Original Research Paper

Volume - 14 | Issue - 04 | April - 2024 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Microbiology

STANDARDIZATION OF PORTABLE CULTURE DEVICE FOR ESTIMATION OF TOTAL VIABLE COUNT FROM FOOD SAMPLES

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ABSTRACT **Background:** Bacterial contamination in food originates from diverse sources, including humans and processing equipment, and often increases during storage, thereby affecting the food quality. Traditional methods for bacterial enumeration involve time-consuming processes, whereas newer techniques such as biosensors offer quicker alternatives at higher costs. Portable culture devices (PCD) have emerged as practical solutions for addressing the need for on-site testing, particularly in resource-limited settings. This study aimed to standardize the use of PCDs to semi-quantify microorganisms in food samples. **Methods:** Viable organisms were successfully semi-quantitatively enumerated using enzyme-chromogenic substrate interactions. **Results:** The device, optimized at a substrate concentration of 1 gL^{-1} and a temperature of 28°C, can detect cell counts of up to 10 CFU/mL. **Conclusion:** Tests conducted on experimentally contaminated food samples, in addition to independent on-site detection without the requirement of typical laboratory facilities, demonstrate the feasibility of Portable Culture Devices (PCDs) as substitutes for conventional culture-based detection methods. They are appropriate for on-the-spot testing in areas with limited resources because they are cost-effective and simple.

KEYWORDS : On-site detection, viable counting, Point of care testing (POCT), Portable Culture Device (PCD), TTC

INTRODUCTION

Food is nutritional and therefore susceptible to spoilage. Foods are nutritious and perishable, and support the growth of microorganisms (Mengistu et al., 2022). Additionally, foods have their own normal flora and can be contaminated with pathogenic and non-pathogenic organisms, unless properly treated (Belina et al., 2021). Food quality depends on chemical, physical, and biological conditions (Mengistu et al., 2022). Bacteria can be introduced into food from various sources including humans, processing equipment, and raw materials (Madoroba et al., 2021)(Sharma et al., 2020). Moreover, microbial numbers can increase during prolonged storage even at low temperatures (Yang et al., 2018)(Baltić et al., 2017).

Existing methods for detecting and enumerating bacteria include microscopic-, culture-based, and culture-independent methods (Hameed et al., 2018). Conventional methods for microbial enumeration involve filtration, serial dilution, and plating and often require 48 h for the results (Apruzzese et al., 2019). Culture-independent methods include the use of bioluminescent and potentiometric biosensors, fluorescence-based probes, oxygen-sensing assays, and flow cytometry (Abbasian et al., 2018). Antigen-antibody-dependent methods, such as enzyme-linked immunosorbent assays and nucleic acid-based polymerase chain reactions, are expensive and cannot distinguish between living and dead organisms (Weng & Neethirajan, 2017).

The number of viable organisms in food serves as a quality indicator for human consumption and export (Mengistu et al., 2022). Often, large batches of exported food are recalled because of non-compliance with relevant quality standards (Belina et al., 2021)(Dada et al., 2021)(Duan et al., 2017). Methods capable of detecting and enumerating live bacteria have applications in food and water analysis industries (Feizi et al., 2016) (Sharma et al., 2020). The most common method for enumerating the microbial load is the plate count technique, which involves sample dilution, plating, and incubation to develop microbial colonies on growth media (Sharma et al., 2020)(Yang et al., 2018)(Hasan et al., 2023). Although simple, this method is labor-intensive and time-consuming, and requires laboratory infrastructure and experienced personnel (Santovito et al., 2021). Moreover, the results may be prone to underestimation owing to cell clumping or inhibition by neighboring cells (Duan et al., 2017). Therefore, there is a need to develop methods that facilitate rapid enumeration of viable counts.

