CHAPTER III

MATERIALS AND METHODS

The present study was designed to investigate the environmental impact on contamination rate of PN admixtures prepared from a separated room, compared with a cleanroom at Ramathibodi Hospital.

3.1 The Validation of Sterile Area

3.1.1 Layout of the Separated Room for PN Admixtures

The separated room located in the preparation area of the Pharmacy Department was designed to be used as PN admixing center. This room was already equipped with air conditioner mounted on the wall. There was also a vertical laminar airflow hood (LAFH) with high efficiency particulate air (HEPA) filter inside. The LAFH was located in the site and avoided from direct exposure to airflow from the air conditioner and the door opening (Figure 2).

3.1.2 Layout of the Cleanroom for PN Admixtures

The new PN admixing center was specified as a cleanroom, located at the first floor, Building 3, which was isolated from the Pharmacy Department. The room was equipped with two vertical LAFHs, in which the only one LAFH was used to prepare PN solutions. The other one was used for I.V. admixture preparation. Layout of the cleanroom is shown in Figure 3.

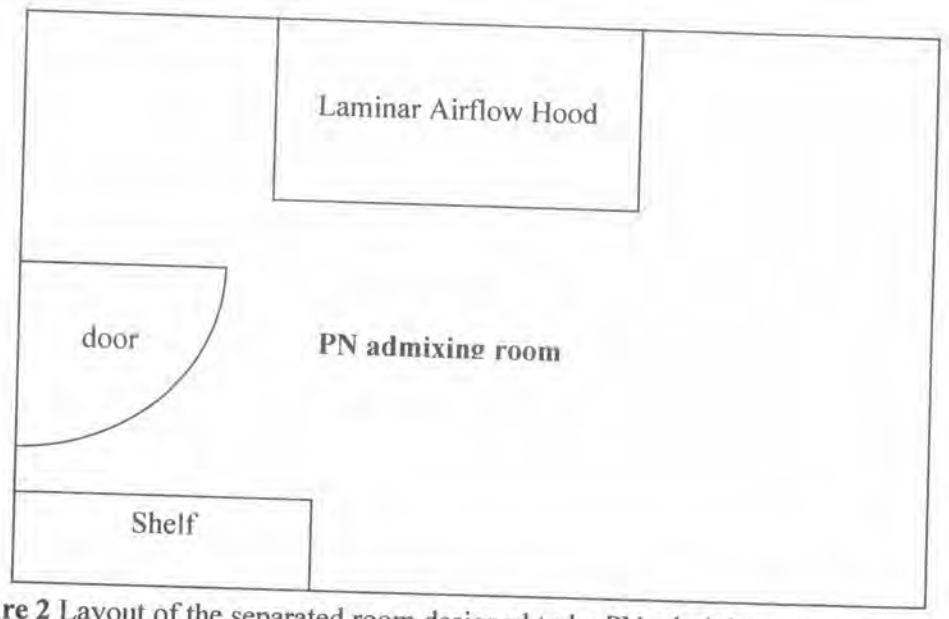

Figure 2 Layout of the separated room designed to be PN admixing center at Ramathibodi Hospital

