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## Use of Rapid Microbial Kits for Regular Monitoring of Food-Contact Surfaces towards Hygiene Practices

Mazni Saad<sup>a,\*</sup>, Toh Poh See<sup>a</sup>,  
Mohd Faiz Foong Abdullah<sup>b</sup>, Norazmir Md. Nor<sup>c</sup>

<sup>a</sup>*Faculty of Hotel and Tourism Management*<sup>b</sup>*Faculty of Applied Sciences*<sup>c</sup>*Faculty of Health Sciences**Universiti Teknologi MARA, 40500 Puncak Alam, Malaysia*

### Abstract

Food business operators should promote safe and healthy food, utilizing simple methods for microbe identification and verification. This study attempts to determine the practicability measurement of hygiene practices in two states of Malaysia. It used an inexpensive and user-friendly microbial kit to evaluate the cleanliness level of Food-Contact Surfaces (FCS). A total of 72 samples in triplicate detected 70% of all coliforms. The prevalent contaminations suggest that food service operators may need to improve the cleanliness of FCS. In conclusion, easy-to-use microbial kits are practical and self-check approach in hygiene and should be made mandatory or alternative for the operator.

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### 1. Introduction

The cooking process is intended to obliterate harmful microorganisms and ensure the prepared food is microbiologically safe for human consumption. However, activities such as adding garnishes, cutting or slicing of cooked food can potentially reintroduce harmful organisms into the food. Such activities will require the use of clean hands and utensils. The preparations should be performed in a clean environment

\* Corresponding author. Tel.: +6-017-878-7543; fax: +6-03-5543-5698.  
E-mail address: [ms\\_mazni1971@yahoo.com](mailto:ms_mazni1971@yahoo.com).

and also on clean food- contact surfaces (FCS) as the final product will not be subjected to the heating process. Therefore, poor hygiene practices can act as a source of contamination. The contamination contributes to food spoilage and the spread of diseases and infections such as food poisoning cases in many situations. Foodborne infections have been associated with outbreaks such as *Caliciviruses (norovirus)*, *Salmonella spp.*, *Hepatitis A*, *Shigella spp.*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi/paratyphi*, *Vibrio cholera 01*, *Yersiniasenterocolitica*, *Giardia lamblia*, *Campylobacter jejuni*, *Cryptosporidium parvum*, *Escherichia coli O157:H7*, *Cyclosporacayetanensis*, and *Entamoebahistolytica* (Michaels et al., 2004). These outbreaks are closely related with bacteria and viruses, parasites, mold, toxins, and contaminants and allergens. In Malaysia, besides cholera, Hepatitis A, typhoid, and dysentery, food poisoning was the most food borne critical factor from 2000 to 2012 (Mazni, Toh, & Mohamed Azam, 2013). Upset stomach, abdominal cramps, nausea, vomiting, diarrhoea, fever and dehydration are the symptoms for food poisoning in short term. In long term effects it can be devastating, and it could be associated with kidney failure, chronic arthritis, brain and nerve damage or death (U.S. Department of Health & Human Services, 2013). Failure to observe the practice of self-hygiene, FCS, and clean environment could lead to serious health consequences in terms of food borne illness. The enforcement of food service hygiene has to comply with the Food Act 1983 and Food Hygiene Regulations 2009 (Mazni et al., 2013). From the preliminary interviews with the Ministry of Health Malaysia, the authorities' inspections often involve laboratory investigations on food premises and food handlers in order to assess the cleanliness level. Unfortunately, these reactive measures do not lead to any added-value for food handlers. As such, there lie many cases of food seizures and actions taken on the organizations. In line with these, this study aims to determine the practicability measurement of hygiene practices. It evaluates the cleanliness level of FCS in critical food services in Perak and Selangor, two states of Malaysia.

