

**CHROMATOGRAPHIC PROFILING OF
SANITIZERS FOR FORENSIC INVESTIGATION**

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CHROMATOGRAPHIC PROFILING OF SANITIZERS FOR FORENSIC
INVESTIGATION

by

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for the degree of
Master of Science (Forensic Science)

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CERTIFICATE

This is to certify that the dissertation entitled Chromatographic Profiling of Sanitizers for Forensic Investigation is the bona fide record of research work done by Patsy Petrus J Sipayang during the period from February 2020 to September 2020 under my supervision. I have read this dissertation and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted as partial fulfilment for the degree of Master of Science (Forensic Science).

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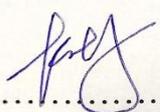
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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledge. I also declare that it has not been previously for concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research and promotional purposes.


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LIST OF ABBREVIATIONS AND SYMBOLS

ABHRs	Alcohol-Based Hand Rubs
ABHS	Alcohol-Based instant Hand Sanitizers
CDC	Centers for Disease Control and Prevention
cm ⁻¹	Wavenumber
Covid-19	Coronavirus Disease 2019
°C	Degree Celsius
<i>et al.</i>	<i>et alia</i> – “and others”
FTIR	Fourier-Transform Infrared Spectroscopy
GC	Gas Chromatography
GC-FID	Gas Chromatography-Flame Ionisation Detector
GC-MS	Gas Chromatography-Mass Spectrometry
H1N1	Influenza A
ICU	Intensive Care Unit
IHS	Instant Hand Sanitizer
IPA	Isopropyl Alcohol
mg	milligram
mL	millilitres
N ₂	Nitrogen
PCA	Principal Component Analysis
PC1	First Principal Component
PC2	Second Principal Component
PDMS	Polydimethylsiloxane
QACs	Quaternary Ammonium Compounds
SARS	Severe Acute Respiratory Syndrome
Sdn Bhd	Sendirian Berhad
SPME	Solid-Phase Microextraction
WHO	World Health Organization
μL	Microliter
μm	Micrometre
x	Multiply

PROFIL KROMATOGRAFI SANITIZER UNTUK PENYIASATAN FORENSIK

ABSTRAK

Kebersihan tangan merupakan syarat penting untuk menjaga kesihatan. Pada masa ini, pensanitasi telah digunakan untuk rutin kebersihan tangan selain kaedah tradisional menggunakan sabun dan air. Oleh kerana semakin banyak pensanitasi diperkenalkan ke pasaran, ada keperluan untuk mengetahui sama ada pensanitasi memenuhi piawai yang tepat. Oleh itu, kajian ini diusulkan untuk membezakan atau menentukan kepelbagaian. Kajian ini bertujuan untuk membezakan pelbagai pensanitasi yang dikumpul dari pasaran tempatan menggunakan teknik kromatografi. Dalam kajian ini, sampel pensanitasi telah diekstrak dengan kaedah Pengekstrakan Mikro Pepejal Fasa (SPME) dan kemudian dianalisis dengan Kromatografi Gas-Pengesan Pengionan Api (GC-FID). Seterusnya, Analisis Komponen (PCA) dan Analisis Gugusan Berhieraki (HCA) telah digunakan untuk memproses data kromatografi untuk memerhatikan sebarang pengelompokan atau pembezaan antara sampel. Daripada profil kromatografi, kesemua sampel menunjukkan kehadiran sebatian alkohol sebagai kandungan utama. Berdasarkan plot skor PCA, plot penyebaran 3D dan dendrogram, dua kelompok utama dapat diperhatikan dan terdapat empat sampel yang tidak termasuk dalam kedua-dua kelompok tersebut. Keempat-empat sampel pensanitasi ini berkemungkinan mengandungi maklumat kromatografi yang berbeza daripada sampel-sampel lain yang diuji dalam kajian ini. Kesimpulannya, kajian ini telah berjaya menjana profil pensanitasi daripada pelbagai jenama dan membezakan sampel tertentu berdasarkan profil kromatografi masing-masing.

CHROMATOGRAPHIC PROFILING OF SANITIZERS FOR FORENSIC INVESTIGATION

ABSTRACT

Hand hygiene had been an important requirement to maintain good health. Nowadays, sanitizer had been used for hand hygiene routine besides the traditional method using soap and water. As more sanitizers were introduced into the market, there is a need to know whether the available sanitizers are meeting the right standard. Therefore, this study was aimed to discriminate the various sanitizers collected from local markets using chromatographic technique. In this study, hand sanitizers samples were extracted by Solid-Phase Microextraction (SPME) method and subsequently analysed by Gas Chromatography-Flame Ionisation Detector (GC-FID). Then, Principal Component Analysis (PCA) chromatographic and Hierarchical Cluster Analysis (HCA) were used to generate the data to observe any clustering or discrimination among the samples. From the chromatographic profiles, all samples showed the presence of alcohol compounds as the main ingredients. Based on the PCA score plot, 3D scatter plot and dendrogram, two main clusters were observed and there were four samples that did not include in both the clusters. These four hand sanitizer samples could have carried different chromatographic information from other samples tested in this study. In conclusion, this study had successfully generated the hand sanitizer profiles from different brands and differentiated certain samples based on their respective chromatographic profiles.