Rapid methods for total viable counting include direct microscopic counting, membrane filtration techniques, and the use of viable stains such as trypan blue and tetrazolium salts, which detect dehydrogenase activity in viable cells (Belina et al., 2021)(Kim et al., 2016)(Cadena-Herrera et al., 2015). Dehydrogenase enzymes play a crucial role in catalyzing the oxidation of organic substances, ultimately forming water (Junillon & Flandrois, 2014). Triphenyltetrazolium chloride (TTC) can be reduced to produce red formazan, allowing quantitative assessment using colorimetric methods (Francisco et al., 2014)(Brown et al., 2013).

Therefore, there is a need to develop rapid and inexpensive food testing and monitoring systems for on-site application (Kerrouche et al., 2020). Portable culture devices (PCDs) offer on-the-spot diagnosis and pathogen detection in environments with limited resources (Agustini et al., 2020)(Nishat et al., 2021)(Mazur et al., 2023). These devices function based on molecular marker-based or culture-based detection systems, meeting the ASSURED criteria that emphasize affordability, sensitivity, specificity, user-friendliness, rapidity, robustness, equipment-free operation, and delivery to underserved populations (Nguyen et al., 2020)(Levy et al., 2020)(Musile et al., 2021). They have also been applied in point-of-care detection systems (Derda et al., 2015)(Bordbar et al., 2021)(Suntornsuk & Suntornsuk, 2020). Furthermore, they are economical for large-scale production and can be operated by individuals without specialized skills in the field (Weng & Neethirajan, 2017)(Xu et al., 2021)(Tang et al., 2016). Funes-Huacca et al. suggested a culture apparatus that included medium-infused paper sheets, a PDMS layer, and tape for the growth and quantification of *E. coli* in remote, resource-constrained settings (Funes-Huacca et al., 2012)(Deiss et al., 2014). Portable culture devices designed with paper and masking tape have also been reported for the detection of *Staphylococcus aureus* (Lokur, 2018). The potential for detecting microorganisms in food samples can also be examined (Noviana et al., 2021)(Akyazi et al., 2018).

This study proposes the construction of a portable culture device with patterned paper, masking tape, PDMS, and cotton pads. The device semi-quantitatively enumerates the number of viable organisms in food samples based on their dehydrogenase activity using TTC as a chromogenic substrate.

MATERIALS AND METHODS

Fabrication of PCD:

The devices were fabricated using patterned Whatman filter paper No. 1, cotton pads as the media reservoir, and masking tape (Nishat et al., 2021)(Ozer et al., 2020).

Preparation of test culture and substrate:

An 18-hour-old saline suspension of *E. coli* MTCC 4040 was used. The strain was obtained from the Microbial Type Culture Collection

(MTCC) and Gene Bank in Chandigarh and maintained on Nutrient Agar (Himedia, Mumbai, India). The optical density of the culture was adjusted to 0.1 at 600 nm wavelength. An appropriate amount of TTC (SRL Chemicals, Mumbai, India) was weighed and dissolved in sterile distilled water.

Loading the devices and recording results:
The devices were loaded with 25 µL of a 1 gL^{-1} TTC solution, 180 µL of nutrient medium, and 20 µL of saline suspension of the test organism, with an optical density of 0.1 at 600 nm. After incubation at 37°C, the devices were visually inspected for color development and scanned using a laser scanner (HP LaserJet Pro M1136 Multifunction Printer Scanner). The intensity was measured using ImageJ® software.

Optimization of detection using a Portable Culture Device:
The fabricated PCD was tested for its ability to estimate the number of viable organisms using a chromogenic substrate. Standardization was performed with respect to the following points.

a. Substrate concentration:
Devices were loaded with 1–5 gL^{-1} of substrate to determine the optimum concentration. All devices were incubated at 37°C for 18 h, and the results were recorded.

b. Incubation temperature and time:
The devices were inoculated and incubated at 37 and 25°C to determine the optimum temperature. Saline suspensions with varying numbers of *E. coli* were prepared by serial dilution. The suspensions were then inoculated onto the PCD. The time required for color development was recorded for 24 hours at two-hour intervals. The cell numbers in the suspension were determined using the Miles and Misra technique (Miles et al., 1938).