## 2. Literature reviews

### 2.1. Food safety aspect

Food safety is indispensable to sustain human life. Yet, providing safety from farm to fork is challenging task to ensure the hazard-free food along the chains (Menkovska, 2011; Payne-Palacio & Theis, 2012). To assure human health and safety, there is a need to identify, assess and control systematically the adverse consequences and their associated probabilities arising from contaminated foods with microbial pathogens (Lammerding & Fazil, 2000).

However, food safety is difficult to define or to measure, and it requires continuous and proper efforts by competent authorities, business operators, scientists, consumers, and sector representatives (Belgian Federal Agency for the Safety of the Food Chain, 2010). Globally, among the proposed measures are (i) through the implementation of standards and guidelines such as ISO 22000, Hazard Analysis Critical Control Point (HACCP) scheme, Good Manufacturing or Management Practices (GMP) scheme (Arvanitoyannis & Varzakas, 2009); (ii) scientific basis for managing risk related to food consumption such as Food Safety Barometer developed by the Scientific Committee of the Belgian Food Safety (Havelaar, Nauta, & Jansen, 2004).

### 2.2. Food-contact surfaces

FCS is where food activities such as garnishing, chopping, deboning, and slicing are taking place. FCS is a priority measurement to control the transmission of foodborne pathogens in ready-to-eat (RTE) food service operation (Cosby et al., 2008). Contaminated food has a direct effect on human health, but contaminated FCS is more critical. This is because it can be one of the factors on food spoilage when the

RTE has direct contact with it. FCS such as plates, cups, cutting boards, food preparation table, and so on can be re-contaminated after routine cleaning procedures. Moreover, it does not go through the heating process before it is served to consumers. Consequently, equipment, utensils and the areas where the food is processed or prepared require full attention in the cleaning or hygiene endeavours.

FCS has been an interest for many studies. For example, Rodriguez et al. (2011) swabbed working tables, cutting boards, sinks, and kitchen faucets for mesophilic aerobic bacteria and *Enterobacteriaceae*. In other study on long-term care facilities in Andalusia, Rodriguez et al. (2011) found high bacteria counts on cutting boards and kitchen faucets. Using the same method, Lahou et al. (2012) enumerated the total viable bacteria count from cutting boards, knives, and spoons. Lahou measured the microbiological performance of a food safety management system in Belgium food service operation. In addition, Cunningham et al. (2011) examined retail food establishments in Minneapolis for total aerobic count bacteria. Their findings revealed that slicer and cutting board both contained the highest average counts of bacteria. In sum, there is a strong bond between FCS and food safety risk.

### 2.3. *E.coli* and Coliform

Coliforms in general, and *Escherichia coli* (*E.coli*) in particular are traditional hygiene indicators of fecal contamination in water and other environmental samples (Enoch et al., 2013; Martinon, Cronin, Quealy, Stapleton, & Wilkinson, 2012). *E.coli* by definition is true fecal bacteria (Atlas et al., 1994; Nkere, Ibe, & Iroegbu, 2011) and coliforms signify most common contamination in fecal wastes (Dorothy & Philip, 1998; Willis, Elviss, Aird, Fenelon, & Mclauchlin, 2012). As a result, *E.coli* and total coliforms are practical surrogates for enteric pathogens when evaluating the hygiene of FCS.

### 2.4. Microbial Kits

Hygiene intervention measures often used the microbial kits to control the micro-organisms in food or FCS. The most common detection of water and food borne pathogens and fecal bacteria indicator are via culturing in a nutrient medium under certain microbiological standard conditions, in which necessitate plenty of time, expense and skilled labour (Atlas et al., 1994). Atlas et al. (1994) also highlighted that there are several problems with viable culture methods such as the tedious process of maintaining viability of bacteria between the time of collection and enumeration, lack of growth of viable, failure to cultivate all living cells of interest, time / days required for detection and confirmation of enteric bacteria, lack of specificity for detection of true fecal coliforms such as *E.coli*, failure to distinguish living from dead cells using direct microscopic counts, and misidentification of organisms due to antigenic cross reactivity using serological procedures.

