CHAPTER V

DISCUSSION

The purpose of this study was to compare the PN contamination rate from 2 different environmental conditions. In addition, cost and unit cost of PN prepared from both sites were compared.

5.1 The Validation of the Sterile Area

Environmental monitoring in critical zones was used as an indirect method for assessing the sterility of the product during aseptic production (Denyer and Baird 2007). The control and monitoring of air particulate contamination was the first step in any routine contamination-control program as it reduced airborne microbial contamination and thereby minimized the risk of contamination. The settling plate method was performed to check the air cleanliness in the LAFH and PN admixing room. It was an acceptable method in detecting airborne microbial contamination as described in USP31 (2008). This method is of limited quantitative value because the results are affected by air currents, settling rate and time of exposure. In addition, the technique could not detect those organisms that remain suspended in air current and hence fail to settle onto the agar surface (Denyer and Baird, 2007; Ramstorp, 2000). It has been argued that this method mimics the deposition of airborne particles into the product directly. Moreover, there are several advantages of this technique. It is less expensive than other standard test method. No sophisticated equipment is necessary and it is easily placed at the sampling point for longer time period. This technique can be routinely utilized for environmental monitoring program and no laboratory personnel required (Whyte, 1996; Rampstorp, 2000).

The result showed that there was significant difference in microorganism growth between both preparation sites, particularly in plate position 2, 4, 5 and 7 in which plate no.6 that served as negative control was found no microorganism growth. In the separated room, several numbers and types of microorganism were found, and most of them were gram positive bacteria and fungi. The main sources were from skin contaminants (such as *Micrococcus spp.*, *Corynebacterium spp.*) and from airborne contaminants (such as *Bacillus spp.*, most of fungi) (Strohl, Rouse, and Fisher, 2001). When compared to the cleanroom, few numbers and types of microorganism were found, and the contamination were mainly from human skin, except *Bacillus spp.* and *Nocardia spp.*, which were usually derived from sources outside clean areas. In a general rule, it is stated that one route that contaminants can come into contact was the transfer of contaminants from the outer environment into the inner.

The transfer of contaminants from the outer environment into the inner environment of a process is one of the most commonly encountered routes of contamination of a product. Transfer of contaminants can take place via the activities of personnel working, even when sitting totally still. The outer skin layer sheds approximately 100,000 particles per minute released into the surrounding air, when moving the number will rise dramatically. Moreover, the contaminants can transfer via the introduction of contaminated media. The use of cleanroom, clean zone and the entire area of contamination control are the basis for the minimization of contamination transfer (Ramstorp, 2000). In addition, only the result from the cleanroom agreed with USP recommendation (USP 31, 2008). The result from the separated room was critically exceeded. When looking backward, this separated room had been constructed more than 20 years with LAFH. It was possible that there

was mechanical damage to a HEPA filter, thus, it may allow dirty air to enter the working area. Some degeneration were observed, mainly was the dust deposit in the sharp corner of the separated room, the position that is hard to clean. This was inferior to the cleanroom in the manner of the design features. Thereby, the results could not be completely compared, because of unequally equipment and material deterioration of both sites. The finding of the present study lead to the conclusion that microbial contamination rate of the cleanroom environment tended to be lower than that of the separated room environment. However, this study had a limited value in the time of settle plates exposing to the environment. Thirty minutes seemed to be less than the recommendation, as the USP recommended more than 1 hour exposition. It was due to a working time adjustment for practically use. In further study, we suggested to extend the exposure time or running settling plate method during the operation period to be an in-process control and monitoring the level of microbial contamination (Sharp, 2005).

In conclusion, microbiological environmental validation should be performed everyday or at least once a month in both separated room and cleanroom. According to Good Manufacturing Practices (GMP) of sterile product, room and workstation air filter testing should be repeated at least annually. Air velocity, room air changes and air particle counts should be tested at least 2-3 times annually. Room air-pressure differentials should be monitored continuously (USP 31, 2008). The workspace cleaning and sanitizing must be strictly renewed and evaluated to reduce microbial contamination (Sharp, 2005).