CHAPTER 1

INTRODUCTION

1.1. Hand Sanitizer

Hand hygiene is an important requirement for a healthy lifestyle. Human uses their hands in almost every single tasks such as eating, washing and many more. Moreover, the maintenance of hand hygiene could help to prevent transmission of infectious bacteria or virus to our body, protecting our health system (Hugonnet *et al.*, 2020). Children and generic groups needed to be taken extra care and cleanliness, particularly due to their relatively lower immunity against bacteria or viruses.

Our surrounding may seem clean but most of the microorganisms could not be seen by the naked eyes. In view of this, it is essential to carry the cleaning before and after we eat or touch something. Infections that are obtained in work and school could easily be transmitted to other family members through contaminated hands. The importance to keep our hands from bacteria or viruses that may lead to certain diseases could be prevented by washing with water and soaps or using sanitizers as an alternative. Recently, the whole world was impacted by the Covid-19 coronavirus outbreak, propelling the usage of sanitizers by our population. To prevent the spread of Covid-19, it was recommended that the public should frequently clean their hands, suggested by the Ministry of Health (2020). With the grows of hand sanitizer ingredients and application of illegal alcohol such as methanol and 1-propanol as substitute of ethanol and isopropanol, the contents of these sanitizers are in question. It is important to ensure that the sanitizers are effective in cleaning up a surface or hand, in fact, they might cause harm to the users if they are not following the correct formulations recommended by the World Health Organisation (WHO).

Hand sanitizer had been used as an alternative for handwashing. It is usually used when water is not available. They can be found mainly in two forms which are liquid and gel (Hayat, 2017). The active ingredients that were usually used as the main ingredients for hand sanitizers are either ethanol or isopropanol. The active ingredients of the hand sanitizer will greatly affect their antimicrobial property. The inactive ingredients that can be found in hand sanitizer includes glycerine, propylene glycol and polyacrylic acid (Moses *et al.*, 2013). Hand sanitizer had shown great demands in many different fields such as health care facilities, school, food processing and any other areas (Aiello *et al.*, 2008; Allegranzi and Pittet, 2007; Bloomfield *et al.*, 2007). WHO had recommended the use of hand sanitizer as their preferred method to clean hands especially in health care facilities where patients are constantly being treated (Pickering *et al.*, 2013; World Health Organization, 2009b). It is also preferred by the hospital as compared to antiseptic soaps for hand hygiene and to apply before a surgery treatment is carried out (Boyce *et al.*, 2000).

Besides alcohol as the basic ingredients for hand sanitizer, quaternary ammonium compounds (QACs) and triclosan could also be used as an ingredient of sanitizer (Hayat, 2017). Alcohols are also known as broad spectrum disinfectant, help to kill bacteria as well as fungi. QACs is an active surfactant includes either benzalkonium chloride or benzthonium chloride and also broad-spectrum antimicrobials that can be found in domestic products. Furthermore, triclosan usually used as an antibacterial and could be found in products such as deodorant soaps, mouth washes and toothpastes.

The hand sanitizer could be profile based on their brands using GC-FID. Chromatographic profiling of hand sanitizers was done by using GC-FID method.

Solid-phase microextraction (SPME) was used as pre-treatment in this study. SPME is always used during headspace analysis (Gürbüz *et al.*, 2006; La Guerche *et al.*, 2006; Ong and Acree, 1999). Instead of diluting the extract, it is possible to directly dilute the sample, saving the time of analysis (Fan and Qian, 2005; Martí *et al.*, 2003). This technique could minimise sample preparation time for further analysis (Plutowska and Wardencki, 2008). This technique mostly relies on the different thickness of fibre coatings that were used for the extraction of sample instead of doing a series of sample dilutions. The limitation of this technique is the availability of the fibre with different thickness.

The task of preparing solutions with different concentrations of odour compound can be time-consuming (Deibler *et al.*, 1999, 2004). These can be solved by using different split ratios of the carrier gas where the injector can be split or splitless depending on the samples being analysed. The initial optimisation of the SPME may be required to condition before it was used for an experiment. This is due to the compositions present in the sample might change, depending on the fibre used, temperature, time used to extract the samples and the volume of the samples needed for the experiment (Fan and Qian, 2005). In this study, different brands of hand sanitizers were analysed and they were profile based on GC-FID data.

1.2 Problem Statement

Covid-19 pandemic had become a serious threat to human health. The need to prevent the transmission of this disease from person to person to be done in order to break the chain. This pandemic is a global concern where government in different countries had taken this issue seriously by making moves such as lockdown, social distancing and focus on enhancing personal hygiene. When the pandemic arises, face

masks and hand sanitizers are needed especially by hospitals to prevent the risk of getting the disease when treating patients. This can help from transmission of the disease from one surface to another. When the bodily fluids of an infected person was present on a surface, it can risk others whom are not infected to become infected with the disease.