The AOAC official method 991.15 use the Colilert test as an alternate approach to inspect *E.coli* and total coliform in water (Linman, Sugerman, & Cheng, 2010). The Colilert test offers a cost-effective means and maximize the protection of public health for many governments, including Malaysia's, since 1998 (Willis et al., 2012). Another type of rapid cleaning validation is The Flash kit (BioControl Systems Inc., USA); a test kit includes FLASH Test devices, hydrating solution and positive control. The design of the kit is to detect protein residues, a nutrient source for microorganisms. The presence of protein residues serves as an indicator of environmental sanitation.

Rapid indicator such as RIDA® COUNT test plate (R-Biopharm AG, Germany) introduce an easy-to-handle and manipulate in the inconvenient conditions and have potential for expanded use in detection of microbes in water and air samples (Mulec, Kristufek, & Chronakova, 2012). Mulec et al. (2012) used RIDA® COUNT test plate to examine the karsts ecosystems which had high organic inputs. The usage of RIDA® COUNT test plate was also successful in the dairies industry in Estonian (Salo, Ehavald, Raaska,

Vokk, & Wirtanen, 2006). The use of RIDA® COUNT test plates method has proven effective in detecting the presence of microbes in water resources. Now, its effectiveness will be tested on solid surfaces.

### 2.5. Practicability identifying pathogenic bacteria

Diagnosis and preparation for subsequent treatment of infectious diseases rapid and reliable identification of microorganisms is a must. For years, the traditional practice of identifying pathogenic bacteria is based on their isolation from clinical samples and propagation on culture medium in the routine laboratory (Trotha, Konig, & Konig, 2001). Similarly, biochemical, morphologic, and serologic tests require growth of the organism. However, Wilson et al. (1991) stated that these procedures are unreliable, impractical, and it has the limited ability to aware of true bacterial in many situations. In short, literatures in principle explain the importance of identifying the actual cause towards hygiene problems and yet the problems arise with the bacteria culturing method.

On the other hand, on-site and in-field detection method remain crucial for immediate prevention of foodborne illness outbreaks. Foodborne illness outbreaks also occur when associations with the consumption of a wide variety of fresh produce including lettuce, alfalfa, artichokes, bean sprouts, cabbage, cilantro, parsley, spinach, watermelon, tomatoes and cantaloupes exist (Park & Oh, 2012). Examples of the fresh produce-associated foodborne illness outbreaks are *Salmonella*, *Shigella*, *Escherichia coli* O157:H7, *Campylobacter*, *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, and *Clostridium botulinum*, which *Salmonella* was one of main causes (Beuchat, 2002; Heaton & Jones, 2008). The study of Park et al. (2013) showed that almost all surface area of fresh produce had uniform bacterial cells.

In case of foodborne illness outbreaks that easily occur along the route from farm to table, Beuchat and Ryu (1997) suggested rapid detection methods in-the-field and on-site at any locations along the food chains. Consequently, Park et al. (2013) introduced the practicability of detecting *S. Typhimurium* by the real-time and on-site detection with minimum sample preparation. It helps in cost-effective and time-effective. The rapid detection methods that on-site and in-field added to the practicability and effectiveness to improve food safety of fresh fruits and vegetables as it demonstrated in this study. From the literature reviewed, current study derives an idea of utilizing rapid indicator of RIDA® COUNT test plate for regular monitoring of FCS towards hygiene practices. Thus, the practicability of easy-to-use microbial kits needs to be assessed in the current food handling practices.

### 3. Material and methods

#### 3.1. Collection of samples

In total, 72 samples of FCS include four samples: dining tables, food trays, cooking pots, and kitchen faucets in six identified food services in the states of Perak and Selangor. In this study, estimation of the total counts bacterial was done by using a contact plate test of RIDA® COUNT test plate for the detection of *E.coli* and total coliforms on FCS as shown in Fig. 1. (a) and (b) below.



