REFERENCES

ภาษาไทย

สุกัลยา ดงสวัสดิ์. 2538. คู่มือการวิเคราะห์ต้นทุนโรงพยาบาลชุมชน. กรุงเทพมหานคร: วิทยาลัยการสาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย.

ภาษาอังกฤษ

Akers, M.J., Larrimore, D.S., Guazzo, D.N., eds. 2003: Parenteral quality control: New York: Marcel Dekker.

Allwood, M. C. 1997. Microbiological risks in parenteral nutrition compounding. Nutrition 13: 60-61.

A.S.P.E.N. Board of Directors and The Clinical Guidelines Task Force. 2002. Guidelines for the use of Parenteral and Enteral Nutrition in Adult and Pediatric Patients. JPEN 23(1) supplement: 1SA -138SA.

Baker, R. D., Baker, S. S., Davis, A. M., eds. 1997. Pediatric Parenteral Nutrition New York: Chapman and Hall.

Brier,K.L., Latiolais, C.J., Schneider, P.J., Moore, T.D., Bueshing, W.J., Wentworth, B.C. 1981. Effect of laminar air flow and clean room dress on contamination rates of intravenous admixtures. Am J Hosp Pharm 38: 1144-1147

Brogden, B. 2004. Current Practice in Administration of Parenteral Nutrition: Venous Access. Br J Nurs 13(18): 1068-1073.

Btaiche, I. F., Khalidi, N. 2004. Metabolic complications of parenteral nutrition in adults, part 1. Am J Health-syst Pharm 61: 1938-1949.

Btaiche, I. F., Khalidi, N. 2004. Metabolic complications of parenteral nutrition in adults, part 2. Am J Health-syst Pharm 61: 2050-2059.

Buchanan, E. C., McKinnon, B. T., Scheckelhoff, D. J., Schneider, P. J., eds.

2002. Principles of sterile product preparation. Bethesda: American society of health-system pharmacists.

Campos, L. C., Lobianco, L. F., Seki, L. M., Santos, R. M., Asensi, M. D. 2007. Outbreak of *Enterobacter hormaechei* septicemia in newborns caused by contaminated parenteral nutrition in Brazil. J Hosp Infect 66(1): 95-97

Creese, A, Parker, D. eds. 2000. Cost analysis in primary health care; a training manual for program managers. Geneva: WHO.

De Chant., R.L, Furtado. D., Godwin, H.N, Domann, D.E.1982. Determining a time frame for sterility of intravenous admixture. Am J Hosp Pharm 39:1305-1308.

Denyer, S.P., Baird, R.M., eds. 2007. Guide to microbiological control in pharmaceuticals and medical devices. 2 nded. Boca Raton: CRC Press.

Driscoll, D. F. 2003. Compounding TPN admixtures: then and now. JPEN 27(6): 433-438.

Farguharson, G., J., Whyte, W. 1999. The design of cleanrooms for the pharmaceutical industry. In Whyte, W. (ed.), Cleanroom design, pp. 79-115. Chichester: John Wiley&Sons.

George R.V, Dockrell, H. M., Zuckerman, M., Wakelin, D., Roitte, I. M., Mim, S. C., et al., eds. 2008. Mim's medical microbilolgy. 4th ed. Philadelphia: Elsevier Limited.

Grant, J. P., ed. 1992. Handbook of total parenteral nutrition. 2th ed. Philadelphia: W.B. Saunders.

Habsah, H., Zeehaida, M., Van Rosenberghe, H., Noraida, R., Wan Pauzi, W. I., Fatimah, I., et al. 2005. An outbreak of *Pantoea spp.* In a neonatal intensive care unit secondary to contaminated parenteral nutrition. J Hosp Infect 51: 213-218.

Hark, L., Morrison, G. 2003. Medical Nutrition and Disease A Case-Based Approach. 3rd ed. Massachusetts: Blackwell Publishing.

Hoffmann K.H., Smith, F.M.,Godwin, H.N, Hogan, L.C., Fortado, D. 1982. Evaluation of three methods for detection bacterial contamination in intravenous solution. Am J Hosp Pharm 39:1299-1302.

Ingcharoensunthorn, P. 2000. Clinical pharmacy activity in parenteral nutrition support for pediatric patients in a newborn surgery ward at the Queen Sirikit National Institute of Child Health. Master's Thesis. Department of Pharmacy, Graduate School, Mahidol University.

Kastango, E. S. 2005. Blueprint for implementing USP chapter 797 for compounding

sterile preparations. Am J Health-Syst Pharm 62: 1271-1288.

Kochevar, M., Guenter, P., Holcombe, B., Malone, A., Mirtallo, J. 2007. A.S.P.E.N. statement on parenteral nutrition standardization. JPEN 31(5): 441-448.