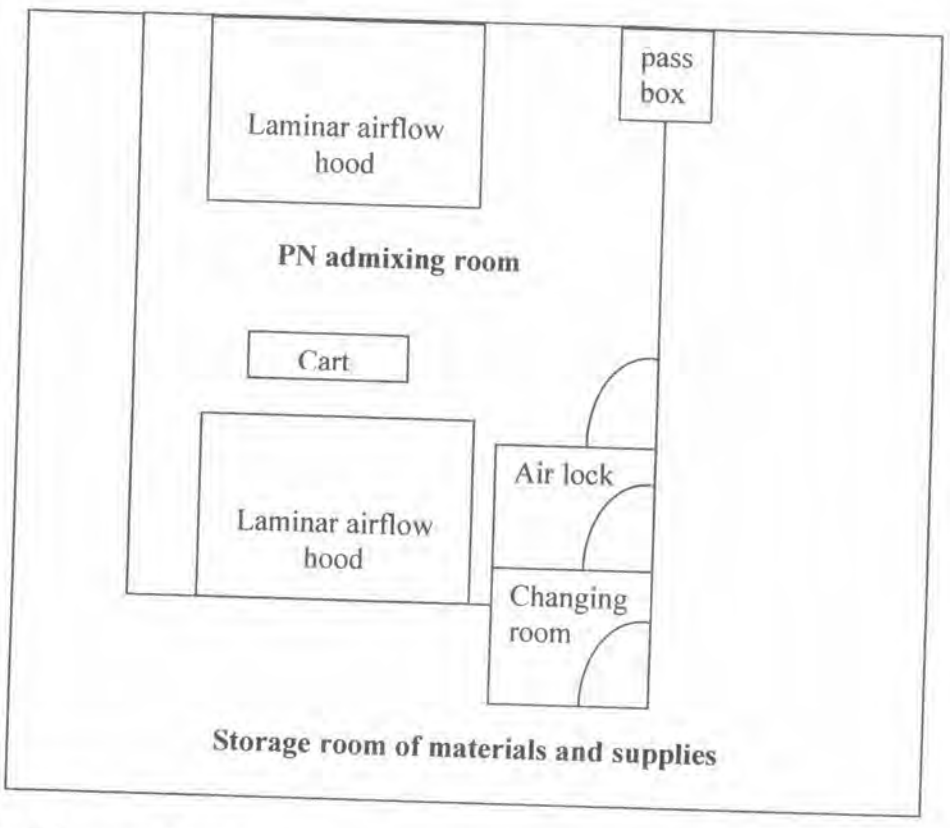

Figure 3 Layout of the cleanroom designed to be the new PN admixing center at Ramathibodi Hospital

3.1.3 Validation of Sterile Area

The clean area of LAFH and admixing room was validated 3 times a week, for 8 weeks. The settling plate method was used for microbiological testing. The equipments, supplies, and procedures were described as follows (Lu, Alto and Prusia, 1983; USP 31, 2008)

Equipment and Supplies

1. Sterile Trypticase Soy Agar™ (TSA)
2. Sterile gown with nose-mouth mask, hair cover, sterile gloves, and slippers supplied from the Department of Central Supply, Ramathibodi Hospital
3. Sterile gauze
4. Distilled water
5. Sterile water
6. 70% ethyl alcohol
7. Disinfectant (Hibiscrub®)
8. 1000 ml Beaker
9. 1000 ml Erlenmeyer flask
10. Stirring rods
11. Hot plate, autoclave, incubator and refrigerator, courtesy permitted for use by the Microbiology Laboratory, Department of Pathology, Ramathibodi Hospital

Procedures

1. Preparation of Trypticase™ Soy Agar (TSA)

Forty grams of TSA powder were suspended in 1000 ml of distilled water and mixed thoroughly. The solution was gently heated with frequent agitation until TSA powder was completely dissolved. Then, TSA solution was transferred to an erlenmeyer flask and autoclaved for 15 minutes at 121 °C, pressure 15 pounds per

square-inch (psi). TSA solution was left to cool down to 60-65 °C, and then poured into sterile petri dishes. After TSA plates were allowed to cool down and harden at room temperature, a few TSA plates were checked for their sterility and performance. The Sterility test was performed by incubating them at 37°C for 24 hours. They would pass the test if no growth of microorganism occurred. The Performance test was performed by inoculating them with 0.5 ml of 10^7 cfu/ml of *Staphylococcus* spp. at 37°C for 24 hours. The media were suitable if colony of microorganism was visualized by naked eye. If the TSA plates passed the sterility test and the performance test, they must be kept in refrigerator (2-8 °C) until use.

2. Validation of Sterile Area

The operator washed his/her hands and arms thoroughly with Hibiscrub®. The shoes were taken off and changed to wear clean slippers before entering the PN preparation room. The operator then wore a sterile gown, hair cover, mask and a pair of gloves inside the room (for separated room) or in a dressing room (for cleanroom). The gloves were wiped with 70% ethyl alcohol for cleansing powder off. Then the operator cleaned all surfaces of LAFH with sterile water, following with 70% ethyl alcohol. When the surfaces of LAFH were completely cleansed from the top to the bottom and from the back of the hood outward to the front, the UV lamp of the LAFH was turned on for 20 minutes. When UV lamp was turned off, the blower of LAFH was turned on.

Six sterile TSA plates were placed in LAFH (location 1-6), one plate in PN preparation room (location 7), as shown in Figure 4 and 5. Plate no.6 served as a negative control. All plates, except plate no.6 were exposed to the flow of air in the LAFH and room for 30 minutes. All plates were covered using aseptic technique, labeled and incubated at 37 °C for 7 days. The type and number of colonies of

microorganisms found in each plate was determined by Microbiology Laboratory, Department of Pathology, Ramathibodi Hospital.