Fig. 1. (a) RIDA® count plate; (b) Natrium Chloride solutions

#### 3.2. Sampling procedure

As for FCS, *E.coli* and coliforms are accessed via RIDA Count® and Colilert Test. To date, rapid indicator such as RIDA® COUNT test plate introduces an easy-to-handle of microbial counts used in water and air samples (Mulec et al., 2012). This study chose samples from clean utensils, which rinsed using ordinary method. Accordingly the swabbing process began with labelling and coding of sample type, date, number of duplicates, and the name of food service on the RIDA® count plates. Subsequently one millilitre of natrium chloride solution was applied to soak the dry-ready-agar on RIDA® count plates for 15 minutes (Fig. 2). The plate applied five times of slight pressures inside the designated sampling area of 10 cm x 10 cm area of FCS; on north-east and north-west, south-west and south-east and finally in the middle of these four. Negative controls for each sample were also made for comparison. Finally, all the RIDA® count plates put in the sterile plastic bags and stored in the cool box. The temperature in the cool box was maintained between 0° to 4°C in order to preserve the integrity of the sample until the sample can be evaluated in laboratory.

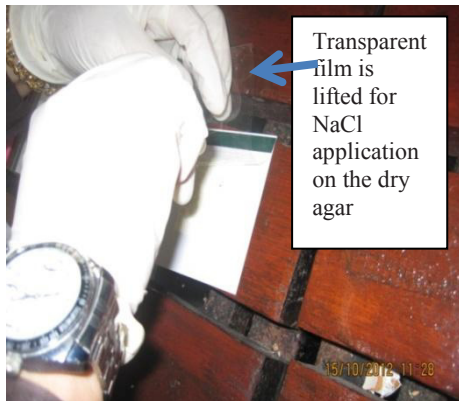


Fig. 2. Swabbing preparation process in Food Service 1, Selangor.  
 Note. From the author personal photo collections.

#### 4. Data analysis

Microbial colonies of FCS were counted as CFU/cm<sup>2</sup>, and the means were computed via SPSS version 21.0 in log<sub>10</sub> CFU/cm<sup>2</sup>. In order to get bacterial colonies counts for *E.coli* and total coliforms, each sample went for incubation process at 35°C for 24 hours. After 24 hours, the observations on RIDA® count plates showed colonies presence with one or two colors. Purple to indigo colonies indicate *E.coli* whereby the light blue colonies signify total coliforms. This study counted the microbial colonies in colony-forming units (CFU) per 10 cm x 10 cm surface (100 cm<sup>2</sup>) and transformed them to log<sub>10</sub> by using SPSS version 21.0.

This research is conducted to identify microbial counts for four types of FCS: dining table tops, food trays, cooking pots, and kitchen faucets. Of these selected samples, it subsequently generated 36 samplings in triplicate (72 sampled sheets for FCS). Microbiological parameters for hygiene indicators in this research are *E.coli* and total coliforms bacteria. Both *E.coli* and total coliforms were able to be isolated from the same source of FCS sample. These tests were done to detect if the 24 units of FCS that went through regular cleaning procedures are truly clean.

#### 5. Results and discussion

This study determined the practicability measurement of hygiene practices in six food service providers. On each visit, microbial analyses of dining table tops, food trays, cooking pots, and kitchen faucets were evaluated. Each surface was swabbed in the area of 10 by 10 cm<sup>2</sup> to detect the presence of *E.coli* and total coliforms. The sampling activities were performed during the food service operation in order to increase the likelihood of finding actual hygiene surface or environment. The approximate surface areas sampled were 100 cm<sup>2</sup> (10 cm x 10 cm). In the next investigation process, this study ensures that only clean utensils submitted for swab purposes. After confirming each surface are clean visually, investigation continues.