Kyle, U. G., Genton, L., Pichard, C. 2005. Hospital length of stay and nutritional status. Curr Opin Clin Nutr Metab Care 8: 397-402.

Leewiriya, P. 1999. Establishment of Parenteral Nutrition Preparation Unit at Lopburi Hospital. Master's Thesis. Department of Pharmacy, Graduate School, Mahidol University.

Llop, J. M., Mangues, I., Perez, J. L., Lopez, P., Tubau, M. 1993. *Staphylococcus saprophyticus* sepsis related to total parenteral nutrition admixtures contamination. JPEN 6: 575-577.

Lu, A., Alto, L. J., Prusia, M.G. 1983. Reliability of the settling-plate method in monitoring laminar-airflow workbenches. Am J Hosp Pharm 40: 271-273.

Lyman, B. 2002. Metabolic complications associated with parenteral nutrition. J Infus Nurs 25(1): 36-44.

Matarase, L. E., Hamilton, C. 1998. Nutrition support: Indications and Efficacy. In: Skippers, A. (ed.). Dietitian's Handbook of Enteral and Parenteral Nutrition, pp. 25-46. Maryland: An ASPEN Publication.

Miller, C.M., Furtado. D., Godwin, H.N, Hogan, L.C., Letendre. D,E. 1982. Evaluation of three methods for detecting low-level bacterial contamination in intravenous solution. Am J Hosp Pharm 39:1302-1305.

Montalvo-Jave, E. E., Zarraga, J. L., Sarr, M. G. 2007. Specific topics and complications of parenteral nutrition. Langenbecks Arch Surg 392: 119-126.

Opilla, M. 2008. Epidemiology of bloodstream infections related to contaminated substances. Am J Infect Control 36: S173.e5-e8.

Payne-James, J., Grimble, G., Silk, D., eds. 2001. Artificial Nutrition Support in Clinical Practice. 1st edition. London: Greenwich Medical Media Limited.

PIC/S Secretariat. 2007. Recommendation on the sterility testing. [online]. Available from: http://www.picscheme.org [2009, March,1]

PIC/S Secretariat. 2009. Recommendation on the validation of aseptic processes. [online]. Available from: http://www.picscheme.org [2009, March,1]

Ramstorp. M., eds. 2000. Introduction to contamination control and clean room technology. Weinheim: Wiley-VCH.

Reily, H. 1998. Parenteral nutrition: an overview of current practice. Br J Nurs 7(8): 461-467.

Rombeau, J. L., Caldwell, M. D., eds. 1986. Parenteral nutrition. Philadelphia: Saunders.

Sakpanich, M. 2002. Establishment of Siriraj Parenteral Nutrition Admixing Center

(SPNAC) and study of parenteral nutrition-related complications in adult patients at Siriraj Hospital. Master's Thesis. Department of Pharmacy, Graduate School, Mahidol University.

Sharp, J., eds. 2005. Good pharmaceutical manufacturing practice: rational and compliance. Boca Raton: CRC Press.

Shikora, S. A., Blackburn, G. L., eds. 1997. Handbook of Nutrition Support :Theory and Therapeutics. 1st edition. New York: Chapman and Hall.

Sinthawat, K. 1995. Establishment and evaluation of centralized parenteral nutrition admixing service for pediatric patients at Pharmacy Department of Ramathibodi Hospital. Master's Thesis. Department of Pharmacy, Graduate School, Mahidol University.

Strohl, W.A., Rouse, H., Fisher, B.D., eds. 2001. Lippin cott's illustrated review: Microbilology. Philadelphai: Lippincott Williams & Wilkins.

Swenson, J.P., Edwards, D., Chamberlain, M., Rault, R., Ivey, MF. 1977. A total parenteral nutrition protocol. Drug intell Clin Pharm 11: 714-720.

Teasley-Strausberg, K. M. 1992. Indications for Parenteral and Enteral Nutrition. In: Teasley-Strausberg, K. M. (ed.). Nutrition Support Handbook, pp. 37-46. Cincinnati: Harvey Whitney Books Company.

Therney, A. J. 1996. Undernutrition and elderly hospital patients: a review. J adv Nurs 23: 228-236.

Thomas, M., Sanborn, M.D., Couldry, R. 2005. I.V. admixture contamination rates: Traditional practice site versus a class 1000 clean room. Am J Health Syst Pharm 62: 2386-2392.

Tresoldi, A. T. 2000. *Enterobacter cloacae* sepsis outbreak in a newborn unit caused

by contaminated total parenteral nutrition solution. Am J Infect Control 28: 258-261.

Trissel,L. A., Gentempa, J.A., Saenz, L.M, Woodard, M. Y., Angeles. C, H. 2007. Effect of two work practice changes on the microbial contamination rates of pharmacy-compounded sterile preparations. Am J Health-Syst Pharm 64; 837-841.

