Sains Malaysiana 54(2)(2025): 473-481
http://doi.org/10.17576/jsm-2025-5402-13

Rapid Detection of Microbial Contamination on Food Contact Surfaces in Restaurants with Long Operating Hours using Mobile Flow Cytometer

(Pengesanan Pantas Pelumusan Mikrob pada Permukaan Sentuhan Makanan di Restoran dengan Waktu Operasi Panjang menggunakan Sitometer Aliran Mudah Alih)

SHAFIZI ABDUL WAHAB[1,2], ALIA ARYSSA MOHD HARIS[1], CHAI LAY CHING[3], NURUL AQILAH MOHD ZAINI[1,5], MOHAMAD YUSOF MASKAT[1,5], CHOK HUI MEI[4], HUDA SABER ABU BAKAR SALEH[4], MOHAMED YUSUF MOHAMED NAZIR[1,5] & SAHILAH ABDUL MUTALIB[1,5,*]

[1]*Department of Food Science, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia*
[2]*Ministry of Health, Kompleks E, Pusat Pentadbiran Kerajaan Persekutuan, 62590 Putrajaya, Malaysia*
[3]*Chancellery Office, Sunway University, Jalan Universiti, 47500 Bandar Sunway, Selangor, Malaysia*
[4]*Premier Diagnostics Sdn. Bhd. 40400 Shah Alam, Selangor, Malaysia*
[5]*Innovation Centre for Confectionery Technology (MANIS), Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia*

Received: 24 September 2024/Accepted: 7 November 2024

ABSTRACT

Cross-contamination on food contact surfaces (FCS) increases the risk of foodborne diseases incidence. Traditional microbial detection methods are time-consuming, prompting the exploration of the rapid method; CytoQuant® mobile flow cytometer which employ perturbation of low-frequency electric fields between intact membrane and cytoplasm. The cross-sectional study used CytoQuant® and total plate count (TPC) involved 30 FCS from six (n = 6) selected restaurants with long operating hours in the Lembah Klang area. Findings showed that the CytoQuant® was able to detect the presence of microbial contamination within 30 seconds without pre-treatment. The results also showed that there were significant differences ($p < 0.05$) between intact cell and particles readings on FCS. Furthermore, even though there are some differences in individual sample readings from the FCS, the mean values across various restaurants showed no significant difference ($p > 0.05$) between CytoQuant and TPC, suggesting that both methods provide comparable intact cell measurements on average. Further microbiological and premise rating analysis showed that all six premises exhibited aerobic, coliform counts and patogen exceeding established thresholds on the cutting board, including detection of salmonella in one of the premises, which correlated with the poor premises rating. This study suggest that employing CytoQuant® on-site ensures rapid and reliable results in monitoring of FCS, reducing the risk of foodborne diseases compared to TPC analysis.

Keywords: Food contact surfaces; *in-situ* measurement; microbial contamination; mobile flow cytometer brand CytoQuant®; rapid detection

ABSTRAK

Pelumusan silang pada permukaan yang bersentuhan makanan (PBM) meningkatkan risiko kejadian penyakit bawaan makanan. Kaedah pengesanan mikrobiologi tradisi memerlukan tempoh masa, justeru, ia mendorong penerokaan penggunaan sitometri aliran mudah alih CytoQuant® yang berdasarkan perbezaan medan elektrik frekuensi rendah antara membran sel dan sitoplasma sebagai salah satu teknologi baharu untuk mengukur pencemaran mikrobiologi di lapangan. Kajian keratan rentas menggunakan sitometri aliran CytoQuant® dan TPC melibatkan 30 permukaan sentuhan makanan daripada enam (n = 6) restoran terpilih dengan waktu operasi yang panjang di kawasan Lembah Klang. Penemuan menunjukkan bahawa penggunaan sitometri aliran CytoQuant® dapat mengesan kehadiran pencemaran mikrobiologi dalam masa 30 saat tanpa rawatan awal. Hasil juga menunjukkan bahawa terdapat perbezaan yang signifikan ($p < 0.05$) antara bacaan sel dan zarah pada permukaan sentuhan makanan seperti papan pemotong, pisau, klip makanan, pinggan dan sudu. Selain itu, walaupun terdapat perbezaan bacaan sel apabila diukur menggunakan CytoQuant® dan TPC pada beberapa PBM, bacaan min bacaan untuk kesemua restoran menunjukkan tiada perbezaan signifikan ($p > 0.05$) antara bacaan sel menggunakan CytoQuant® dan analisis TPC. Analisis mikrobiologi dan penarafan premis lanjutan menunjukkan kesemua enam premis mencatatkan bilangan aerobik, koliform dan patogen melebihi ambang yang ditetapkan pada papan pemotong, termasuk pengesanan *Salmonella* spp. di salah satu premis yang berkorelasi dengan penarafan premis yang rendah. Oleh itu, penggunaan

CytoQuant® di lapangan memastikan keputusan yang cepat dan boleh dipercayai dalam pemantauan permukaan sentuhan makanan dan mengurangkan risiko kejadian penyakit bawaan makanan berbanding dengan analisis TPC.