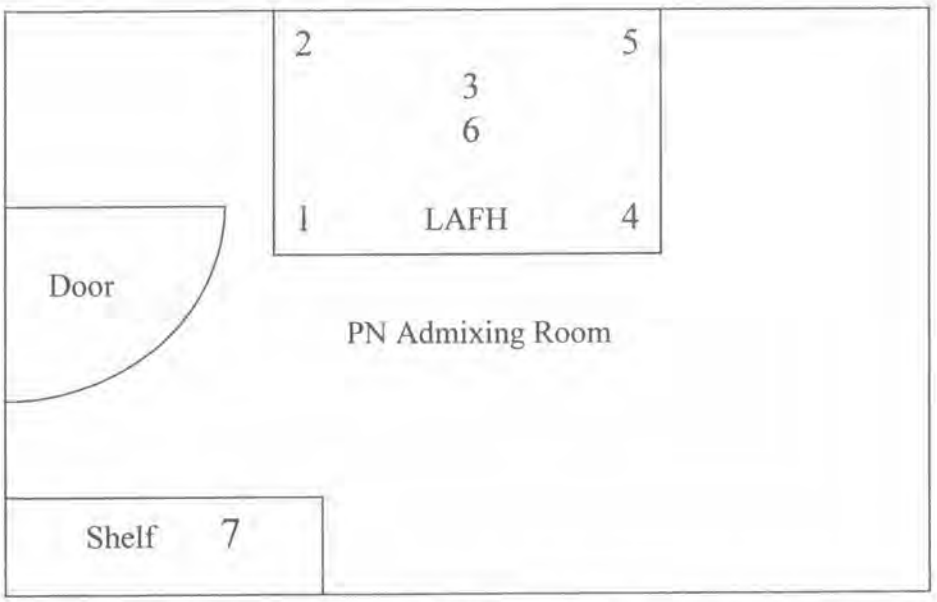

Figure 4 Position of TSA plates in LAFH and the PN preparation room in the separated room
Abbreviations: 1=plate no.1, 2=plate no.2, 3=plate no.3, 4=plate no.4, 5=plate no.5, 6=plate no.6, 7=plate no.7

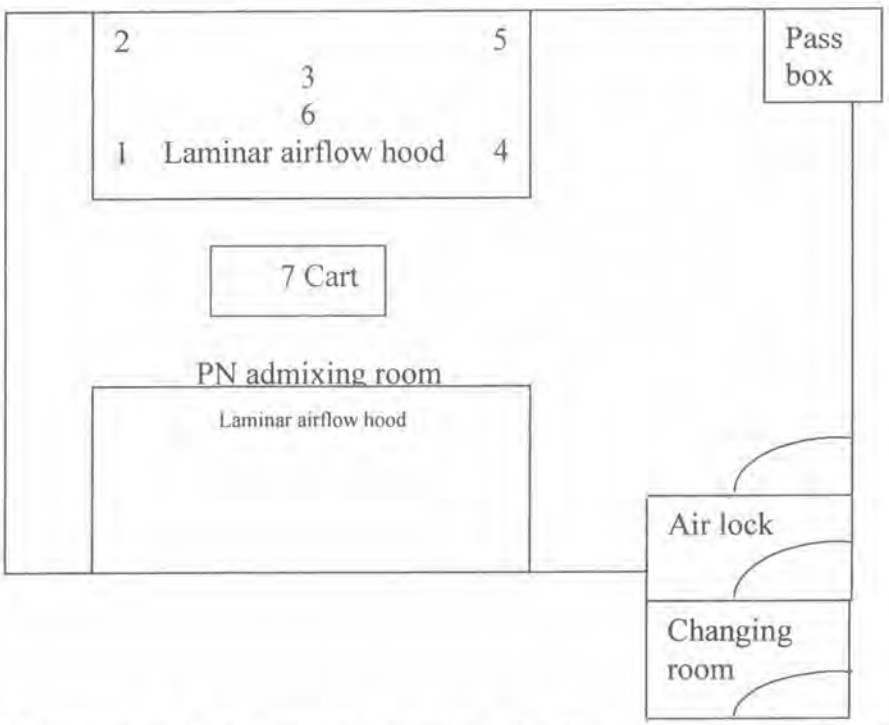

Figure 5 Position of TSA plates in LAFH and the PN preparation room in the cleanroom
Abbreviations: 1=plate no.1, 2=plate no.2, 3=plate no.3, 4=plate no.4, 5=plate no.5, 6=plate no.6, 7=plate no.7

3.2 Sterility test of Parenteral Nutrition (USP 31, 2008)

The PN solution prepared from each PN admixing unit was tested for sterility everyday for 8 weeks by direct inoculation method. The procedures were described as follows (USP 31, 2008).