U.S. Pharmacopoeia National Formulary USP XXXI, NF XXVI. 2008. Rockville: The United States Pharmacopeial Convention.

Vonberg, R. P., Gastmeier, P. 2007. Hospital-acquired infections related to contaminated substances. J Hosp Infect 65: 15-23.

Wershil, B. K. 1986. Infection and Parenteral Nutrition. In: Lebenthal, E. (ed.). Total Parenteral Nutrition Indications, Utilization, Complications, and Pathophysiological Considerations, pp. 341-350. New York: Raven Press.

Worthington, P., Gilbert, K. A., Wagner, B. 2000. Parenteral Nutrition for the Acutely Ill. Crit Care Nurse 11(4): 559-557.

Whyte,W. 1996. In support of settle plates. PDA J Pharm Sci technol 51(4): 201-204.

Whyte,W., Baikey, P.V., Tinker. J., McCubbin. I., Young, L., Jess, J. 1982. An evaluation of the routes of bacterial contamination occurring during aseptic pharmaceutical manufacturing. J Parenter Sci Technol 36(3): 102-107

APPENDICES

Appendix A

Media and Microbiology techniques

1. Media

1.1 Casein-Soybean Digest Agar (Trypticase Soy Agar): pH 7.3

	grams per liter
Peptone(tryptic digest of USP Casein)	17.0
Papaic digest of soy meal (phytone peptone)	5.0
NaCl	5.0
Agar	15.0
Distilled water q.s. to	1000 ml

Casein-soybean digest agar will support growth of the same range of organisms as the corresponding broth. It can be used as a base for blood agar or chocolate agar. With or without blood, it is a useful all-purpose agar medium to be used wherever total aerobic plate counts are desired (e.g., urine cultures) either by the pour plate method or by surface streaking of calibrated loops to tempered plates. Casein-soybean digest agar plates with or without blood are also useful as settling plates to determine the number of bacteria in the air either while working at a laminar flow hood (e.g., when doing tissue culture work or performing an aseptic fill) or for general monitoring of the microbial environment for contamination control in the lab, ward, or operating room. Addition of glucose must be considered if it is to be considered nutritionally similar to TSB.

1.2 **Blood Agar: pH 7.3 ±**

Any all-purpose medium such as nutrient agar, brain heart infusion agar, or soy-bean casein digest agar to which 5% defibrinated blood has been added to is called blood agar. The most commonly employed supplement is whole citrated or defibrinated sheep blood. Goat, horse, or rabbit blood may be employed. Human blood can be employed but care must be taken to test for the presence of streptococcal antistreptolysin antibodies. The secretion of extracellular hemolysins produces complete lysis of the crythrocytes around the colony (beta-hemolysis), or incompleter hemolysis, as shown by a green discoloration of the hemoglobin around the colonies (alpha-hemolysis). Failure to affect the cellular integrity of erythrocytes, as seen by no change in the color of the agar surrounding

of the colony, is also of diagnostic value (gamma-hemolysis). All blood agar should be freshly prepared, preincubated at 35 to 37° C for sterility, and quality control checked with human strains (*Streptococcus pyogenes* for beta-hemolysis, *Streptococcus viridans*, or *Streptococcus pneumoniae* for alpha-hemolysis).

The presence or absence of beta-hemolysis is useful not only for the streptococci and staphylococci but is a useful diagnostic aid for speciations of Gram-negative rods and spore-forming members of the genus Bacillus and other groups. Typical hemolytic reactions can be inhibited by the presence of carbohydrates; accordingly, any blood agar base employed must not contain glucose.

1.3 MacConkey's Agar: pH 7.1 +

	grams per liter
Peptone	17.0
Proteose (polypeptides)	3.0
Lactose	10.0
Bile salts	1.5
NaCl	5.0
Agar	13.5
Neutral red (vital dye)	0.03
Crystal violet	0.001
Distilled water q.s. to	1000 ml

MacConkey's agar shares two things in common with EMB: (1) Gram-negative rods are selected and differentiated with concurrent inhibition of Gram-positive organisms and (2) the diagnostic color changes are related to fermentation of lactose, but are not related to the inclusion of a conventional pH indicator. The medium is more selective than EMB and relies upon a bile salt mixture and crystal violet to inhibit the Gram-positive forms. It is especially useful for the detection of enteric organisms; lactose-fermentating strains produce brick red colonies while lactose-negative forms (*Salmonella, Shigella, Pseudomonas, Acinetobacter, Proteus*) produce pink or colorless colonies. Typical strains of *Escherichia coli* also show a zone of opaque red precipitation around the colony, whereas, other

lactose-fermenters will show the red colony with a pale pink periphery and no pericolonial precipitate. As with EMB agar, extremely glycolytic strains may produce intracolonial acidity that concentrates the neutral red dye (red colonies). On this medium, lactose-negative colonies often produce a pale yellow discoloration of the agar. Bacteriuria (10^5 organisms/ml in urine) is diagnosed on this medium by direct streak with a calibrated loop.