Hand sanitizer is one of the essential needs during pandemic. At the early stages of pandemic, hand sanitizers were greatly depleted from the market due to high demands. During the pandemic season, more hand sanitizers with different brands were introduced to the market. Therefore, this study was focused to discriminate the hand sanitizers collected from local markets. The chromatogram profiles that recovered from different brands of hand sanitizer were compared to detect whether it could be differentiated between them which could aid in forensic investigation of fraud and misuse of hand sanitizers.

1.3 Aim and Objectives

The aim of this study was to discriminate the hand sanitizer collected from local markets based on their chromatographic profiles. The objectives of this study were as follows:

- i. To determine the composition of different brands of hand sanitizers with SPME-GC-FID method.
- ii. To discriminate different brands of hand sanitizers based on multivariate analysis.

1.4 Significance of Study

Hand sanitizer had been in high demand during the Covid-19 pandemic. The emergence of newly and different brands of hand sanitizers can be seen throughout the world. These new introduced hand sanitizers were bought by many people and in high amount. Therefore, the need to profile these newly introduced hand sanitizers obtained from local markets were crucial. The profiling done in this study could also aid in discriminating the samples and to trace the samples back to their respective source if they are encountered as forensic evidence.

The manufacturer of these hand sanitizers might resort to fraud by manufacturing hand sanitizers, not complied as formulations published by WHO (World Health Organization, 2015). To make matter worse, the labelled on the hand sanitizer might not be coincide with the appropriate amount of alcohol in them. Those who bought the product thinking it can help them to reduce the risk of getting Covid-19 might even be in high risk due to the hand sanitizers used. This can be very dangerous for them and also the people around them due to the lacks of alcohol concentration in the hand sanitizer. According to World Health Organization (2009b), the appropriate amount of alcohol in hand sanitizer should be between 60% to 95% for them to be effective against virus. Therefore, the need to profile these hand sanitizers is of great importance. Additionally, it is hoped that the detection of hand sanitizers from any surfaces from an individual or place could aid in forensic investigation to link between the suspect, victim and the crime scene.

CHAPTER 2

LITERATURE REVIEW

2.1 WHO Formulations

According to WHO (2015), the recommended sanitizers' formula include 96% ethanol, 3% hydrogen peroxide, 98% glycerol and sterile distilled water. Besides, it was also recommended to replace 96% ethanol by 99.8% isopropyl alcohol and other reagents remain the same. Alcohol-based hand rubs (ABHRs) had been commonly used as hand sanitizers in many countries (Cindy White *et al.*, 2003). Some active ingredients such as povidone-iodine, quaternary ammonium compounds (QAC), chlorhexidine or triclosan may be added in the formulation for hand sanitizers to increase their effectiveness. Povidone-iodine can be as an antiseptic for skin disinfection, QAC can be used against gram-positive and gram-negative bacteria and triclosan also works well with gram-positive bacteria. Alcohols had been the main composition in the production of hand sanitizers, as they can be used for protein denaturation and the ideal concentrations to be an effective sanitizers are ranging from 60% to 80% (Ayliffe *et al.*, 1988; Larson, 1999; Reybrouck, 1986). If the concentrations of alcohol is higher than 80%, it was said to be less effective because protein needed water to undergone denaturation (Block, 1977). On the other hand, less contents of alcohol might not be able to facilitate the denaturation of protein. Alcohol can be used to kill bacteria and fungi that may be present in food or other surroundings. However, it may not be applied to some viruses, bacterial spores and protozoan oocytes (Block, 1977).

The ABHRs were compared with 60% isopropanol in term of their respective effectiveness (European standard, 2013). Both ethanol and isopropanol are quite

similar in their behaviours, but some studies reported ethanol to be more effective against viruses compared to isopropanol due to stronger and broader viricidal activity (Dharan *et al.*, 2003; Kramer *et al.*, 2002). The formulation in the forms of solution and gels was studied. It had been found that ethanol in solution form was better than ethanol in the form of gel by its effectiveness, unless ethanol gel had undergone some formulations and had been tested. This is due to the fact that the solutions form meet the EN 1500 requirement within 30 seconds of applications compared to gel form. Both of the studies focus on testing of solutions and gel sanitizers. (Dharan *et al.*, 2003; Kramer *et al.*, 2002).

2.2 Effectiveness of Sanitizers

Direct contact between individual can transmit the virus easily and this had been proved in a laboratory simulation (Bean *et al.*, 1982). Hand-to-hand contact can be resulted in transmission of virus (Bean *et al.*, 1982; Reed, 1975). However, there are some environmental factors that need to be considered, such as relative humidity and moisture in our hands that will also influence the survival of the virus (Irwin *et al.*, 2011; McDevitt *et al.*, 2010).

According to Ochwoto *et al.* (2017), an effective hand rub could reduce the microbes on the hands by 50%. Four out of fourteen hand sanitizers that were studied in Kenya market had shown effectiveness above the 60% Isopropyl alcohol (IPA) reference standard (Ochwoto *et al.*, 2017). These four sanitizers are different based on their active ingredients containing alcohol with chlorhexidine, ethyl alcohol with hydrogen peroxide, 70% denatured alcohol with 70% isopropyl alcohol and 70% ethanol, respectively. However, seven out of fourteen hand sanitizers were found less effective towards the bacteria such as *Escherichia coli*, *Staphylococcus aureus* and

Pseudomonas aeruginosa. In this study, ethanol-based gel formulations were found more effective as compared to isopropyl alcohol-based gel formulations which showed different result from other studies.