All positive tests according to Mulec et al. (2012) is noteworthy as they indicate the presence faecal origin at significant levels on the test surfaces. While scholars highlighted that there is no standard food safety standard for surface cleanliness based on bacteria detection (Casey & Alach, 2004), it is also not explicitly stipulated in Malaysia Food Act 1983. Therefore, this research decided the pass-fail threshold

of  $1.0 \log_{10}$  CFU/cm<sup>2</sup> (250 CFU/100 cm<sup>2</sup>), following Cunningham et al. (2011). According to Cunningham et al. (2011), other previous scholars such as Buckalew (1996), Malik et al. (2003), Dancer (2004), Cooper et al. (2007), and Lewis et al. (2008) had also determined any greater value than  $1.0 \log_{10}$  CFU/cm<sup>2</sup> for total coliforms is not suitable for use in food preparation.

### 5.1. Microbial analyses (Prevalence of *E.coli* and Coliforms)

Following incubation for 24 hours, the observations on RIDA® count plates showed colonies presence with one or two colors. Typical purple to indigo colonies designates *E.coli* whereby the light blue colonies mean total coliforms. As shown in Table 1, this study collected swabs of dining table, food tray, cooking pots, and kitchen faucets in six food services establishments. FCS samples with total coliforms counts exceeded of  $1.0 \log_{10}$  CFU/cm<sup>2</sup> were considered to fail the hygiene test (Cunningham et al., 2011).

Our findings revealed that the microbial counts of coliform were prevalent, indicating hygiene issues exist in every food service establishments. The swab test recorded about 70% of the FCS contaminated with coliforms, in which 54% of them was highly affected. Thus, they are not suitable for use in food preparation. On the other hand, the sampled FCS did not contain *E.coli* except for cooking pots in Food Service 6. The result shows serious hygiene issues for the same unit of sample. Table 1 showed the widespread of unhygienic FCS in all food service establishments. Specifically, the results implied that washing and sanitation steps have not been effective in almost all FCS.

Table 1. Critical microbial counts for *E.coli* and coliform on sampled food-contact surfaces

Sample Area	Counts ( $\log_{10}$ CFU/cm <sup>2</sup> )											
	FoodService 1		FoodService 2		FoodService 3		FoodService 4		FoodService 5		FoodService 6	
	EC	TC	EC	TC	EC	TC	EC	TC	EC	TC	EC	TC
Dining table tops	-	++	-	++	-	+	-	++	-	++	-	++
Food trays	-	++	-	++	-	+	-	++	-	++	-	-
Cooking pots	-	-	-	+	-	-	-	++	-	++	+	++
Kitchen faucets	-	-	-	-	-	++	-	+	-	-	-	-

Note. - non detection for *E. coli* (EC), Total Coliforms (TC); + TC <  $1.0 \log_{10}$  CFU/cm<sup>2</sup>; ++ TC ≥  $1.0 \log_{10}$  CFU/cm<sup>2</sup>



Fig. 3 illustrates the results of hygiene level for each sample of FCS. Overall, the results are less than desirable hygiene levels. Dining table tops contained 58% faecal contamination; 33% in food trays; 33% in cooking pots, and 8% in kitchen faucets. FCS contaminated samples in the areas of six food services indicate that foodservice operations need to improve the FCS cleanliness. The microbial counts for table tops and kitchen faucets are contradicted with the study of Rodriguez et al. (2011), in which they obtained the opposite results. Nevertheless, the overall high contamination on the FCS corroborates with Lahou et al. (2012) findings.

On the other hand, these findings here are shown to be applicable for regular monitoring as well as to find critical points where special concentration for the quality of food safety. This is in agreement with the studies of Salo et al. (2006) and Mulec et al. (2012). Thus, the FCS requires improved cleaning activities. These must be more elaborated than simple cleaning using hot water or disinfectant spray after removal of the surface residues. In addition, triangulation measures of hygiene practices are crucial in assuring the quality of food safety (Mazni et al., 2013), and one way is through periodic inspections for verification purposes. Accordingly, the verification of hygiene level creates the curiosity and excitement of the inspection activities and participation among the food handlers (Faizi, Azaria, & Maleki, 2013).