Kata kunci: Pelumusan mikrobiologi; pengesanan pantas; pengukuran di lapangan; permukaaan sentuhan makanan; sitometri aliran mudah alih CytoQuant®

INTRODUCTION

The incidence of foodborne diseases is often associated with unhygienic food premises, unsanitary food handling practices as well as contaminated food equipment and contact surfaces that contribute to cross-contamination in food (Ahmed et al. 2017; Al Banna et al. 2022; Zulfakar, Sahani & Hamid 2018). The main concern for restaurants with long operating hours is the cleanliness level as they operate the longest period. In this study, long hours operating restaurant was classified if the premise operate more than 18 hours per day (Shafizi et al. 2024). Food contact surfaces (FCS) are among the important elements that need to be constantly monitored in avoiding food contamination at the same time as the food is safe to be consumed. This is due to the contact surface of food that plays a role in controlling the spread of foodborne disease pathogens (Cosby et al. 2008). Unsanitary equipment handling practices during food handling can result in food contamination. According to Lani et al. (2014), food contact surfaces such as cutting boards, knives, plates, spoons and other equipment that have been contaminated with bacteria are at high risk of causing cross-contamination to food if hygiene practices are neglected.

Microbiological contamination occurs when pathogens polluted ready-to-eat food either by direct or indirect contact over the surface of equipment or machines, including indirect surfaces such as floors and walls in the processing area (Keeratipibul et al. 2017). According to Hultman et al. (2015), microorganisms present in the form of biofilm on the surface are difficult to get rid of, causing constant contamination and the presence of biofilm can increase the adhesion of pathogenic bacteria on the surface. While visual inspection is a good first step in assessing cleanliness level, it is lacking in what is necessary which is to detect the presence of pathogenic microorganism as well. Microorganism can be detected through its unique properties of cytoplasm and cell membrane, by influencing the electrical field in a different way from other particles. Intact cells and non-conductive particles provide this principle most clearly using metallic (conductive) particles. The conductivity of metallic particles will permit the electrical field to pass through unimpeded regardless of the frequency of the electric field. To the contrary, non-conductive particles such as polystyrene will resist the electrical field, so the current will only move in the liquid medium, which leads to a measurable volume displacement correlating with the particles in the flow channel (Ilea, Soptica & Widmann 2022).

Traditional microbiological methods often offer less preventive control and preoperational actions as lab results take days. ATP tests, while simple and fast, quantify biological residues, which are not a meaningful proxy for disinfection efficacy. Thus, there is a need for fast and accurate quantification of bacteria concentration in food safety. The purpose of the quantifications is to ensure that the total bacteria concentrations will not pose a risk to the consumers. Current practice for microorganism swabs is only for the case of food poisoning in identifying the root cause. This practice happens by collecting samples of FCS involved, then send to the laboratory to be analysed using plate counting. This method is well established and reliable, extremely easy and precise, but is notoriously slow and only shows the presence of culturable bacteria (Bertelsen et al. 2020). Thus, innovation in technology detecting bacteria and its properties is crucial in improving food safety. Existence of new technology of fast and accurate measurement of bacteria, especially *in-situ,* will be able to lead to new set of restaurant cleanliness checklist considering the ceiling limit for bacteria detection, so to improve steps taken in food poisoning and food-borne diseases preventive measures.

CytoQuant® is a mobile flow cytometer which provides *in-situ* measurement. It enables immediate verification of microorganism contamination either for food premise inspection specifically on verification of cleaning and disinfection procedures or any other purposes such as for the detection of bacteria and residue particles on surfaces. This mobile flow cytometer has the functionality to make on site verification on the effectiveness of cleaning and disinfection processes at food production sites such as food factories and restaurants. Measurements can be carried out before and after cleaning and disinfection activities. The device was made in Austria and has been introduced to the market of food service industry since 2022. The device applied the concept of impedance flow cytometry where it detects the unique electromagnetic properties of bacterial cell membrane and cytoplasm which differ them from any other particles (Ilea, Soptica & Widmann 2022). Impedance is defined as the complex ratio of the voltage to the current in an alternating/direct current circuit (Xu et al. 2016). Flow cytometry involves electrical field to count cells suspended in a fluid and to determine some of their physical or chemical properties. Intact cell and particles per mL can be calculated from 1.5×10^4 and 5.00×10^3, respectively, with maximum reading of 1.00×10^7 for both parameters. Therefore, instead of a laser, an impedance

flow cytometer employs an alternating current, the varying frequencies of which enable the device to detect, measure the size of and separately count membrane-intact cells and other particles. Immediate reading is the main advantage of this device as it can read within 30 seconds. Moreover, the measurement is not influenced by temperature and disinfectants so to avoid results variability. It is also requiring a very simple test procedure and does not require a specific place nor specific training. Its test results can be saved, exported and documented easily. This device provides precise counts of intact cells and particles (Ilea, Soptica & Widmann 2022).