The effectiveness of hand hygiene products had been conducted by Grayson *et al.* (2009) using 20 volunteers. The virus that had been used to compare the effectiveness between four different types of hand hygiene products were influenza A (H1N1). The hand hygiene mentioned above are soap and water as well as three different types of sanitizers containing alcohol. A significant decrease of the virus can be observed after the three sanitizer products were used. Moreover, sanitizers containing alcohol demonstrated small but significance decrease if compared to soap and water due to the criteria of ethanol being able to kill bacteria or viruses but the researcher did not mentioned which is the best out of the three sanitizers used.

Previous studies also reported that alcohol products can be affected by method of delivery in terms of their cleaning effectiveness. This had been reported by Kramer *et al.* (2002) where liquid was more effective compared to gel due to faster rate against virus or bacteria and shorter dried period. The friction during hands washing with soap and water as well as sanitizers was one of the interest to determine the removal of virus and bacteria from hands in this study. Butz *et al.* (1990) also studied about the bacterial reduction after the use of traditional method of water and soap, antiseptic soaps where triclosan or chlorhexidine gluconate was added, as well as hand wipes containing alcohol (30% w/w alcohol). The result showed that both traditional method of soap and water as well as cleansing with alcohol hand wipes gave the similar effect, but the hand wipes were more convenient especially when there was lack of water supply. The limitation in the study was that the hand wipes used contained low concentration of alcohol and the viruses were not been tested.

To assess the effectiveness on cleaning, the environmental factors, host, method of application of the test product and different instruction from the manufacturer on how to use or handle the products may cause some variations to the result obtained (Elaine L. *et al.*, 2012). The time for the virus to persist in an environment and on the host should be tested. The appropriate method to use the product might influence the effectiveness of the products. The controlled laboratory may not produce an accurate result of the viral viability in infection that occur naturally as well as the definite viral loads in a clinical setting. However, the study had met all the requirements needed to be used in health care setting despite the factors that need to be considered. The products that contain 60%-90% of ethanol had been proven to be effective against viruses.

2.3 Preventive Measure

Similar to Influenza A (H1N1) pandemic that occurred in 2009, two measures had also been used in the present Covid-19 pandemic includes respiratory hygiene and proper handwashing technique (Aledort *et al.*, 2007). The measures mentioned were effective especially as non-pharmaceutical public health interventions. The pandemic in 2009 had a large impact on health-related perceptions and behaviour of an individual where self-protection becomes crucial to protect oneself and the people around their surroundings. According to Agüero *et al.* (2011), approximately 80% of respondents had adopted one of the two preventive measures during the H1N1 pandemic. These self-protection behaviours could be observed to be persisted by most of the population even after the pandemic. However, there had also been a slight decrease of using these preventive measures after the declining phase of these pandemics.

According to Agüero *et al.* (2011), the respondents whom purchase face masks were 3.9% in the first wave of the 2009 H1N1 pandemic while 1.9% on the second wave. It was lower as compared with other countries for the pre-pandemic phase where Malaysia shown 8% followed by European countries at 7% and USA at 5% (Goodwin *et al.*, 2009; Steel Fisher *et al.*, 2010). Previous study done by Lau *et al.* (2010) showed a prevalence differences in wearing face masks as preventive measures for Asian where the range of the ranking was between 22% and 89% which was higher compared to Spain which only shown 7%. These percentage could also be seen for social distancing where only 4% of the Spanish population was observed to avoid crowded places which was lower as compared to Asian countries where the population showed the percentage of 55% avoidance from crowded places (Lau, Griffiths, Choi, and Tsui, 2010). These behaviours might be related to the public concern where severe acute respiratory syndrome (SARS) was a great threat before H1N1 arises (Tang, 2003).

The study done by Agüero *et al.* (2011) focused on the perceptions and beliefs which include the perceived susceptibility towards the H1N1 pandemic, the effectiveness of the preventive measures used during the pandemic and the usefulness of information obtain from the government for the health behaviour prevention. The credibility of information given by the government was crucial in the adoption of preventive measures (Cava *et al.*, 2005). The government must act swiftly to prevent the diseases from spreading by introducing and emphasizing the used of preventive measures to stop the chain of pandemic from becoming even worse.

2.4 Settings

There are many different settings where sanitizers can be used. Different target groups are involved in different settings. The following section covers four different

settings, namely there are home, school, health care and military. Home setting mainly involves parents as well as caregivers of the children. School setting involves students and teachers. Health care setting involves with doctors, nurses and patients while military setting focuses with the recruits.

