doi.org/10.3390/app10072527
26. Kim, S. I., Kim, H. J., Lee, H. J., Lee, K., Hong, D., Lim, H., Cho, K., Jung, N., & Yi, Y. W. (2016). Application of a non-hazardous vital dye for cell counting with automated cell counters. *Analytical Biochemistry*, *492*(3), 8–12. https://doi.org/10.1016/j.ab.2015.09.010
27. Law, J. W. F., Ab Mutalib, N. S., Chan, K. G., & Lee, L. H. (2015). An insight into the isolation, enumeration, and molecular detection of Listeria monocytogenes in food. *Frontiers in Microbiology*, *6*(NOV), 1–15. https://doi.org/10.3389/fmicb.2015.01227
28. Law, J. W. F., Mutalib, N. S. A., Chan, K. G., & Lee, L. H. (2014). Rapid methods for the detection of foodborne bacterial pathogens: Principles, applications, advantages and limitations. *Frontiers in Microbiology*, *5*(DEC), 1–19. https://doi.org/10.3389/fmicb.2014.00770
29. Madoroba, E., Magwedere, K., Chaora, N. S., Matle, I., Muchadeyi, F., Mathole, M. A., & Pierneef, R. (2021). Microbial communities of meat and meat products: An exploratory analysis of the product quality and safety at selected enterprises in South Africa. *Microorganisms*, *9*(3), 1–26. https://doi.org/10.3390/microorganisms9030507
30. Mata, G. M. S. C., & Vanetti, M. C. D. (2012). Comparison of Conventional and Rapid Methods for Salmonella Detection in Artisanal Minas Cheese. *Journal of Food Research*, *1*(3), 178. https://doi.org/10.5539/jfr.v1n3p178
31. Mazur, F., Tjandra, A. D., Zhou, Y., Gao, Y., & Chandrawati, R. (2023). Paper-based sensors for bacteria detection. *Nature Reviews Bioengineering*, *1*(3), 180–192. https://doi.org/10.1038/s44222-023-00024-w
32. Meldrum, R. J., Ellis, P. W., Mannion, P. T., Halstead, D., & Garside, J. (2010). Prevalence of listeria monocytogenes in ready-to-eat foods sampled from the point of sale in wales, United Kingdom. *Journal of Food Protection*, *73*(8), 1515–1518. https://doi.org/10.4315/0362-028X-73.8.1515
33. Mengistu, D. A., Mulugeta, Y., Mekbib, D., Baraki, N., & Gobena, T. (2022). Bacteriological Quality of Locally Prepared Fresh Fruit Juice Sold in Juice Houses of Eastern Ethiopia. *Environmental Health Insights*, *16*. https://doi.org/10.1177/11786302211072949
34. Miles, A. A., Misra, S. S., & Irwin, J. O. (1938). The estimation of the bactericidal power of the blood. *Journal of Hygiene*, *38*(6), 732–749. https://doi.org/10.1017/S002217240001158X