Fig. 3. Microbial counts for coliforms are shown in  $\log_{10}$  CFU/cm<sup>2</sup>

## 6. Implications of the study findings

From the findings, there is evidence of *E.coli* and coliform on FCS. The presented data requires further attention from the respective food providers and authorities. Managers should take note on this matter since the contamination of *E.coli* and coliform are indicative of faecal contamination. Some pathogenic strains of *E.coli* responsible for a syndrome are closely related to diarrheal, which is caused by serious food poisoning in humans. In relation to this, proper hygiene practices shall enhance the operations of food service. To enhance safety in the food service industry; regular hygiene measures of microbial counts should be considered in earnest. While traditional mechanisms using culturing in a nutrient medium are tedious and require a skilful person, dependant on the authorities, and yet the tests do not cover FCS, the user-friendly microbial kit used in this study may be the solution for ordinary food



handlers to preserve food safety for human consumption. The use of the user-friendly microbial kits in monitoring of FCS increased food operators' participation and hygiene pro-social behaviour because it's practical and convenient. Despite the size or type of the food service operations, the proposed measurement has the potential to inculcate good food self-hygiene practices, clean environment, and health safety culture as a priority in the workplace.

More importantly, lapses in hygiene practices are usually due to carelessness, oversights, and lackadaisical attitudes. Most workers are paid a monthly wage, and have no incentive to exceed requirements. The microbial test scheme proposed in this work is easy to use, inexpensive and provide empirical data that are easy to observe and interpret. By implementing regular hygiene checks using a scientific method such as this, it is expected that the workers will now feel the need to better observe hygiene practices. Thus, the method may bring about a change in attitude and ensure better compliance to food safety guidelines.

This study has wide implications in terms of academic contributions, particularly in food safety literature review. These microbial investigations were successfully conducted on solid materials using rapid indicator swab kits. Over the years, the use of the swab kits was only used in the detection of microbes in water mediums. In addition, this study can help pave the way for improvements on Food Act and Regulations in Malaysia. So far the Malaysian Food Act 1983 specifies the food and water standards for coliforms organism and *E.coli*, but it does not account for solid materials such as FCS. Yet, FCS has often been suggested as another important factor in food safety problems. In addition to this, RIDA® count plates (R-Biopharm AG, Germany) is only an example of rapid indicator kits issued in Germany. The invention may be replicated and produced in Malaysia to save costs. The findings of this study have offered the local authorities, especially the Ministry of Health and the Municipality, with important information so that these authorities could create and implement effective strategies to encourage preventive measures in handling hygiene issues.

## **7. Limitation and future research**

This study revealed the shortcoming of the method used. During the visit, the researchers were not allowed to enter the kitchen side simply because we are not immunised. As a result, the swab activities can only be done on kitchen equipments brought out to the dining hall. The investigation did not include heavy-duty or large equipments such as tilting fry-pans, steam-jacketed kettles, steamers, and reach-in refrigerators. It is recommended that future studies should take these equipments into account as they would have higher potential of food residual due to the complicated cleaning process. Another area worth studying is the recording of time taken for the investigation and comparing it with other studies done on FCS. This study provides the opportunity for future researchers to further complement the present findings. There are more areas of interest to study in terms of training on effective cleaning and sanitizing.

## **8. Conclusion and recommendation**

The microbial test scheme such as RIDA® COUNT test plate that is proposed in this work represents a valuable tool for monitoring hygiene practices at the food service premise; especially to detect the cleanliness of the FCS that has underlined the foodborne outbreaks in the country for years. As the design of this kit filled the gap of the hygiene expectations, this study recommends it for use in promoting food safety in accordance with the requirements of the Food Act 1983 and food hygiene regulations 2009. By including a microbial test that is easy to use and interpret, weaknesses in hygiene practises can be swiftly pinpointed and allowing for immediate correction steps to be taken. Such regular monitoring is expected

to prompt a change in the workers' attitude and behaviour to ensure better compliance. It's obvious that the use of the scientific kit can be commercialized as a multi-purpose test-kit.

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