2.4.1 Home Setting

Transmission rates between children was considered high due to many reasons (Goldmann, 2000). These reasons include exchanging bodily fluids between them when in contact which can occur during play time, children that potentially suffer from contagious disease may not be separated from other children and the staff responsible for taking care of these children may also face some challenges with the children's and surrounding hygiene (Goldmann, 1992, 2000). These children that may have been infected from other children may transmit them to their other family members and the cycles becomes continues to get bigger (Hall *et al.*, 1976; Haug *et al.*, 1978; Rodriguez *et al.*, 1979). The transmission can be easily done through contaminated hands (Ansari *et al.*, 1991; Butz *et al.*, 1993; Daniel and Musher, 2003; Dennehy, 2000; Gwaltney and Hendley, 1978; Gwaltney *et al.*, 1978; Hendley *et al.*, 1973; Keswick *et al.*, 1983). A simple way to avoid such transmission is by washing hands with water and soaps. According to Niffenegger (1997) , there had been a decrease in illness rate in child care centres after the handwashing intervention was introduced. There was a study in Pakistan where diarrhoea incidence in households had been reduced due to a proper hygiene being introduced (Luby *et al.*, 2004).

Families that have children and parents that hired caregivers due to work use hand sanitizers with their advantages on the ease of use as well as for the purpose of maintaining good hygiene (Sandora *et al.*, 2020). Most of the caregivers use hand

sanitizer due to their availability and cheap. The hand sanitizers can kill bacteria and viruses present on the hands and also contains emollients which tend to be gentler on hands compared to water and soaps (Sandora *et al.*, 2020). However, there are some inconsistencies reported, as some of the caregivers might wash their hand after going to the bathroom and changing diapers sometimes not after wiping the children's nose. In view of this, it was found difficult to investigate the effectiveness of hand sanitizer in the study. In fact, the study was also limited by the documentation from the caregivers instead of using microbiological method to analyse the infection in relation to the amount of hand sanitizer applied during the study (Sandora *et al.*, 2020).

In the same study, the commonly found virus in gastrointestinal illness (GI) known as rotavirus cannot be simply killed using the common method of washing hands with soap and water (Sandora *et al.*, 2020). However, the use of hand sanitizer containing alcohol can effectively kills the virus. Therefore, it is an utmost important for caregiver to use hand sanitizers when dealing with children to prevent any diseases that may transmit from the caregiver to the children and *vice versa*.

Above described factors had contributed to the use of sanitizers in home setting. Moreover, with the recent Covid-19 outbreak, the demand and use of hand sanitizers had achieved the highest levels with the aim to clean all the surfaces potentially to be contacted by any individual. At the early stage of the outbreak, such sanitizers had even sold out in most of the places, including in Malaysia.

2.4.2 School Setting

The elementary school group was prone to the infection caused by microorganism (Guinan *et al.*, 2002; Neuzil *et al.*, 2002). These outbreaks had resulted in an increased in absenteeism in both teachers and students, healthcare expenditures

for both teachers and students also increased which in turn caused the students' learning environment to decrease as well (Neuzil *et al.*, 2002). It has been estimated by the United States CDC that the average school-aged child had been absent for one week annually from school were due to some illness-related absenteeism in 1995.

The effectiveness of handwashing and promotion of proper hand hygiene had been proved to be effective. On contrary, some studies that had been carried out in school settings prove that routine handwashing had been lacking (Guinan *et al.*, 1997; Pete, 1986). According to Guinan *et al.* (1997), the handwashing technique using soap and water can be observed from the range of 8 to 29 percent in the school-aged children. These low percentage may be due reasons such as insufficient time during the day and the difficult to access proper washing facilities in the school environment.

The attempt to fight the obstacles of having to do routine handwashing in the school environments leads to the use of alternative hand hygiene routine which is hand sanitizers (Meadows and Saux, 2004). The concern regarding this matter is that programs can be carried out where the evidence of effectiveness is not present in school environment. So, it is of utmost important to investigate the evidence that can be found regarding the effectiveness of antimicrobial rinse-free hand sanitizer programs that can help to reduce the absenteeism of students cause by communicable diseases.

According to Cramer and Carol (1999), it is important to prevent any infectious diseases from transmitting from one student to another as it had been a concern for parents of those children. Two of the most common infectious diseases that can be found in school environment are respiratory and diarrheal illnesses. These infectious may happen in low rate but sometimes an outbreak could occur, leading to an increase of absenteeism and public health authorities may need to interfere when these cases do

happen. Hands are the main cause for transmission from one to the other to occur. Therefore, the right technique for hand hygiene need to be implement as a defence against the risk of transmission (Early *et al.*, 1998; Guinan *et al.*, 2002; Larson, 1988).

2.4.3 Health Care Setting

The routine handwashing had been a basic measure in the world of healthcare where it has been recorded at the mid-nineteenth century (Best and Neuhauser, 2004; Embry, 2002). A Hungarian obstetrician named Ignaz Semmelweis applied routine handwashing in addition with chlorinated lime for the staff in the maternity ward to reduce the outbreak of puerperal fever and proved to be reliable as the mortality rate had been reduced significantly from 13-18 percent to 2 percent. These significant findings had cause implementation of routine handwashing by hospitals ever since (Larson, 1988, 1999).