Intact cells are special in the sense that they resemble both non-conductive and metallic particles, depending on the frequency of the electric field. At low frequencies, the isolating quality of a cell membrane prevents the electric field from penetrating it, leading to the displacement as with non-conductive particles. Higher frequencies, however, can partially penetrate the membrane; as such, cells are similar in conductivity to metallic particles. To overcome this problem, CytoQuant® which has microelectrodes, generates fields at both low and high frequencies, allowing the device to detect these changes in conductivity and resistance and thus attribute them in precise numbers to either intact cells or other particles. The detector identifies the target as a bacterium based on the varying degree of impedance or conductivity at these frequencies. The result will show separate counts of intact cells and other particles (Ilea, Soptica & Widmann 2022). Therefore, the purpose of this study was to test the functionality of this mobile flow cytometer in measuring the total reading of the intact cells and particles in the field as well as the accuracy of the CytoQuant® readings in comparison to the TPC method.

MATERIALS AND METHODS

SAMPLING

This cross-sectional study was done in the period of three months started in December 2022 and ended in February 2023. This study involved six selected (n = 6) restaurants with long operating hours located in the Lembah Klang area. Long-hours operating restaurant refers to the register list of 24-h food premises by local authority which include restaurant that operate for more than 18 h but excluding fast food restaurant. The food contact surface (FCS) selected were the ready-to-eat (RTE) cutting board, knives, food clips, dinner plates, and spoons. All swab samples were taken between 10.00 am and 11.00 am in each premise before being placed into icebox for further analysis.

IN-SITU CYTOQUANT® MEASUREMENT

Measurement was done following the instructions in the manual (Ilea, Soptica & Widmann 2022). The CytoQuant® consists of a CountCell component and three types of consumables which include CytoQuant® Storage Vial, CytoQuant® Swab Kit and CytoQuant® Cleaning Vial. Prior to using the CytoQuant® CountCell, start-up procedure was done by first inserted the CytoQuant® Cleaning Vial to ensure that the device is clean and ready to use. Swab kit was opened carefully and the swab was taken out of the vial for swab. The cotton swab was pressed on the targeted FCS and move in *zigzag* motion across the entire surface area of approximately 100 cm^2 (10 cm × 10 cm). Then, the swabbing procedure was repeated by turning over the cotton swab surface to the other side and swabbed on the same food contact surface in direction changed to 90°.

Upon completion, the swab was inserted back into its vial containing 3 mL buffer solution, screwed to close and shaken to form suspension of bacteria and residues. The vial was then inserted into the CytoQuant® and the reading was taken after 30 s. The results displayed on the screen were recorded as Intact Cells per mL (IC/mL) and Particles per mL (P/mL). This procedure was done for five FCS; RTE food chopping board, knifes, food clips, plates, and spoons. The swab samples were then placed an ice-box with a controlled temperature of 0 °C to 4 °C. The samples were then taken back to the Microbiology Laboratory, Universiti Kebangsaan Malaysia (UKM), for Total Plate Count (TPC) analysis within 24 h.

TOTAL PLATE COUNT (TPC)

All swab samples were analysed according to Bacteriological Analytical Manual (Maturin & Peeler 2001). Swab samples from the vials were diluted using buffered peptone water (Merck, USA) up to 10^{-5}. Plates were labeled according to the premise identification (ID), type of food contact surface, sampling date and time as well as sample dilution factor. The method used to determine colony count was a spread plate method. A total of 100 µL was transferred onto Plate Count Agar (PCA) (Oxoid, UK) using micropipette and incubated at 35 °C for 48 h. The TPC of bacteria was determined and calculated as CFU/mL units.

MICROBIAL SAMPLING TECHNIQUES

The sampling method was applying surface swabs on cutting boards using a 3M™ Environmental Scrub Sampler (ESS). Cutting boards were selected from ready-to-eat (RTE) food areas from each restaurant. Swabbing of the cutting board surfaces was carried out using 3M™ ESS containing 10 mL 3M™ Wide Spectrum Neutralizer, in accordance with the manufacturer's recommendations. The swabbing process involved pressing the ESS down and flexing the stick to ensure complete contact with the surface. The area sampled was approximately 10 × 10 cm. The procedure was repeated by flipping the device to the other side and altering the sampling direction to 90° (3M, U.S.A). The collected swabs were placed into a sample bag and secured, then stored in an ice box maintained

at a temperature between 0 °C and 4 °C. Samples were transported from restaurants to the laboratory and analysed within 24 h.