5.2 Sterility Test of Parenteral Nutrition

The sterility of a product was defined by the absence of viable and actively multiplying microorganisms when tested in specific culture media (PIC/S, 2007). This test was performed on the end product and must still be regarded as an essential quality control test, especially, in case of aseptic preparations (Denyer and Baird, 2007). However, the sterility test cannot be used to demonstrate the sterility of the entire batch. Appropriate sampling and proper manufacturing control can reduce this limitation.

An ideal test of sterility should be nondestructive, uncomplicated and able to detect all levels of contamination. USP 31 (2008) specifies two basic methods for performing sterility tests, the direct inoculation method and the membrane filtration method. The latter is the method of choices when feasible. Nevertheless, the direct inoculation method was selected to use in this study, as it was simple and inexpensive. It exist a lower probability of inadvertent contamination and does not require great operating skill and equipment. Only small volume of sample is required for this method. Disadvantage of this method is the lower sensitivity in detecting low-level contaminations when compared with the membrance filtration method (Akers, M, Larrimore, and Guazzo, 2003). This inferior point would be solved by the use of double-strength broth method that could detect low-level bacterial contamination (Hoffman, et al. 1982; Miller, et al. 1982), and the transfer of sample in sterility test must be completed within 40-60 minutes after preparation (De Chant, et al., 1982). USP 31 (2008) suggested that minimum volume used in sterility test was 10% of total content, but not less than 20 ml in case of total content greater than 100 ml. This standard was not practical in this study because the excessive loss of a final product. Previous studies (Sinthawat, 1995; Leewiriya 1999; Ingcharoensunthorn, 2000;

Sakpanich, 2000) showed that 6-10 ml of final products could support growth of microorganisms. Thus, 10 ml of a final product was tested for sterility in this study. The number of final products to be tested for sterility was according to USP requirement. In addition, samples were selected from the beginning, every 5 unit of preparations and the last of preparation for determining the time frame of preparation as described in GMP (Sharp, 2005; PIC/S, 2007)

This sampling method was consistent with the sterility recommendation (PIC/S, 2007). There were 3,699 and 3,632 bottles prepared from the separated room and cleanroom respectively. Seven hundred and forty-eight bottles from the separated room and 728 bottles from the cleanroom were sampled. It was found that 7 samples (0.94%) prepared from the separated room were contaminated while there was no microorganism found in any samples prepared from the cleanroom. This result showed the significant difference in contamination rate between both areas. This finding was similar to the study of Brier et al. (1981) who compared the effect of LAFH and clean tabletop on contamination rates of intravenous admixtures. They found that the contamination of admixtures compounded in LAFH was significantly less than those compounded on a clean tabletop. This suggested that the most important factor is the environment when one adheres to aseptic technique.

Thomas, Sanborn, and Couldry (2005) compared the contamination rates of medium-risk intravenous admixtures in a traditional practice site and a class 1000 cleanroom by simulated media fills method. They found no significant difference between both sites and concluded that the key affecting variable was the aseptic technique of personnel. This result might be argued that the simulated media fills method was recognized as a test of operator's aseptic technique. In this test situation,

they would take more than their usual care, and thus the normal possibility will not be precisely simulated (Sharp, 2005).

Types of microorganisms found in sterility test were considered with the results of settle plates. The results showed that type of microorganism found in a final product was similar to that found in the environment in that period. It showed a strong relationship between the air cleanliness and the product quality. This finding was similar to the study of Whyte et al. (1982). They studied the role of microbial airborne during aseptic filling, and found that there was a significant increase in vial contaminations associated with deterioration in air quality. They found a strong association between the type of bacterial isolated from the air and those found in the vial. The result confirmed the fact that product contamination by air and people were primary sources of contamination in clean area.

Although there was contamination in PN products, none of the patients who received contaminated PN in this study developed sign of sepsis. It might be caused by the variables in each individual, such as infecting dose, age, sex, nutritional status and genetic background. Therefore, it is uncommon for a microbe to cause disease in all infected individuals (George, et al., 2008).