In health care settings such as hospital and clinics, it is necessary to have good hand hygiene. Therefore, washing hands with soaps and water is of importance. In certain instances, the requirement of a sink to carry out the task may not be sufficient in term of time and accessibility. It is easier for the hospital to provide alcohol-based sanitizers to the personnel in the health care settings. Sanitizers can be placed beside the patients' bed and door for easy access.

Use of alcohol-based hand rubs (ABHRs) was preferred as compared to antimicrobial soap due to their effectiveness and less time needed (Miller *et al.*, 2006; Didier Pittet *et al.*, 2000). It had been a great tool to increase the compliance of health care workers. Some studies reported that the use of ABHR had variations of three and 144 times per hour for the compliance of healthcare workers (Didier Pittet, 2001; Didier Pittet *et al.*, 2000). There was reported with a range of 0.13-6.25 rubs per

patient, per nurse and per hour in the adult intensive care units (Girou and Oppein, 2001; Hugonnet *et al.*, 2020; D Pittet *et al.*, 1999) .

Some studies reported on the decrease of blood concentration, probably due to the exposure of ABHR. It had been observed that the absorption of ethanol after the use of hygienic and surgical hand disinfection had achieved 31.5 mg and 154.2 mg, respectively (Kramer *et al.*, 2007). Different organs might have also distributed different concentration of ethanol throughout the body system due to their solubility in water. Organs such as brain, liver, lungs had the highest concentration of ethanol which might be due to the inhalation rate as well as tidal volume with an efficiency of 30% to 80% (Standards, 2006; Tardif *et al.*, 2004).

Hautemanière *et al* (2013) used wooden dummy to study the exposure of ethanol through inhalation. The result showed that ethanol value was higher in wooden dummy as compared to the volunteers. The rate of evaporation was found faster on human skin than the wooden dummy. ABHR will stop only when the skin had dried according to the hand rub protocol. This could be related with the evaporation time and amount of ethanol used on both skin and wooden dummy. The total evaporation time might also differ due to the mechanical friction by volunteers that could subsequently increase the temperature and decrease the time of evaporation. On the other hand, this might not be the case for the wooden dummy placed on a hot plate with constant temperature. Alcohol was easily volatilised due to increase in temperature, facilitating the evaporation process.

In the Intensive Care Unit (ICU), there are high risk for patient to face with cross transmission. Therefore, compliance within the hospital and health care setting must be carried out. They must have access with good hand hygiene supplies. The supplies such as hand sanitizer must be placed where it is convenient for the workers

to access. According to Bischoff *et al.* (2000), the compliance could be done by providing alcohol based hand rub dispenser. Firstly, a ratio of 1 dispenser per 4 beds followed by 1 dispenser per bed was investigated. The results showed that an increase of compliance was recorded due to easy access of dispensers. It was also reported that compliance could be achieved where each health care personnel was provided with dispensers and bottles that contain alcohol-based solutions. Usually, the non-compliance for the hand hygiene was due to time-consuming and also the strategy for disinfection using no water. In fact, hand washing is the standard used in healthcare settings. However, the alternative method using hand rub solution was promoted to improve hand hygiene (Hugonnet *et al.*, 2020).

Non-compliance to good hand hygiene practice could cause adverse effect to patient (Larson, 1999). This is particularly importance in ICU where high risk infection could occur. A clinical trial had been carried out to compare the adverse effect that could occur between unmedicated soap and alcohol based hand gel (Boyce *et al.*, 2000). The result showed that the unmedicated soap could cause irritation and dryness of skin while alcohol based hand rub gel did not show any adverse effect to the skin. The ingredient such as emollients potentially found in the hand rub and hand lotion that might contribute to the absence of adverse effect on the skin. Health care workers that use water and soap to wash their hands had detected with 52 colony forming units on their fingertips. However, it was noted that there was limitation in this study as no control group was used. To conclude, good hand hygiene is a high priority and should be implemented in every hospital.

According to study done by Ataei *et al.* (2013) in three hospitals in Iran, there had been some lacking to conduct proper hand hygiene routine. Only 62% of the public hospitals had at least the product for hand hygiene. These products consist of water

and soap and an alcohol dispenser. Unlike public hospitals, private and teaching hospitals have hand-wash basins located at all the patient rooms. However, public hospitals only had a single hand-wash basin available in each wards and limited supply of paper towels were provided. Alcohol dispensers were found in places such as nursing trolleys or fixed on the wall of patient rooms, but the location did not cover all the necessary points where alcohol dispensers were needed.

The hospitals in the study have different type of policies, economies and infrastructure (Ataei *et al.*, 2013). Private hospitals had better facilities if compared with teaching and public hospitals. However, it has lowest compliance towards a proper hand hygiene routine which may be due to lower incomes and hierarchical management system being lacking which caused the staff in the private hospitals to have less motivation to attend training courses that were needed to conduct a good hygiene routine. Further studies were suggested to investigate the reasons of lack of compliance in private hospitals. The less compliance to use alcohol rub might be due to their lack of knowledge added with the concern of the drying effect of alcohol on the skin. There had been no objections on religious level concerning the use of alcohol rubs as disinfection and permission to use them in medical field had been publicize widely.