Samples were diluted from 10^{-1} to 10^{-5} using buffer peptone water (Merck, U. S. A) and then vortexed (Velp Scientifica, Italy). Following this, 1 mL of each dilution was inoculated onto the 3M™ Petrifilm for Aerobic Count Plate (AC), *Escherichia coli*/Coliform Count Plate (ECC), *Salmonella* Express Plate (SALX) and *Staph* Express Count Plate (STX) (3M, U.S.A). The incubation followed the AOAC Official Method for aerobic bacteria (AOAC 990.12), *E. coli* and coliform (AOAC 991.14), *Salmonella* spp. (AOAC 2014.01), and *S. aureus* (AOAC 2003.07), and colony counts were reported as log CFU/cm^2. Colonies were classified as 'satisfactory' for < 1.30 $\log_{10}$ CFU/cm^2 (aerobic), < 1.00 $\log_{10}$ CFU/cm^2 (coliform, *E. coli, S. aureus*), while 'unsatisfactory' for ≥ 1.30 $\log_{10}$ CFU/cm^2 (aerobic), ≥ 1.00 $\log_{10}$ CFU/cm^2 (coliform, *E. coli, S. aureus*), following the methods of Marzano and Balzaretti (2011) and Sneed et al. (2004). For *Salmonella* spp., results were reported as 'detected' or 'not detected'.

RESTAURANTS INSPECTION

The inspection was carried out using the Food Premise Inspection Form (JKT/KS/1), based on the Guidelines on Food Premises Grading System established by the Ministry of Housing and Local Government (KPKT 2014). This inspection assessed the level of cleanliness and assigned grades of A, B, and C. The inspection form (JKT/KS/1) comprises seven main components and thirty-one evaluation items. The main components assessed include the food preparation area, food serving areas, food handlers, water supply system, sanitation system, building structure and maintenance, and other significant factors. Grading was based on the inspection score, with Grade A (86 - 100%) designating 'very clean', Grade B (71 - 85%) rating as 'clean', Grade C (51 - 70%) as 'less clean', while a score below 51% received an ungraded classification.

DATA ANALYSIS

The data were analysed using IBM SPSS statistical software version 20 (Armonk, New York: IBM Corp). Analysis of descriptive statistical data described values in the form of mean and standard deviations. The analysis of statistical data used were Kruskal Wallis, Man-Whitney U tests and analysis of variance (ANOVA) with Tukey's pairwise comparison. In all analyses, differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

CytoQuant® MEASUREMENT

CytoQuant® has the ability to measure the total reading of the intact cells and particles on food contact surfaces (FCS) by swabs. Table 1 shows the intact cell and particle readings of five FCS using the CytoQuant®. Result shows that CytoQuant® was able to detect intact cell and particle levels for all 30 readings on each FCS in six of restaurants with long operating hours. Based on Table 1, the highest recorded reading for intact cell is 1.00×10^7, detected on all food contact surface (FCS) except for the spoon, while the lowest reading is 1.40×10^4, referring to FCS except for the cutting board and knife. The highest particle reading observed was 5.00×10^6, detected across all food contact surfaces (FCS) except for the spoon, which had the lowest reading at 1.40×10^4.

The analysis showed that there was a significant difference ($p < 0.05$) between the intact cell readings and the particle on the FCS using CytoQuant®. Study found that CytoQuant® has the ability to measure intact cell reading ranges 1.40×10^4 to 1.00×10^7 IC/mL and particle from 1.40×10^5 to 5.00×10^7 P/mL. These indicates that the equipment has a high level of sensitivity to detect intact cell and particles in the samples. Generally intact cells are defined as cells with an intact cell wall, irrespective of their state (such as stressed, or viable but not culturable) or required growth conditions (aerobic or anaerobic, pH, nutrition, salt concentration, temperature, lag-time, incubation time). Since no solid or liquid media can isolate the full range of microbial diversity, and considering that the majority of microbes are non-culturable (Panaiotov et al. 2021), it is important to note that intact cell counts and plate counts represent two fundamentally different parameters. CytoQuant® additionally classifies any objects that are not bacteria and pass through the microfluidic flow cell as residue particles. Particle counts are a direct indication of cleanliness (Ilea, Soptica & Widmann 2022).

Based on the observation in the study premises, there were several factors that affected the quantity of different intact cell and particle readings among all five different FCS. Among them were the condition and types of material of FCS, level of hygiene and the cleaning procedures practiced. Cutting boards used were all made of plastic materials and had rather rough surface if compared with the other utensils. FCS with rough surface area are more likely to trap dirt as they are more difficult to be cleaned. While knives, chopsticks and spoons are made up of stainless-steel materials that facilitate effective washing and clean surface conditions. This is in line with the statement of Whitehead and Verran (2006), who reported increased substratum surface roughness affects the retention of microorganisms on that surface.

The cutting boards were also seen with poor hygiene as there were food leftovers as well as other utensils such as knives and chopsticks were placed on them after used. FCS with poor hygiene level are at higher risk of being more contaminated with bacterial overload as well as particles. This statement is in line with the findings of a study done by De Filippis et al. (2021), which stated that the bacterial

TABLE 1. Intact cell and particle readings on food contact surfaces using CytoQuant®