2. Microbiology Methods

Inoculation of media

All specimens should be cultured as soon as possible on suitable primary isolation media, the choice of which depends on (1) the nature (origin) of the specimen, (2) the result of a Gram stain, and (3) the physician's request, e.g., for fungal culture. At least one liquid medium, e.g., thioglycollate broth, and one non-selective plating medium, e.g., blood agar, should be employed. All media must be at room temperature or at 37° C before they are inoculated.

2.1.1 Inoculation of liquid media

If the inoculum is liquid, use a sterile Pasteur pipet for inoculation, although a loop may also be employed. In general, liquid media are used to (1) foster the growth of a small number of bacteria, (2) dilute antibacterial agents in the specimen (antibiotics, antibodies, etc.) and (3) depending on the choice of the liquid medium, stimulate the growth of certain bacteria (e.g., anaerobes in thioglycollate broth).

Hold all tubes almost horizontally. Loosen cap (or cotton plug) and hold between fingers to avoid contamination. Sterilize loop by heating it until it is red hot: allow it to cool, obtain inoculum, and transfer it into liquid. It is important to flame the entire length of the loop before inoculation of a liquid medium. Replace cap or cotton and mix by tapping on tube.

Incubate liquid medium at 35-37° C under 3-5% CO_2 until growth occurs. Check for growth every 12 hr with Gram stain. If growth occurs, subculture to solid medium. If no growth occurs after 1 week, the medium may be discarded and 'no growth' should be reported.

Liquid media are examined for turbidity, sediment, arrangement of

colonies (floating, floccules, chains, etc.) pellicle formation, color development, and gas and odor production.

2.1.2 Inoculation of solid media

Four techniques are used for inoculation of solid media: (1) streak plate, (2) pour plate, (3) streak-pour plate, and (4) slant.

Streak Plate: The purpose of the streak plate is to isolate organisms in pure culture. Collect specimen in sterile (flamed) loop that is slightly bent so that it will glide smoothly over the surface of the medium. Deposit inoculum at one edge and streak back and forth over the area, progressing across the agar until one fourth of the plate surface is covered. Flame the loop, streak at right angles to the originally inoculated agar, and cover the second quarter of the plate. Flame loop again and repeat procedure until third and fourth quadrants are covered. Instead of flaming the loop, which is time consuming, two loops may be used alternately or the loop may be used to cut deeply twice into the agar before progressing from one quadrant to the next. Whichever method is used, a small area of the agar should be undercut with the loop to streak the undersurface of the agar. Turn plate upside down to prevent contamination by water condensation. Incubate at 35-37° C under 3-5% CO_2. Examine for growth every 12 hr. Keep medium for 1 week before reporting 'no growth.

Examine solid media for size, shape, color, outline, and consistency of colonies, changes in the medium, and odor.

Aerosol contamination of the plates must be minimized by holding the plates vertically when streaking. If the medium is accidentally cut into with the streaking loop, interrupt the streak and start anew, using material from the starting area.

For subculture and bacterial identification choose well-isolated colonies only. Prepare a gram stain of each morphologically different colony and subculture to a suitable differential medium. Touch only the dome of the center of a colony with the end of a straight wire (after flaming and cooling) and transfer the inoculum to a suitable differential medium (see below). If colonies are not adequately isolated after overnight incubation, transfer a small amount of the entire growth with a loop to a second nonselective plate. Streak out the inoculum to obtain adequate isolation of colonies.

Pour Plate: The pour plate is used to determine the approximate number of viable organisms in a liquid medium. They are reported as number of colonies per milliliter of inoculum. Blood agar pour plates are also excellent for determining the type of hemolysis produced by streptococci.

Melt a tube of 10 ml agar in water bath, cool to 45° C, and inoculate tubes with measured volume of suitably diluted inoculum. Pour inoculated agar into sterile Petri dish. Distribute evenly by tilting plate and cool, leaving the top half of the Petri dish partially open for a few minutes to allow vapor to escape. Incubate plate at 35-37° C and increase CO_2 tension to 3-5%.

Streak-pour Plate: Streak a blood agar plate as described for a streak plate and cover streaked surface with melted blood agar. This is a good method for studying hemolysis.

Slant: Slants are tubes containing media gelled in such a way that the upper surface is slanted. Handle and hold slant as described for liquid media. Heat entire length of inoculating loop until red hot and allow to cool inside the tube holding the inoculum. Pick up inoculum with loop and transfer the material to the bottom of the slant, which is inoculated by zigzagging across the entire surface toward the mouth of the tube. Flame mouth of tube and replace cap or cotton plug.