The need to improve hospital infrastructure is also of important especially in public hospitals where most of the patients were being treated. The availability of alcohol rub must be sufficient to accommodate the staff and patients in the hospitals. However, the alcohol rubs provided at the appropriate location will only show a good result if the hospital workers motivated to use them. Other studies had mentioned better compliance rates if the alcohol rub were used compared to when only the handwashing facilities were being provided (Didier Pittet *et al.*, 2000; World Health Organization,

2009a). The need for an extensive training and education, promotion regarding the proper hand hygiene technique and regular evaluation towards the hospital workers might be the key to improve compliance towards hand hygiene routine. This may pose some challenge towards private hospitals which may not had an academic resource or learning materials.

Alcohol-based hand sanitizers were also said to be effective for H1N1 viruses and also help to reduce the respiratory illnesses such as an influenza infections (Aiello *et al.*, 2010; Grayson *et al.*, 2009; Cindy White *et al.*, 2003). However, during the 2009 H1N1 outbreak the use of alcohol-based sanitizers as a preventive measure was low due to lack of awareness towards the effectiveness of these products. This can be observed by the study done by Murray *et al.* (2009) about the usage of sanitizer during H1N1 pandemic which showed that less than 20% of people made good use of alcohol dispenser that can be found in hospitals.

The behaviours of the people not using the alcohol-based hand sanitizer need to be change gradually to fight against the diseases. When the actual health threat already present, a strategically-placed environmental cues to action could be an effective tool to promote adherence towards the use of alcohol-baes hand sanitizer (Janz *et al.*, 2002). The environmental cues had been proposed by the original Health Belief Model to motivate health behaviour (Hochbaum, 1958). Signs can be placed near the point of use such as the location where alcohol dispenser were placed which may act as a reminder towards the people to frequently use the alcohol-based hand sanitizer provided in the hospital for their own protection as well as for those around them (Naikoba and Hayward, 2001).

2.4.4 Military Setting

In a military training, health is a major concern especially when dealing acute respiratory disease (McDevitt *et al.*, 2010). The military recruits could expose themselves to the surroundings containing environmental and biological agents that may cause them to be more susceptible to diseases (Gdalevich *et al.*, 1999; Gunzenhauser, 2003; Lee *et al.*, 1995). This can be happened especially at the surroundings that are unfamiliar to the recruits. Moreover, the extensive outdoor training or climate change, stress due to sudden changes in living environment as well as communal living conditions where the recruits share their facilities could possibility decrease their body immunity (Billings and Billings, 2004; O'Neil Snoddy Jr. and Henderson, 1994). Therefore, any disease arisen during the training could affect the training intensity and time for the recruits, and the prevention of disease and its transmission must be taken into consideration. The military is required to get an effective method that is not costly to prevent such disease.

Hand hygiene is one of the approach that could be used to prevent diseases (Boyce and Pittet, 2002; Ryan *et al.*, 2001; Cindy White *et al.*, 2003). The implementation of hand hygiene in different settings such as school campus, naval basic training and health care had shown great decrease in diseases. From 1996 to 1998, the Great Lakes Naval Training Centre had implemented the hand hygiene where they need to wash their hands five times per day and the result showed a decrease in respiratory illnesses (Ryan *et al.*, 2001). However, the subjects also faced the same problems as in the healthcare where limited amount of sink and time have led to challenges in maintaining good hygiene. The author had suggested an approach as prevention method which was the alcohol-based instant hand sanitizers (IHS).

The previous studies had shown that hand hygiene was an efficient tool to be used by the military even though there are some constraints due to time and locations as in the health care settings (Kaplan and McGuckin, 1986; Whitby and McLaws, 2004). The effectiveness of IHS had been proven by undergoing multiple clinical studies (Dyer *et al.*, 2000; Guinan *et al.*, 2002; Hammond *et al.*, 2000; Terrence *et al.*, 2005; Catherine White *et al.*, 2001; Cindy White *et al.*, 2003). The Centres for Disease Control and Prevention had also recommended the use of IHS for hand hygiene but not when hands are soiled by visible objects such as soils (Boyce and Pittet, 2002). This had been advised during the revision of the Guideline for Hand Hygiene Settings in 2002. The guidelines clearly stated that IHS are more effective as standard for hand hygiene routine if compared to the traditional method of using antimicrobial soaps to remove bacteria (Boyce and Pittet, 2002; Dyer *et al.*, 2000; Guinan *et al.*, 2002; Hammond *et al.*, 2000; Terrence *et al.*, 2005; Catherine White *et al.*, 2001; Cindy White *et al.*, 2003).

For military setting, it had been suggested that hand hygiene regimen need to be developed properly. The uses of IHS by the military setting can be great advantage to reduce illnesses during training. A questionnaire analysis had been used by Sheehan *et al.* (2007) to determine the current knowledge on the efficacy of hand hygiene regimen. It showed an increase in both intervention groups where the IHS was introduced and the aftermath. From the study, the primary intervention group was reported with a higher illness rate as compared to the secondary intervention group, which did not compile with the expectation. It was expected that hand hygiene education should provide more compliance which in turn decrease the illness rate. Therefore, a more detail evaluation for the intervention activities need to be carried out to determine the effectiveness of IHS in military setting during its implementation.