Premise	Mean (IC/mL)											
	Cutting board		Knife		Food clips		Plate		Spoon		*P value*	
	Intact Cell	Particle	Intact Cell	Particle	Intact Cell	Particle	Intact Cell	Particle	Intact Cell	Particle	Intact Cell	Particle
1	8.90×10^{4}	4.28×10^{4}	2.90×10^{4}	1.98×10^{6}	2.10×10^{4}	8.80×10^{5}	2.50×10^{4}	3.80×10^{5}	1.40×10^{4}	4.70×10^{5}		
2	1.00×10^{7}	5.00×10^{6}	1.00×10^{7}	5.00×10^{6}	2.80×10^{5}	4.28×10^{6}	1.40×10^{4}	2.00×10^{5}	1.40×10^{4}	1.50×10^{5}		
3	1.00×10^{7}	5.00×10^{6}	1.00×10^{7}	5.00×10^{6}	1.40×10^{4}	2.60×10^{5}	1.00×10^{7}	5.00×10^{6}	1.40×10^{4}	1.40×10^{5}		
4	4.30×10^{5}	1.52×10^{6}	2.10×10^{5}	2.09×10^{6}	1.00×10^{7}	5.00×10^{6}	2.30×10^{4}	3.50×10^{5}	1.40×10^{4}	6.20×10^{5}	0.003*	0.002*
5	7.80×10^{4}	4.49×10^{6}	6.50×10^{4}	3.91×10^{6}	1.40×10^{4}	1.71×10^{6}	4.00×10^{4}	2.70×10^{5}	1.80×10^{4}	1.80×10^{5}		
6	1.00×10^{7}	5.00×10^{6}	1.00×10^{7}	5.00×10^{6}	1.40×10^{4}	6.60×10^{5}	1.40×10^{4}	3.50×10^{5}	1.40×10^{4}	2.20×10^{5}		

*Significant $p < 0.05$

population on the surface of the food contact is dependent on their composition and hygienic conditions. Next, the FCS cleaning method including the cleaning frequency of the FCS by food handlers should also be emphasised. Cleaning FCS regularly with proper procedures using detergent or disinfectant will lower the probability of bacterial detection and any particle presence.

TOTAL PLATE COUNT ANALYSIS

Total Plate Count (TPC) is the cultural method used to determine the number of bacterial colonies or intact cell reading as compared to reading by CytoQuant®. Table 2 shows the intact cell readings of five FCS using the TPC method. The analysis showed that the highest mean readings was recorded by the plate with a reading of 4.70±1.10 × 10^7 CFU/mL, while the lowest reading was also recorded by plate with a value of 4.30±1.50 × 10^2 CFU/mL. Measurement using TPC or cultural method is a traditional method in determining the presence of bacteria present in FCS. For the determination of TPC, colonies of white or creamy yellow were counted after an incubation at 35 °C for 48 h. TPC taken into account the presence of bacteria, which was determined as intact cell by CytoQuant®, along with the count of particles as advantage. The findings showed that the range of intact cell readings in the FCS studied ranged from 4.30 × 10^2 to 4.70 × 10^7 CFU/mL after considering the dilution factors used. As indicated in Table 2, there is a significant difference ($p < 0.05$) value of TPC among the FCSs for each premises. While comparison among column also showed significant difference ($p < 0.05$) of TPC value among the premises for different FCS. Therefore, the traditional TPC method is a reliable method to make comparison with any other method or new equipment for microbial detection.

COMPARISON ON THE READING OF INTACT CELL USING CytoQuant® AND TOTAL PLATE COUNT ANALYSIS

The result of intact cell readings from food contact surfaces measured by CytoQuant® TPC showed some differences in individual sample. However, mean values across various restaurants showed no significant difference ($p > 0.05$) between CytoQuant and TPC, with a Mann-Whitney U test yielding a p-value of 0.88. This suggests that both methods provide comparable intact cell measurements on average.

This results is in line with the finding from Romer Labs (Ilea, Soptica & Widmann 2022) who also reported that there was an association or no significant difference between the results of the two methods. However, CytoQuant® leads in the means of technology, mobile and quick measurement time compared to the TPC method. The major drawbacks of current method are that it is time consuming, labour intensive and costly when involve many samples to be analysed are involved.

The revolution of CytoQuant® to be suited in food industry monitoring is supported by several factors that indicate a gap in the measurement of bacteria with current method. The highest concern is on the term of 'great plate count anomaly' which refers to observation that most of the microbes seen in the microscope cannot be grown under laboratory conditions. This means that microscopic cell counts are significantly higher than corresponding counts of 'colony forming units' on agar plates. Examples illustrating on how this phenomenon happened: while 50% of the microorganisms of the oral flora can be cultured with agar plates, most of the gastrointestinal flora cannot be cultured at all. The reasons for this are numerous. For instance, aerobic plate count methods rely on very general media, which do not support the growth of most bacteria groups (Ilea, Soptica & Widmann 2022).