If the slant consists of a butt and a slanted portion, handle as above but inoculate the butt first by stabbing the needle to the bottom of the medium and then streak the surface.

Isolation

Isolation is done in a sequential manner as follows.

- **Broth culture:** The first step for isolation and purification of bacteria involves development of 24 hour culture containing viable cells. The sample is added to 15 ml of sterile nutrient broth and incubated at 37°C for 24 hours. The advantage is that all cells become metabolically active including the spores, which germinate to produce viable vegetative cells. It also increases the number of inherent microbes.
- **Serial dilution:** Since the earlier step results in increase of number of cells, next the bacterial suspension is diluted by serial dilution technique.

One ml of this 24 hour culture is added to 9.0 ml of sterile DW to get 10^{-1} dilution. From this tube, one ml suspension is once again added to 9.0 ml of DW. In this manner, by stepwise repeated dilution bacterial suspensions ranging from 10^{-1} to 10^{-10} are obtained. (See chapter 3 for details).

- **Culture of microbes:** The bacteria are inoculated on nutrient medium by Streak plate method. 20 ml of liquid medium is poured into Petri dishes and cooled till it solidifies. One ml of bacterial suspension from successive dilutions is streaked onto the surface of solidified agar with the help of a transfer loop and incubated at 37 °C for 48 hrs. The plates are observed for colony formation. Cells from a single colony are picked and mixed in sterile broth / normal saline/ sterile distilled water and the whole procedure is repeated till pure cultures develop i.e. all colonies in the Petri plate are identical. Identification of these in these done by standard methods.

2.3 Identification

Identification of the pure isolates is done by methods involving direct microscopic examination, study of cultural characteristics as well as physiological and biochemical properties of the isolate.

The following characteristics are studied for identification

1. Morphology
2. Staining reactions
3. Growth on differential media
4. Colony characteristics
5. Biochemical properties
6. Antibiotic susceptibility
7. Serology
8. Pathogenicity
9. Flow cytometry
10. Phage typing
11. Protein and lipid analysis
12. Genome analysis
13. Comparison of nucleotide sequences

2.3.1 Direct microscopic examination

Bacteria are visualized under the compound light (bright field) microscope, for observing size, shape, and presence or absence of motility, spore formation and capsulation.

A. **Motility:** Movement is studied by hanging drop method. Vaseline is applied around the depression of the cavity slide. One drop of unstained, live bacterial isolate is aseptically transferred to a clean cover slip and the cavity slide is inverted over it. The slide is then turned right side up so as to suspend the liquid containing bacterial isolate in the well for microscope observation. An unstained wet film or hanging drop preparation is examined under the light microscope for observation of motility. An unstained wet film may also be examined under phase contrast microscope for demonstration of motility of spirochetes against a dark background.

B. **Staining Reactions:** In order to study the morphology of bacteria, cells are killed by heat and fixed on the slide. These fixed bacteria are then stained and studied for size, shape, arrangement, spore formation, capsulation, flagellation, etc. In all staining reactions age of the culture is important. In older cultures, staining characteristics either vary or are not brought out well.

- **Gram stain:** Place a drop of sterile water on a microscope slide. Make a light suspension of test culture in the water. Allow to air-dry and heat fix the film. Cool and flood with crystal violet for 60 seconds. Wash with water. Flood with Lugol's (or Gram's) iodine for 60 seconds. Wash as before and drain off excess water. Decolourise with acetone and wash off immediately. Counterstain with dilute carbol fuchsin for 30 seconds. Wash and blot dry. Gram-positive cells appear purple and negative appear red.
- **Ziehl-Neelsen Stain:** Flood a fixed slide with strong carbol fuchsin solution. Heat the slide until it steams and keep steaming for 5 minutes but do not boil. Do not allow the slide to dry out. Wash the slide with sterile water. Treat with 3% acid alcohol for 10 minutes or until only a suggestion of pink remains on the film.

Wash the film with water. Counterstain for 15-30 seconds with methylene blue. Wash and blot dry. Acid-fash bacilli appear bright red while tissue cells and other bacteria stain blue.

- **Endospore staining:** Several days old culture of bacteria is stained and examined for endospore formation. Endospore staining is done by preparing a smear on a clean slide and heat fixing it. The smear is flooded and kept saturated with 5% malachite green while heating continuously for 5 minutes. Subsequently, smear is rinsed with water and counterstained with safranin for 30 seconds. The slide is once again washed, dried and investigated for endospore formation. Cells with endospore appear green while vegetative cells appear red.
- **Spore Staining by modified Ziehl-Neelsen method:** Stain the fixed film with strong carbol fuchsin for 3-5 minutes. Heat until steam rises. Wash with water. Treat with 0.25% sulphuric acid for 15-60 seconds. Wash with water. Counterstain with 1% aqueous methylene blue for 5 minutes. Wash and blot dry. Bacterial spores are seen as red structures, vegetative cells stain blue.
- **Capsule staining:** Bacterial cultures are stained with India ink. Place the cover slip over the test culture and remove excess ink. Slide is then observed for capsule stain. Capsule forms a clear halo around the cell against a dark background.
- **Capsule staining (Relief staining with eosin):** Place a drop of broth culture on one end of a microscope slide. Add one drop of eosin solution and leave for one minute. Take a second slide and draw its edge back to contact the stained suspension. Holding the second slide at 45 degrees spread a thin layer of fluid along the first slide by a continuous forward movement. Allow the file to air-dry then examine under oil-immersion. Background material and cells stain red. Capsular material appears as an unstained halo around the cells.