A pharmacy's actual contamination rate will be the result of all of the contamination risk factors, including the nature and complexity of the compounding operation, the quality of the preparation environment and the preparation skills. Despite the best environmental control, strictly aseptic technique must be performed to prevent the contamination occurring from personnel and manufacturing process. Also, it can reduce touch contamination that was frequently reported as an important route in product contamination (Trissel et al., 2007). PIC/S (2009) recommended the

validation of aseptic processes by simulated media fills method which would be, at least, annually tested for patient safety implications (Sharp, 2005).

In conclusion, this study showed the relationship between air cleanliness and quality of final product. Cleanroom is a system that can provide a cleaner environment than separated room, thus, better microbiological quality of PN can be confided by cleanroom system. For further study, sterility test should be performed by more media types because no single media can support the growth of all microorganisms (Akers, 2003). In addition, sterility test could be replaced by media fill simulation test.

5.3 Cost and Unit Cost Analyses of PN Prepared from the Separated Room and Cleanroom

In the present study, PN cost analysis was calculated from total direct cost which was simplified and could be adjustable for Ramathibodi Hospital and others. Total direct cost was divided into 3 main parts: labor cost, capital cost and material cost. The labor cost was the full cost of employing personnel represented by the individual's gross earning including salary, overtime wage with any supplemental benefits. For the examples, contributions to health insurance, social security and pension plans, hardship bonuses, holiday and sick pay, cost of travelling and meeting may have been deducted. In this study, monthly salary and benefit cost of each personnel was kept to be the private data of Human Resources Department, so the researcher could not be informed. Daily personnel's overtime wage was used for estimating the total incomes of each individual. However, the labor cost found in this study may be underestimated due to a few reasons: 1) each personnel's salary, overtime expense and benefit used could not equalization, even in the same position,

for example, nine pharmacists in Ramathibodi Hospital turned a role in PN preparation, hence, individual's salary, overtime wages and other benefits was different; 2) Monthly salary was depended on working experiences, so labor cost tended to be increasing every year; 3) The labor cost found in this study mainly included the personnel directly had a job in PN processing. There were indirect personnel involvement such as engineers and technicians who had a role in maintenance, ward clerk for the PN transportation, etc.

The capital cost included the input that last for more than one year such as, vehicle, space, equipment, etc. Estimated working life of each item must be a calculated factor. The straight-line method was used in calculated depreciation value, which was the simplest and most practical used (สุกัลยา คงสวัสดิ์, 2538). Nevertheless, item costs in this study, particularly in the separated room were presented in historical cost. The appropriate cost concept described as the current cost should be used, not the original purchase price. Thus, past-cost value in this study should be changed into present value by future cost estimation (Creese and parker, 2000). Therefore, if several costs were estimated into future cost, total capital cost would be increased.

The material cost was the cost of materials used up in the course as direct inputs into PN processing. Nutrient cost in this study was calculated separately for 3 formulas: Pediatric PN, adult PPN and adult TPN for the more accurate unit cost of each formula. However, there were some material losses in the period of study, so the material cost presented in this study tended to be underestimated.

Most of all resources presented in this study were the shared cost of 3 formulas except the nutrient cost. Then, cost allocation must be determined. In this study, the factor used to determine was the volume of each formula preparation. Furthermore, the cleanroom in Ramathibodi Hospital was build for the purpose of

preparing sterile product preparation including IV admixtures. Cost allocation should be calculated by the preparation number of PN preparations and IV admixtures. In this study, it was assumed that the number of preparations was equal, so almost capital cost presented in the cleanroom period was a half of total cost. Finally, 3 PN formulas were calculated. The comparison of unit cost was done. It was found that the cost of PN prepared from the cleanroom was higher than that from the separated room (22.50, 5.88 and 19.57% for pediatric PN, adult PPN and adult TPN respectively) according to the mainly difference from area development cost, in which cleanroom construction were complicated due to the system approach. Moreover, the unit cost presented in this study was not accurate, as the indirect cost was not examined in this study. Therefore, if this cost was also examined, unit cost of production would increased.

Cost per unit of a PN formulas preparation in this study was based on the number of labors, preparation site, preparation volume, instrumental and material uses. Consequently, the cost per unit cannot be readily applicable to other hospital where the production capacity was different from Ramathibodi Hospital. It was suggested that the further study should include indirect cost calculation over one full year to avoid any distortions that might be caused by seasonal effects.