Other than that, it is the issue of viable but non-culturable (VBNC) microorganisms. Generally, bacteria in the VBNC state do not multiply but are still alive, as shown by their metabolic activity. According to Oliver (1995), 'VBNC' can be defined as a metabolically active bacterial cell that crossed a threshold in this way, for known or unknown reasons, and became unable to multiply in or on a medium normally supporting its growth. Most relevant fact is that they can become culturable after resuscitation and thus proliferate in food. Moreover, some pathogenic

TABLE 2. Intact cell readings on food contact surfaces using plate count agar method

Premise	Mean (CFU/mL)				
	Cutting board	Knife	Food clips	Plate	Spoon
1	$3.10±0.90×10^{3\ bB}$	$4.30±2.30×10^{4\ bC}$	$3.03± 0.95×10^{3\ bC}$	$2.30±0.60×10^{4\ bB}$	$1.13±0.96×10^{6\ aC}$
2	$1.67±0.58×10^{4\ bB}$	$4.00±1.00×10^{5\ bBC}$	$1.30± 0.58×10^{6\ bA}$	$4.70±1.1×10^{7\ aA}$	$3.30± 0.26×10^{6\ bB}$
3	$1.60±1.20×10^{6\ aA}$	$6.06±1.50×10^{5\ abBC}$	$7.00± 1.70×10^{5\ abAB}$	$1.10± 0.28×10^{4\ bB}$	$1.20±0.10×10^{5\ abC}$
4	$1.99±0.27×10^{6\ aA}$	$2.70±0.70×10^{6\ aA}$	$1.10±0.06×10^{5\ bBC}$	$2.70±0.58×10^{4\ bB}$	$6.70±5.70×10^{2\ bC}$
5	$4.00±1.70×10^{4\ bB}$	$2.40±0.12×10^{4\ bC}$	$2.40±1.3×10^{5\ aBC}$	$6.00±1.90×10^{4\ bB}$	$3.60±3.60×10^{4\ bC}$
6	$1.90±0.11×10^{5\ cB}$	$1.10±0.06×10^{6\ bB}$	$6.70±5.8×10^{2\ cC}$	$4.30±1.50×10^{2\ cB}$	$5.80±0.61×10^{6\ aA}$

*a-c Values with different letters in the same row are significant with $p < 0.05$; *A-C Values with different letters in the same column are significant with $p < 0.05$

bacteria will not grow in the absence of a host and need only to survive in food until ingestion to cause illnesses. There are many reasons why bacteria can go into the VBNC state, these include starvation, incubation outside of the optimal temperature range for growth, elevated osmotic concentrations, levels of oxygen concentration, or exposure to white light (Ilea, Soptica & Widmann 2022).

Furthermore, CytoQuant® is able to measure the presence of all types of bacteria including anaerobic and microaerophilic bacteria meanwhile this bacterial in which current practiced method for these particular groups of bacteria is via cultivation in a very specific condition such as in sealed jar with commercial gas-generating envelopes as they require lower level of oxygen. Generally, all bacteria can be divided into three groups: obligate, aero-tolerant and facultative. As their names indicate each of them has special requirements regarding the air, or more precisely, the oxygen, surrounding them. Obligate anaerobes such as *Clostridioides difficile* are harmed by oxygen and will die shortly after exposure. Aero-tolerant bacteria such as *Clostridium botulinum* cannot make use of oxygen and will neither die nor grow in its presence. Facultative anaerobes can use oxygen but do not need it for growth, as is the case with *E. coli*. There is microaerophilic group of bacteria such as *Campylobacter* that needs some oxygen to grow, though in much smaller amounts (1 - 2%) than in normal air but can be inhibited in aerobic conditions (Ilea, Soptica & Widmann 2022).

Another advantage of CytoQuant® is that it is capable of detecting psychrotrophic bacteria. This is based on its ability to make measurements without being affected by the influence of ambient temperature. This is contrary with current method which needs some consensus on methodologies for psychrotrophic and aerobic bacteria in culture method as the methods synopses up to 100 applicants (Jay 2002). Psychrotrophic bacteria grow at temperatures as low as 0 °C, with optimal and maximal growth temperatures above 15 °C. The psychrotrophic groups of bacteria commonly found in food are the Gram-negative genera *Pseudomonas, Aeromonas, Achromobacter, Serratia, Alcaligenes, Chromobacterium,* and *Flavobacterium* as well as Gram-positive genera such as *Bacillus, Clostridium, Corynebacterium, Streptococcus, Lactobacillus,* and Microbacteria. *Listeria monocytogenes* and some strains of *Clostridium botulinum* are also known to be able to proliferate at refrigeration temperatures (Ilea, Soptica & Widmann 2022).

Above all, CytoQuant®'s ability to break down the biofilm layer and subsequently calculate the total presence of bacteria is surely another level of advantage, as current practice of bacterial biofilm detection requires very detailed laboratory analysis while the general method is not able to detect. In fact, the vast majority of bacteria are able to form biofilms. Biofilms are composed of monocultures or of several different microorganism species. Bacterial biofilms are often defined as communities of surface-attached bacteria and are typically depicted with a classic mushroom-shaped structure characteristic of *Pseudomonas aeruginosa* (Sauer et al. 2022). Microorganisms which form or thrive in biofilms are more resistant to disinfection. Some researchers have suggested that the complex structure of mixed biofilms renders them to be more stable and more resistant to cleaning chemicals. The initial population that binds to the surface can change the properties of that surface, allowing for those that come later to adhere via cell-to-cell association; in some cases, the attachment of a second species may increase the stability of the biofilm population. For example, studies showed that *L. monocytogenes* is more likely to adhere to steel in the presence of *Pseudomonas*. Other effects of biofilms such as the corrosion of metal surfaces are a further critical concern (Ilea, Soptica & Widmann 2022).