C. **Measurement of cell size:** is done by using a micrometer.

2.3.2 Cultural characteristics

Colony characters of three-days old pure cultures are studied on solid medium.

1. **Colony size:** Pinpoint / Extremely small / Pinpoint to small / 5-10 mm
2. **Colony shape:** Evenly circular / Irregular/ Notched/ Thread like or Filamentous/ Rhizoidal/ Wavy/ Punctiform/ Circular
3. **Colony margin:** Entire/ Undulate/ Lobate/ Curled/ Serrate/ Filamentous/
4. **Colony elevation:** Flat/ Raised/ Convex/ Pulvinate/ Umbonate
5. **Colony surface:** Smooth; Shiny glistening/ Routh; dull, granular or matte/ Wrinkled; folder/ Dry: powdery, brittle/ Mucoid; slimy or gummy
6. **Colony consistency:** Butyrous or buttery. Viscous, Stringy, Rubbery, Dry brittle or powdery
7. **Optical features:** Opaque/ Transparent/ Translucent/ Opalescent
8. **Chromogenisis or pigmentation:** Production and retention of water-soluble intracellular pigments due to which colony is clored. E.g. pink colonies of Flectobacillus major, red colonies of *Serratia marsecens*, golden colonies of *Staphylococcus aureus*.
9. **Production and diffusion of water-soluble pigments:** which colour the medium. e.g. bluish green color of medium due to secretion of pyocyanin by *Psedudomonas aeruginosa*.
10. **Production and diffusion of sparingly water-soluble pigments:** which accumulate as colored crystals near the colony. e.g. green crystals of chlororaphin secreted by *Pseudomonas chlororaphis*.
11. **Production and diffusion of water-soluble and fluorescent pigments:** due to pigment medium glows white or blue green when exposed to UV rays. e.g. secretion of fluorescent pyoverdin by *Pseudomonas aeruginosa*.
12. **Amount of growth in broth:** Scanty/ Moderate/ Abundant.
13. **Oxygen requirement:** Bacteria are inoculated into nutrient broth to study the oxygen requirement of pure isolates. Depending on growth they are grouped as:
    - Aerobic: Growth on surface of medium

- Microaerobic: Dispersed growth
- Anaerobic: Growth at bottom of medium.

14. **Hemolytic behavior:** Hemolysis of RBCF by bacteria is studied by culturing them on blood agar. Based on the hemolytic behavior they are classified as:
    1. Alpha hemolytic: complete hemolysis of RBC
    2. Beta hemolytic: partial hemolysis of RBC
    3. Gamma hemolytic: non hemolytic

2.3.3 Biochemical and physiological properties

Bacteria and other unicellular microbes lack extensive digestive system. Their nutrition is dependent on substances absorbed by the cell membrane. Since, macromolecules cannot pass through the membrane they have to be broken down into simpler substances by enzymatic activity outside the cell. The microbes therefore secrete extracellular enzymes to degrade polysaccharides, proteins, lipids etc. The breakdown products are smaller in size, have low molecular weight and can be easily absorbed by the general body surface and later used for metabolic activity within the cell. All organisms have a preference for a particular substrate and secrete enzymes accordingly, therefore detection of exo-enzyme secretion is a good method to identify and differentiate microbes. Presence or absence of enzyme activity is studied by providing the substrate in the medium. Digestion of the substrate is an indication of positive reaction. In some cases the reaction is clearly visible as change in either color or property of medium, but in cases where the reaction is invisible indicator dyes are introduced in the medium. The dyes changes color according to pH of medium. Any change in biochemical composition of the medium is then reflected by change in color of the dye.

2.3.4 Antibiotic Sensitivity Testing

The sensitivity of the organism isolated from the patient to antimicrobial agents is tested by placing filter paper discs impregnated with the antibiotic on culture plates preceded with the organism to be tested and by judging the degree of sensitivity by the size of inhibition zones resulting after overnight

incubation, during which adequate interaction between the bacterium and the drug takes place.