Determination of Viable counts using the Miles and Misra technique:
Test saline suspensions were prepared and serially diluted. Ten microliters of the dilution were inoculated in one quadrant of a Nutrient Agar plate (Himedia, Mumbai, India) in triplicate. The plates were then incubated at 37°C for 24 h. The colonies were counted and the CFU/mL for each dilution was determined (Miles et al., 1938).

Proof-of-concept studies for estimating the viable count of E. coli from spiked food using PCD:
Dairy products such as milk and Curd, Juice, and seafood such as shrimp and egg yolk were used for the analysis. Solid food samples (50 g) were homogenized in 450 mL of sterile physiological saline, autoclaved, and spiked with 10 ml of a randomly selected dilution of saline suspension of *E. coli* at an optical density of 0.1 at 600 nm. Liquid samples such as milk and egg yolk were similarly spiked. The devices were inoculated with spiked samples, incubated, and the results were recorded. Dilutions of the spiked samples were plated on Nutrient Agar to determine the viable counts using the Miles and Misra method.

Estimation of the viable count of food using PCD:
Dairy products such as milk and Curd, Juice, and seafood such as shrimp and egg yolk were used for analysis. Solid food samples (50 g) were homogenized with 450 mL of sterile physiological saline, inoculated onto the devices, incubated, and the results were recorded. Liquid samples such as milk and egg yolk were inoculated onto the devices and incubated, and the results were recorded. Dilutions of the samples were also plated on Nutrient Agar to determine viable counts using the Miles and Misra technique.

RESULTS AND DISCUSSION

Optimization of substrate concentration

Fig 1. Optimisation of Substrate concentration (From left to right: Devices inoculated with substrate concentration ranging from 1-5 gL^{-1})

All devices exhibited a pinkish-red color, as shown in Fig. 1. The color displays a gradient of intensity. The mean grey values for 1 gL^{-1}, 2 gL^{-1}, 3 gL^{-1}, 4 gL^{-1}, and 5 gL^{-1} were found to be 55.48±0.74, 57.43±0.73, 63.50±0.45, 65.24±0.85, and 68.11±0.88, respectively. However, in accordance with ASSURED criteria, 1 gL^{-1} of substrate was selected for further studies.

Optimization of Incubation Temperature

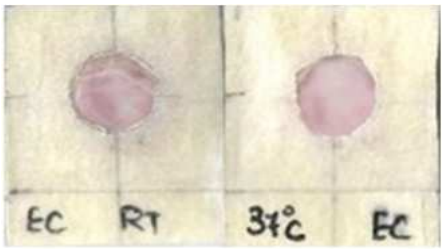

Fig 2. Optimisation of temperature (From left to right: Devices incubated at 25°C and 37°C)

To determine the optimum temperature, the devices were incubated at 37 and 25°C. Pinkish-red coloration was observed in all the devices, as shown in Fig. 2; however, ImageJ analysis did not reveal any significant differences in color intensity. The mean grey value for the devices incubated at 37°C was 61.70±1.09, and at 25°C was 66.61±1.27. These results align with the potential of the proposed application for on-site detection as the device functions equally efficiently at both temperatures. However, these devices cannot be used at temperatures that do not support microorganism growth.

Detection time and limit of detection

Table 1. The mean gray value measured using ImageJ for devices inoculated with cell numbers ranging from 10^8 to 10^1 CFU/mL and incubated for 24 h. (Standard deviation = ± 0.09 to ±1.32)

Time (in h) / Cell number CFU/mL	8	10	12	14	16	18	20	22
10^8	55	58	60	66	66	66	68	70
10^7	0	55	57	61	64	65	66	68
10^6	0	0	54	55	61	63	66	65
10^5	0	0	0	53	57	61	62	65
10^4	0	0	0	0	54	56	59	63
10^3	0	0	0	0	0	54	56	59
10^2	0	0	0	0	0	0	53	56
10^1	0	0	0	0	0	0	0	53