35. Nishat, S., Jafry, A. T., Martinez, A. W., & Awan, F. R. (2021).

Paper-based microfluidics: Simplified fabrication and assay methods. *Sensors and Actuators, B: Chemical*, *336*, 129681. https://doi.org/10.1016/j.snb.2021.129681
36. Olstein, A., Griffith, L., Feirtag, J., & Pearson, N. (2013). Paradigm diagnostics salmonella indicator broth (PDX-SIB) for detection of salmonella on selected environmental surfaces. *Journal of AOAC International*, *96*(2), 404–412. https://doi.org/10.5740/jaoacint.11-373
37. Rahimi, E., Abdos, F., Momtaz, H., Torki Baghbadorani, Z., & Jalali, M. (2013). Bacillus cereus in infant foods: Prevalence study and distribution of enterotoxigenic virulence factors in Isfahan Province, Iran. *The Scientific World Journal*, *2013*. https://doi.org/10.1155/2013/292571
38. Rosenquist, H., Smidt, L., Andersen, S. R., Jensen, G. B., & Wilcks, A. (2005). Occurrence and significance of Bacillus cereus and Bacillus thuringiensis in ready-to-eat food. *FEMS Microbiology Letters*, *250*(1), 129–136. https://doi.org/10.1016/j.femsle.2005.06.054
39. Santovito, E., Elisseeva, S., Bukulin, A., Kerry, J. P., & Papkovsky, D. B. (2021). Facile biosensor-based system for on-site quantification of total viable counts in food and environmental swabs. *Biosensors and Bioelectronics*, *176*(December 2020), 112938. https://doi.org/10.1016/j.bios.2020.112938
40. Sharma, N., Singh, K., Toor, D., Pai, S. S., Chakraborty, R., & Khan, K. M. (2020). Antibiotic resistance in microbes from street fruit drinks and hygiene behavior of the vendors in Delhi, india. *International Journal of Environmental Research and Public Health*, *17*(13), 1–12. https://doi.org/10.3390/ijerph17134829
41. Sharma, S., Sharma, V., Dahiya, D. K., Khan, A., Mathur, M., & Sharma, A. (2017). Prevalence, Virulence Potential, and Antibiotic Susceptibility Profile of Listeria monocytogenes Isolated from Bovine Raw Milk Samples Obtained from Rajasthan, India. *Foodborne Pathogens and Disease*, *14*(3), 132–140. https://doi.org/10.1089/fpd.2016.2118
42. Suntornsuk, W., & Suntornsuk, L. (2020). Recent applications of paper-based point-of-care devices for biomarker detection. *Electrophoresis*, *41*(5–6), 287–305. https://doi.org/10.1002/elps.201900258
43. Tang, F., Xiong, Y., Zhang, H., Wu, K., Xiang, Y., Shao, J. B., Ai, H. W., Xiang, Y. P., Zheng, X. L., Lv, J. R., Sun, H., Bao, L. S., Zhang, Z., Hu, H. B., Zhang, J. Y., Chen, L., Lu, J., Liu, W. Y., Mei, H., … Sun, Z. Y. (2016). Visual detection technique for efficient screening and isolation of Salmonella based on a novel enrichment assay using chromatography membrane. *European Journal of Clinical Microbiology and Infectious Diseases*, *35*(3), 353–361. https://doi.org/10.1007/s10096-015-2543-2
44. Tiwari, S., Shukla, V., & Lokur, A. (2024). Standardization of portable culture device for estimation of total viable count from food samples. *Indian Journal of Applied Research*, *1*, 1–4.
45. Tomás, D., Rodrigo, A., Hernández, M., & Ferrús, M. A. (2009). Validation of real-time PCR and enzyme-linked fluorescent assay-based methods for detection of Salmonella spp. in chicken feces samples. *Food Analytical Methods*, *2*(3), 180–189. https://doi.org/10.1007/s12161-009-9082-3
46. Weng, X., & Neethirajan, S. (2017). Ensuring food safety: Quality monitoring using microfluidics. *Trends in Food Science and Technology*, *65*, 10–22. https://doi.org/10.1016/j.tifs.2017.04.015
47. Xu, J., Chen, X., Khan, H., & Yang, L. (2021). A dual-readout paper-based sensor for on-site detection of penicillinase with a smartphone. *Sensors and Actuators, B: Chemical*, *335*(February). https://doi.org/10.1016/j.snb.2021.129707
48. Yang, D., Lu, A., Ren, D., & Wang, J. (2018). Detection of total viable count in spiced beef using hyperspectral imaging combined with wavelet transform and multiway partial least squares algorithm. *Journal of Food Safety*, *38*(1), 1–13. https://doi.org/10.1111/jfs.12390
49. Yoshitomi, K. J., Jinneman, K. C., Orlandi, P. A., Weagant, S. D., Zapata, R., & Fedio, W. M. (2015). Evaluation of rapid screening techniques for detection of Salmonella spp. from produce samples after pre-enrichment according to FDA BAM and a short secondary enrichment. *Letters in Applied Microbiology*, *61*(1), 7–12. https://doi.org/10.1111/lam.12422

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