2.3.5 Animal Pathogenicity

Various experimental models used in diagnostic microbiology laboratory are guinea pig, rabbit, armadillo and monkey. Various routes of inoculation are: intradermal, subcutaneous, intramuscular, intraperitoneal, intracerebral and intravenous. Oral and nasal routes can also be used. The identification of the organism is carried out on the basis of clinical and postmortem findings and cultural characteristics.

2.3.6 Serological Methods

Antibodies are highly selective in terms of the surface proteins to which they bind. Thus by studying antibody – antigen binding it is possible to distinguish one bacterial species from the other, or even one strain from the other on the basis of their surface proteins. Serological methods detect antibodies by the following methods – Agglutination tests, ELISA, and Western blots.

2.3.7 Flow Cytometry

This technique uses methods, which analyze cells suspended in a liquid medium by light, electrical conductivity or fluorescence as they pass through a small orifice.

2.3.8 Phage Typing

Bacteriophages are species – specific. Infection by phages is used to distinguish bacterial species and strains.

2.3.9 Protein Analysis (gel electrophoresis, SDS-PAGE, establishment of clonality)

Identification is done on the basis of the size and other differences between proteins among different organisms by separating proteins using gel ecectrophoresis.

2.3.10 SDS-PAGE

Sodium dodecyl sulphate (also known as sodium lauryl sulphate) – Polyacrylamide gel electrophoresis can detect small differences between isolates and also establish clonality.

2.3.11 Comparison of Nucleotide Sequences

Methods like Southern blot, nucleic acid hybridization, RFLP,

DNA fingerprinting are used to identify bacteria by determining the exact nucleotide sequence in the genome of different species, strains etc.

References

O'Leary, W. (ed.). 1990. Practical Handbook of Microbiology. Boca Raton: CRC Press.

Sharma. K. (ed.). 2007. Manual of Microbiology: tools and techniques. 1st ed. Kent: Anshan Ltd.

Appendix B

Result of settle plates

Table B-1 Result of microorganism culture on TSA plates in the separated room

	Laminar Airflow Hood						Preparation room
Plate No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
22-Oct-07	NG	NG	2 cfu *Staph. Coag.neg.*, 1 cfu *Micrococcus* spp., 1 cfu *Corynebacterium* spp.	1 cfu *Micrococcus spp.*, 1 cfu *Corynebacterium spp.*	1 cfu *Corynebacterium spp.*	NG	2 cfu *Staph. Coag.neg.*, 2 cfu *Micrococcus spp.*, 2 cfu *Pseudomonas spp* 2 cfu *K. pneumoniae*
24-Oct-07	NG	1 cfu *Asp. flavus*	1 cfu *Aspergillus spp.*	1 cfu *Bacillus spp.*, 5 cfu *Staph. Coag.neg.*, 1 cfu *Micrococcus spp.*	NG	NG	NG
26-Oct-07	NG	NG	NG	1 cfu *Pseudomonas spp* 1 cfu *Aspergillus spp.*, 1 cfu *Paecilomyces spp.*, 1 cfu *Micrococcus spp.*	NG	NG	4 cfu *K. pneumoniae* 4 cfu *Micrococcus spp.*, 1 cfu *Corynebacterium spp.* 3 cfu *Staph. Coag.neg.*, 1 cfu *Asp. Fumigatus*, 6cfu Hyaline fungi,
29-Oct-07	2 cfu *Corynebacterium spp.*	NG	1 cfu *Aspergillus spp.* 1 cfu *K. pneumoniae*	4 cfu Hyaline fungi, 1 cfu *Staph. Coag.neg.*, 2 cfu *Corynebacterium spp.* 3 cfu *Bacillus spp.*,	NG	NG	1 cfu *Aspergillus spp.*, 4 cfu *Micrococcus spp.*, 6 cfu *Corynebacterium spp.* 6 cfu *Staph. Coag.neg.*,
31-Oct-07	NG	NG	NG	4 cfu *Micrococcus spp.*, 2 cfu *Corynebacterium spp.* 2 cfu *Staph. Coag.neg.*, 1 cfu Hyaline fungi	NG	NG	NG
2-Nov-07	1 cfu *Aspergillus spp.*, 2 cfu *K. pneumoniae*	1 cfu Hyaline fungi 1 cfu *Bacillus spp.*	NG	1 cfu Hyaline fungi, 1 cfu *Micrococcus spp.*	2 cfu *Bacillus spp.*	NG	3 cfu *K. pneumoniae* 5 cfu *Micrococcus spp.*, 3 cfu *Bacillus spp.*
5-Nov-07	NG	NG	NG	NG	NG	NG	3 cfu *K. pneumoniae* 3 cfu *Micrococcus spp.*, 1 cfu *Bacillus spp.*, 3 cfu *Corynebacterium spp.*