Table 2. User friendly interpretation chart

Time (in h) / Cell number CFU/mL	8	10	12	14	16	18	20	22	24
10^8	1	1	2	2	3	3	4	4	5
10^7	0	1	1	2	2	3	3	4	4
10^6	0	0	1	1	2	2	3	3	4
10^5	0	0	0	1	1	2	2	3	3
10^4	0	0	0	0	1	1	2	2	3
10^3	0	0	0	0	0	1	1	2	2
10^2	0	0	0	0	0	0	1	1	2
10^1	0	0	0	0	0	0	0	1	1

Table 1 shows the mean gray intensities of the devices measured using ImageJ software. The results indicated that the intensity of the color was directly proportional to the cell number and inversely proportional to incubation time. Fig 3 shows the devices inoculated with 10^8-10^1 CFU/mL after 24 h of incubation. It is evident from these results that the time required for color development was 8-24 h, for 10^8-10^1 CFU/mL respectively, which is equal to that required for conventional plating methods. The minimum number of viable cells was 10^1 CFU/mL. Therefore, the LOD of the semiquantitative method was 10^1 CFU/mL, which is less than the permissible limit for most exportable foods. Cell counts greater than 10^8 cells were detected in less than 8 h. This makes the device suitable for estimating the viable counts in food samples. The research aims to develop a detection system for on-site testing; therefore, a user-friendly interpretation chart is recommended, as presented in Table 2.

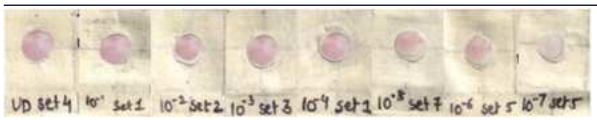

Fig 3. Determination of Limit of Detection (From left to right: Devices inoculated with 10^8-10^1 CFU/mL after 24 h of incubation at 25°C)

Proof-of-concept studies for estimating the viable count of *E. coli* from spiked food using PCD:

Viable counts of food samples spiked with *E. coli* were determined by PCD. The counts were correlated with those obtained for spiked samples using the conventional Miles and Misra technique. Table 3 presents the results. The time of appearance of the color was correlated with the cell number, as shown in Table 3. The cell numbers estimated using both methods were similar. This scorecard-based readout is useful for onsite detection.

Table 3. Results of spiked samples using PCD and Miles and Misra for determination of viable counts from samples.

Spiked Sample	Time of development of colour of PCD (h)	Corresponding cell number from the scorecard (CFU/mL)	Viable count (CFU/mL)
Milk	8	10^8	1.67×10^8
Shrimp	10	10^7	3.14×10^7
Juice	12	10^6	6.5×10^6
Dahi	14	10^5	4.23×10^5
Egg yolk	18	10^3	2.16×10^3

Estimation of viable counts in food using PCD

Food samples with permissible limits were selected and collected from local locations in Mumbai. The PCDs were then inoculated with the samples. Viable counts of the samples were determined using the Miles and Misra method. Table 4 and Fig 4 present the results. All samples contained living bacteria. The estimated numbers of viable organisms using both the conventional and portable culture devices were similar. The food that must be exported has permissible limits. Thus, estimation of viable counts in food samples is important. PCDs can be used to rapidly determine the microbial load of food before export, to avoid rejection.

Fig 4. Results of samples using PCD for determination of viable counts from samples.

Table 4. Determination of viable counts in samples using PCD and the Miles and Misra technique.

Sample	Time of development of colour of PCD	Corresponding cell number from the scorecard (CFU/mL)	Viable count (CFU/mL)	Permissible Limits (CFU/mL)
Milk	8 h	10^8	1×10^8	3×10^4
Shrimp	10 h	10^7	5.18×10^7	5×10^6
Juice	12 h	10^6	5.5×10^6	5×10^1
Dahi	20 h	10^2	4.23×10^2	1×10^7
Egg yolk	22 h	10^1	1.13×10^1	Nil

CONCLUSIONS

A rapid method for semi-quantitative estimation of the viable counts of organisms in food was developed using TTC on a Portable Culture Device. The device was standardized for the substrate concentration, incubation time, and temperature at an optimum concentration of 1 gL^{-1}. The optimal time and temperature were determined as 8 h and 25°C, respectively.