A more thorough research needs to be studied to identify an effective educational strategy to help in promoting a good hand hygiene routine in military population.

In the study, the use of traditional method, namely soap and water has decreased during military training due to less amount of running water and soap available in the military environment. Indirectly, it led to a positive impact in the promotion of hand sanitizer. A follow-up study should be conducted to obtain more information on the impact, although it might be limited by the inability to randomise the group due to the military setting. Additionally, non-blinded primary and secondary intervention groups could also cause competitiveness and bias. Bias can also occurred when the trainees is more conscious of their health and resort to take a preventive action, affecting the result of the study (Sheehan *et al.*, 2007).

2.5 Forensic Perspectives

Alcohol-based sanitizer had been proven to be cost effective in breaking the chain infection. Note also that the cost to provide alcohol sanitizers and the dispensers could be expensive in some instances (Kampf, 2018). This is true during the Covid-19 crisis where all these sanitizers were sold in very high price as compared to the price before the high demand. Moreover, certain manufacturers might resort to decrease the alcohol concentration in the sanitizer or change the composition within the sanitizers which could result in failure to meet the criteria as hand sanitizer to effectively kill and remove bacteria. With such activities, the manufacturers would gain more benefit from their sales. Indirectly, such irresponsible acts could lead to unconscious spread of the bacteria, or virus with cases of Covid-19, where the users have assumed that the application of sanitizers have at least cleaned up a surface, but in fact no cleaning is possible with that sanitizer. It is one of the concerns from the perspectives of forensic

science as it could be considered as fraud, especially to the customer of buyers that had bought the hand sanitizer as their safety concern towards their health.

According to Miller *et al.* (2006), the effect of prolonged usage of alcohol-based instant hand sanitizers (ABHS) on blood alcohol concentrations was not yet been studied thoroughly. The study focus on a case report where 38 years old physician had shown negative blood ethanol level even though ethanol-based ABHS are used repetitively. However, previous study had been carried out on the dermal absorption of alcohol, specifically on isopropyl (Turner *et al.*, 2004). The study confirmed that isopropyl alcohol could be absorbed through the intact skin of adult humans. It was important to study such effect, as to recommend the uses of sanitizers to those frequent users, such as the personnel in health care and military settings described in the previous section. With the study, these personnel would be aware on the accumulated usages of the alcohol to avoid the long term chronic, if any. Negative results on the serum ethanol level even though the individual had been using ABHS frequently for 2 hours were evident in previous literature (Turner *et al.*, 2004).

Infrared spectroscopy was previously used to analyse the content of sanitizers (Elmer, 2020). Using the technique, Beer-lambert law was used to generate a calibration curve. The model for the ethanol-based sanitizer was created based on the area of a peak at 1045 cm^{-1} where the C-O stretch was located and could be predicted as the presence of primary alcohol. For isopropanol-based sanitizers, the peak will be evident at 1131 cm^{-1} where the C-O stretch was located as a secondary alcohol. It is a fast and reliable method that can be used to determine the alcohol content based on the functional group, and further analysis was required on questioned sample suspected of containing alcohol products. It was also suggested that GC should be used after infrared spectroscopy for confirmation purpose (Elmer, 2020).

Hand sanitizers could be found and known as alcohol-based sanitizers (Dhandapani, 2020). The major components found in alcohol-based sanitizers included isopropanol and ethanol products, and they are mainly volatile in nature. Therefore, the method used to analyse the alcohol-based sanitizers was gas chromatography (GC). The analytes of interest could be separated according to their respective boiling point and their polarity. The results had showed that the analyte of interest contain hydroxyl group which was known to be present in alcohol group that can be found in alcohol-based sanitizers (Dhandapani, 2020).

GC techniques could help to determine the concentration of alcohol in the samples upon classification on their effectiveness towards the viruses and bacteria on hands. These methods could subsequently aid to identify whether a product is a fraud product containing no or limited sanitizing composition. As mentioned in previous sections, the high demand of hand sanitizers due to Covid-19 pandemic had caused numerous hand sanitizers to be sold in the market, but they are unknown if they are genuine or fake products. The seller or supplier may use this opportunity to increase the price twice or three times from the original price, taking the advantage to gain huge profit. More severely, they may also be sold counterfeit products, and this greatly impacted the users where it might cause harm if the formulation is not accepted or not recommended. For example, the product sold may not contain 60% or 70% of alcohol which can kill germs on our hands but the seller labelled as it is. Their acts not only harm themselves if the authorities found solid evidence against them but also could harm the people that had bought and used the products. The customer who bought the products could feel secure in protecting themselves from the virus but due to the dishonesty of the seller, no protection was gained. Therefore, as a customer, one must

be smart in choosing the correct and approved hand sanitizers by buying at trusted stores, especially with the availability and accessibility of online shopping recently.