Equipment and supplies

1. Soybean Casein Digest Medium USP (TSB)
2. Sterile gown with nose-mouth mask, hair cover, and gloves, supplied from the Department of Central Supply, Ramathibodi Hospital
3. Sterile gauze
4. Distilled water
5. Sterile water
6. Tap water
7. 70% ethyl alcohol
8. Disinfectant (Hibiscrub®)
9. Sterile disposable 10-ml syringes
10. Sterile disposable 18 G needles
11. 50-ml vials with rubber closures and aluminum caps
12. 1000-ml beaker
13. LAFH
14. Hot plate, autoclave, incubator and refrigerator, courtersy permitted for use by the Department of Microbiology, Ramathibodi Hospital

Procedures

1. Preparation of Trypticase™ Soy Broth (TSB) medium

Double strength TSB medium was prepared by suspending 60 grams of TSB in 1000 ml of distilled water. The solution was mixed thoroughly and warmed

slightly until TSB was completely dissolved. Then, the 10 ml of double strength TSB medium was transferred into each of 50 ml vial. Each vial was closed with rubber closure and capped with aluminum cap. The TSB vials were autoclaved at 121 °C, pressure 15 psi for 15 minutes. All vials were left to cool down in room temperature. Tests for sterility and performance of the media as described above were conducted. If the TSB media pass those tests, they were kept in refrigerator (2-8 °C) until use.

2. Sampling of PN admixtures

The double strength TSB vials were taken out of refrigerator and left in room temperature. The outer surfaces of vials were wiped with 70% ethyl alcohol and taken into LAFH. After the preparation was finished, 10 ml of PN solutions that were sequenced in 1^{st}, 5^{th}, 10^{th}, 15^{th},...., and the last bottles were immediately transferred into TSB vials by aseptic technique.

3. Culture

PN samples were kept in the incubator at 37 °C for 7 days. Two additional TSB medium-filled vials were used as control. One vial served as a negative control in which 10 ml of sterile distilled water was inoculated. The other served as a positive control in which 10 ml of tap water was inoculated. Both vials were incubated and observed during 7 days. The sterility of culture media was accepted if there was no microorganism growth in the negative control vial. The ability of TSB culture media to support growth of microorganisms was accepted if there was microorganism growth in the positive control vial.

4. Identification of microorganisms

When a media turns cloudy, it can assume the growing of microorganism in that sample. The identification of microorganism must be done. The cloudy media was streaked over a large surface area of a sterile blood agar plate for supporting the

was streaked over a large surface area of a sterile blood agar plate for supporting the growth of a wide range of bacteria, and a sterile MacConkey agar plate for isolation and differentiation of gram-negative organisms. They were incubated at 37°C for 24 hours. This step was done by Department of Microbiology, Ramathibodi Hospital.

3.3 Statistical Analysis

The difference between 2 sites as number of plates that found microorganism growth and number of contaminated samples in the sterility test was performed by Chi-Square method, with significant level at 5%.

3.4 Cost and Unit cost Analysis of PN Preparation

The investment cost per milliliter (ml) of adult PPN, adult TPN and pediatric PN prepared from the separated room and the cleanroom were estimated.: (สุกัลยา คงสวัสดิ์, 2538)

3.4.1 Capital Cost consisted of cost, lifetime and quantities of heavy equipment used in PN preparation. The depreciation rate was a factor in calculating the remaining value by the formula as followed:

$$\text{Heavy equipment depreciation rate (Baht/year)} = \frac{\text{Heavy equipment cost (Baht)}}{\text{Life time (years)}}$$

3.4.2 Material Cost consisted of cost and quantities of nutrients and chemicals, office and household applications, wasteful materials. The electricity and water supply cost estimation must be taken into account.

3.4.3 Labor Cost consisted of the personnel salary and overtime wages, inclusion of another personnel related to the operation such as labour, housekeeper, engineer, etc. The working hours of each person were estimated for accurate results.

3.4.4 Unit Cost Analysis

The sum of capital cost, labour cost and material cost were the total cost of all PN prepared in those period. The cost allocation was calculated from each formula preparation ratio in those periods as the following equations:

$$\text{Cost allocation} = \frac{\text{each PN formula preparation (ml)}}{\text{Total PN preparation (ml)}}$$

The cost allocation was used for correcting the exact cost of each PN formula preparation as the formula below:

$$\text{Unit cost of PN (Baht/ml)} = \text{Cost allocation X} \frac{\text{Sum of PN preparation cost (Baht)}}{\text{Total PN preparation (ml)}}$$