This proof-of-concept was established by detecting *E. coli* in spiked food samples, demonstrating that the device can be used to semi-quantify the viable counts of organisms in food samples. The intensity of the color developed can be read using ImageJ software or any other image analysis software for mobile phones. However, a user-friendly score card can also be used for interpretation in resource-constrained settings. Additional samples are required to thoroughly test the devices. Moreover, on-site applicability of the detection system requires rigorous testing.

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. Akyazi, T., Basabe-Desmonts, L., & Benito-Lopez, F. (2018). Review on microfluidic paper-based analytical devices towards commercialisation. *Analytica Chimica Acta*, *1001*, 1–17. https://doi.org/10.1016/j.aca.2017.11.010
4. 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
5. 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
6. 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
7. 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
8. 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
9. 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
10. 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
11. Deiss, F., Funes-Huacca, M. E., Bal, J., Tjhung, K. F., & Derda, R. (2014). Antimicrobial susceptibility assays in paper-based portable culture devices. *Lab on a Chip*, *14*(1), 167–171. https://doi.org/10.1039/c3lc50887k
12. 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
13. 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
14. 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
15. 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
16. Funes-Huacca, M., Wu, A., Szepesvari, E., Rajendran, P., Kwan-Wong, N., Razgulin, A., Shen, Y., Kagira, J., Campbell, R., & Derda, R. (2012). Portable self-contained cultures for phage and bacteria made of paper and tape. *Lab on a Chip*, *12*(21), 4269–4278. https://doi.org/10.1039/c2lc40391a
17. 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
18. 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
19. Junillon, T., & Flandrois, J. P. (2014). Diminution of 2,3,5-triphenyltetrazolium chloride toxicity on Listeria monocytogenes growth by iron source addition to the culture medium. *Food Microbiology*, *38*, 1–5. https://doi.org/10.1016/j.fm.2013.07.005
20. 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
21. 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
22. Levy, A. F., Labrador, A., Knecht, L., & van Dyken, J. D. (2020). An inexpensive, high-throughput µPAD assay of microbial growth rate and motility on solid surfaces using saccharomyces cerevisiae and escherichia coli as model organisms. *PLoS ONE*, *15*(10 October), 1–16. https://doi.org/10.1371/journal.pone.0225020
23. Lokur, A. (2018). Vegetos Portable Culture Device for. *Vegetos An International Journal of Plant Research and Biotechnology*, *31*(3), 119–122. https://doi.org/10.5958/2229
24. 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
25. 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
26. 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
27. 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
28. Musile, G., Agard, Y., Wang, L., De Palo, E. F., McCord, B., & Tagliaro, F. (2021). Paper-based microfluidic devices: On-site tools for crime scene investigation. *TrAC - Trends in Analytical Chemistry*, *143*, 116406. https://doi.org/10. 1016/j.trac.2021.116406
29. Nguyen, V. T., Song, S., Park, S., & Joo, C. (2020). Recent advances in high-sensitivity detection methods for paper-based lateral-flow assay. *Biosensors and Bioelectronics*, *152*(January), 112015. https://doi.org/10.1016/j.bios.2020.112015
30. 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
31. Noviana, E., Ozer, T., Carrell, C. S., Link, J. S., McMahon, C., Jang, I., & Henry, C. S. (2021). Microfluidic Paper-Based Analytical Devices: From Design to Applications. *Chemical Reviews*, *121*(19), 11835–11885. https://doi.org/10. 1021/acs. chemrev.0c01335
32. Ozer, T., McMahon, C., & Henry, C. S. (2020). Advances in Paper-Based Analytical Devices. *Annual Review of Analytical Chemistry*, *13*, 85–109. https://doi.org/10. 1146/annurev-anchem-061318-114845
33. 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
34. 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
35. 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
36. 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
37. 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
38. 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
39. 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