ENUMERATION AND DETECTION OF BACTERIAL LOADS ON CUTTING BOARD AND RATING OF FOOD PREMISES

Analysis of intact cell counts using CytoQuant® and traditional TPC methods (Tables 1 & 2) showed the cutting boards recorded the highest cell counts. Thus, further study prompted a focused microbiological investigation of cutting boards from all study premises (Table 3). The results showed that all six premises exhibited aerobic and coliform counts exceeding established thresholds, suggesting deficiencies in the sanitation practices, potentially stemming from inadequate cleaning, disinfection protocols, or both. The observation that *E. coli* and *S. aureus* counts remained below established thresholds in all samples suggests the presence of effective control measures targeting these specific pathogens, but also indicates that broader spectrum contamination is still occurring. The detection of *Salmonella* spp. in one of the premises further highlights the heterogeneity of microbial contamination across food preparation sites and emphasizes the inherent limitations of relying solely on indicator organisms for comprehensive food safety assessment. However, the discrepancy between significantly elevated levels of general bacterial contamination (indicated by aerobic and coliform counts) and the relatively low counts of specific foodborne pathogens warrants further investigation to ascertain the composition of the microbial community and pinpoint the sources of contamination, especially on the highly-touch surface in food preparation such as cutting board. Cutting boards serve as a critical highly-touch surface in food preparation, facilitating the direct transfer of microorganisms from raw to ready-to-eat foods. Their porous nature and frequent exposure to diverse food matrices create a favorable environment for microbial colonization, making them ideal for assessing hygienic practices and contamination risk. The observed data from this study might also correlate with lower food premise inspection scores, reflecting the impact of sanitation practices on overall hygiene and safety.

Correlation between the bacterial loads with food premise rating scores, which asses the cleanliness and

TABLE 3. Bacterial load on cutting board using petrifilm method and rating of food premise inspection (n = 6)

Premise	Log_{10} CFU/cm^2				Presence	Food premise rating
	Aerobic count	Coliform	*E. coli*	*S. aureus*	*Salmonella* spp.	
1	≥ 1.30	≥ 1.00	< 1.00	< 1.00	Not detected	70%
2	≥ 1.30	≥ 1.00	< 1.00	< 1.00	Not detected	67%
3	≥ 1.30	≥ 1.00	< 1.00	< 1.00	Not detected	78%
4	≥ 1.30	≥ 1.00	< 1.00	< 1.00	Not detected	67%
5	≥ 1.30	≥ 1.00	< 1.00	< 1.00	Not detected	66%
6	≥ 1.30	≥ 1.00	< 1.00	< 1.00	Detected	59%

hygenic practice in all food premises was also reported (Table 3). These ratings are based on the Food Premise Inspection Form (JKT/KS/1) in accordance with the Guidelines on Food Premises Grading System established by the Ministry of Housing and Local Government (KPKT 2014). Based on Table 3, the food premise rating scores across all study sites ranged from 59% to 78%. Only one premise achieved a clean status (71% - 85%), while the remaining premises fell into the less clean category (51% - 70%). Thus, this data suggests a positive correlation between the bacterial loads as well as cleanliness and hygenic practice in all food premises.

CONCLUSIONS

The findings reported that CytoQuant® has the ability to measure intact cells and particles readings on food contact surface (FCS) in study premises. The findings also showed that CytoQuant®'s intact cell readings were generally recorded higher than Total Plate Count (TPC) method, thus, proved better sensitivity levels. Therefore, CytoQuant® is suitable to be used as the latest technology for cleanliness level indicators in monitoring the level of bacterial load on equipment FCS in food processing environment. The use of CytoQuant® will be able to give a significant impact to immediate implementation of preventive measures against the risk of foodborne diseases, and at the same time fulfilling the need for a fast and accurate quantification of microorganism and particle detection in food safety field.

ACKNOWLEDGMENTS

This work was funded and supported by Premier Diagnostics Sdn. Bhd (ST-2023-026). The authors would like to acknowledge the ethics of all the agencies involved such as Universiti Kebangsaan Malaysia (UKM) and Majlis Perbandaran Kajang (MPKj) for granting permission to carry out the research. Authors are also thankful for the restaurant owners who participated in this research study.

REFERENCES

Ahmed, Z., Afreen, A., Hassan, M.U., Ahmad, H., Anjum, N. & Waseem, M. 2017. Exposure of food safety knowledge and inadequate practices among food vendors at Rawalpindi; the fourth largest city of Pakistan. *Journal of Food and Nutrition Research* 5(1): 63-73.

Al Banna, M.H., Khan, M.S.I., Rezyona, H., Seidu, A-A., Abid, M.T., Ara, T., Kundu, S., Ahinkorah, B.O., Hagan, J.E.J., Tareq, M.A., Begum, M.R., Chowdhury, M.F.T. & Schack, T. 2022. Assessment of food safety knowledge, attitudes and practices of food service staff in Bangladeshi hospitals: A cross-sectional study. *Nutrients* 14(12): 2540.

Bertelsen, C.V., Franco, J.C., Skands, G.E., Dimaki, M. & Svendsen, W.E. 2020. Investigating the use of impedance flow cytometry for classifying the viability state of *E. coli*. *Sensors* 20(21): 6339.

Cosby, C.M., Costello, C.A., Morris, W.C., Haughton, B., Devereaux, M.J., Harte, F. & Davidson, P.M. 2008. Microbiological analysis of food contact surfaces in child care centers. *Applied and Environmental Microbiology* 74(22): 6918-6922.

De Filippis, F., Valentino, V., Alvarez-Ordonez, A., Cotter, P.D. & Ercolini, D. 2021. Environmental microbiome mapping as a strategy to improve quality and safety in the food industry. *Current Opinion in Food Science* 38: 168-176.

Hultman, J., Rahkila, R., Ali, J., Rousu, J. & Bjorkroth, K.J. 2015. Meat processing plant microbiome and contamination patterns of cold-tolerant bacteria causing food safety and spoilage risks in the manufacture of vacuum-packaged cooked sausages. *Applied and Environmental Microbiology* 81(20): 7088-7097.

Ilea, C., Soptica, F. & Widmann, S. 2022. *Flow Cytometry and Verification of Cleaning and Disinfection in Food Manufacturing Facilities,* edited by Davis, J. Austria: Romer Labs Division Holding GmbH.

Jay, J. 2002. A review of aerobic and psychrotrophic plate count procedures for fresh meat and poultry products. *Journal of Food Protection* 65(7): 1200-1206.

Keeratipibul, S., Laovittayanurak, T., Pornruangsarp, O., Chaturongkasumrit, Y., Takahashi, H. & Techaruvichit, P. 2017. Effect of swabbing techniques on the efficiency of bacterial recovery from food contact surfaces. *Food Control* 77: 139-144.

Lani, M.N., Mohd Azmi, M.F., Ibrahim, R., Alias, R. & Hassan, Z. 2014. Microbiological quality of food contact surfaces at selected food premises of Malaysian heritage food ("Satar") in Terengganu, Malaysia. *The International Journal of Engineering and Science* 3(9): 66-70.

Marzano, M.A. & Balzaretti, C.M. 2011. Cook-serve method in mass catering establishments: Is it still appropriate to ensure a high level of microbiological quality and safety? *Journal Food Control* 22(12): 1844-1850.

Maturin, L. & Peeler, J.T. 2001. *Chapter 3: Aerobic Plate Count.* https://www.fda.gov/food/laboratory-methods-food/bam-chapter-3-aerobic-plate-count

Ministry of Housing and Local Government (KPKT). 2014. *Guidelines on Food Premises Grading System in the Local Authority Area*, Secretary General's Circular of the Ministry of Local Government Development, No. 2 of 2014.

Oliver, J.D. 1995. The viable but non-culturable state in the human pathogen *Vibrio vulnificus*. *FEMS Microbiology Letters* 133(3): 203-208.

Panaiotov, S., Hodzhev, Y., Tsafarova, B., Tolchkov, V. & Kalfin, R. 2021. Culturable and non-culturable blood microbiota of healthy individuals. *Microorganisms* 9(7): 1464.

Sauer, K., Stoodley, P., Goeres, D.M., Hall-Stoodley, L., Burmolle, M., Stewart, P.S. & Bjarnsholt, T. 2022. The biofilm life cycle: Expanding the conceptual model of biofilm formation. *Nature Reviews Microbiology* 20(10): 608-620.

Shafizi, A.W., Sahilah, A.M., Chai, L.C., Razalee, S. & Aishah, E. 2024. Microbial assessment on cutting boards and cleanliness levels of restaurants with long operating hours around Klang Valley, Malaysia. *International Food Research Journal* 31(3): 658-669.

Sneed, J., Strohbehn, C., Gilmore, S.A. & Mendonca, A. 2004. Microbiological evaluation of foodservice contact surfaces in Iowa assisted-living facilities. *Journal of the American Dietetic Association* 104(11): 1722-1724.

Whitehead, K.A. & Verran, J. 2006. The effect of surface topography on the retention of microorganisms. *Food and Bioproducts Processing* 84(4): 253-259.

Xu, Y., Xie, X., Duan, Y., Wang, L., Cheng, Z. & Cheng, J. 2016. A review of impedance measurements of whole cells. *Biosensors and Bioelectronics* 77: 824-836.

Zulfakar, S.S., Sahani, M. & Hamid, N.H.A. 2018. Microbiological assessment of food contact surfaces in residential college cafeterias at a local university in Malaysia. *Jurnal Sains Kesihatan Malays*ia 16: 33-38.

*Corresponding author; email: sahilah@ukm.edu.my