Abbreviations: Oct=October, Nov=November, Dec=December, 07=2007, NG=No microorganism growth cfu= colony forming unit
Staph. Coag. Neg. =Staphylococcus coagulase negative , Hyaline fungi=Hyaline fungi(non-spore),*Asp =Aspergillus* , *K = Klebsiella*

Table B1 Result of microorganism culture on TSA plates in the separated room (continued)

	Laminar Airflow Hood						Preparation room
Plate No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
7-Nov-07	NG	1 cfu *Staph. Coag.neg.*,	1 cfu *Micrococcus spp.*	1 cfu *Bacillus spp.*, 1 cfu *Staph. Coag.neg.*, 1 cfu *Micrococcus spp.*	NG	NG	1 cfu *Bacillus spp.*, 2 cfu *Acremonium spp.*, 2 cfu *Micrococcus spp.*
9-Nov-07	NG	NG	2 cfu *K. pneumoniae*	1 cfu *Corynebacterium spp.*	NG	NG	3 cfu *Micrococcus spp.* 3 cfu *Bacillus spp.*, 3 cfu *Corynebacterium spp.* 3 cfu *Staph. Coag.neg.*, 1 cfu *Aspergillus spp.*, 1 cfu *Curvularia spp.*
12-Nov-07	NG	NG	1 cfu *Pseudomonas spp.*	2 cfu *Bacillus spp.*, 3 cfu *Staph. Coag.neg.*, 6 cfu *Micrococcus spp.* 1 cfu *Asp. Niger*	1 cfu *Micrococcus spp.*	NG	2 cfu *Bacillus spp.*, 1 cfu *Acremonium spp.*, 6 cfu *Micrococcus spp.* 2 cfu *Staph. Coag.neg.*,
14-Nov-07	2 cfu *Staph. Coag.neg.*, 4 cfu *Micrococcus spp.* 1 cfu Hyaline fungi 2 cfu *Corynebacterium spp.*	2 cfu *Bacillus spp.*, 2 cfu *Staph. Coag.neg.*, 4 cfu *Micrococcus spp.*	3 cfu *Bacillus spp.*, 2 cfu *Staph. Coag.neg.*, 3 cfu *Micrococcus spp.* 1 cfu Hyaline fungi	3 cfu *Bacillus spp.*, 3 cfu *Staph. Coag.neg.*, 5 cfu *Micrococcus spp.* 1 cfu Hyaline fungi 1 cfu *Acremonium spp.*, 1 cfu *Asp. Niger*	8 cfu *Bacillus spp.*, 4 cfu *Staph. Coag.neg.*, 2 cfu *Micrococcus spp.* 1 cfu *Acremonium spp.*, 1 cfu *Asp. Niger* 6 cfu *Corynebacterium spp.*	NG	2 cfu *Bacillus spp.*, 3 cfu *Staph. Coag.neg.*, 8 cfu *Micrococcus spp.*

Abbreviations: Oct=October, Nov=November, Dec=December, 07=2007, NG=No microorganism growth cfu= colony forming unit
Staph. Coag. Neg.=Staphylococcus coagulase negative, Hyaline fungi=Hyaline fungi(non-spore),*Asp=Aspergillus, K = Klebsiella*

Table B-1 Result of microorganism culture on TSA plates in the separated room (continued)

	Laminar Airflow Hood						Preparation room
Plate No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
16-Nov-07	NG	NG	NG	4 cfu *Bacillus spp.*, 3 cfu *Staph. Coag.neg.*, 4 cfu *Micrococcus spp.* 1 cfu Hyaline fungi 2 cfu *Corynebacterium spp.* 1 cfu *Aspergillus spp.*	1 cfu *Bacillus spp.*,	NG	4 cfu *Bacillus spp.*, 4 cfu *Staph. Coag.neg.*, 4 cfu *Micrococcus spp.* 1 cfu *Acremonium spp.*, 4cfu *Corynebacterium spp.*
19-Nov-07	NG	NG	NG	3 cfu *Staph. Coag.neg.*, 4 cfu *Micrococcus spp.* 2 cfu *K. pneumoniae*	NG	NG	2 cfu *Bacillus spp.*, 2 cfu *Staph. Coag.neg.*, 6 cfu *Micrococcus spp.*
21-Nov-07	NG	1 cfu *Micrococcus spp.*	NG	1 cfu *Staph. Coag.neg.*, 2 cfu *Micrococcus spp.*	NG	NG	3 cfu *Staph. Coag.neg.*, 6 cfu *Micrococcus spp.* 3 cfu *Corynebacterium spp.* 1 cfu *Acremonium spp.*, 1 cfu *Aspergillus spp.*
23-Nov-07	NG	NG	NG	3 cfu *Bacillus spp.*, 3 cfu *Staph. Coag.neg.*, 4 cfu *Micrococcus spp.* 3cfu *Corynebacterium spp.*	NG	NG	1 cfu *Bacillus spp.*, 4 cfu *Staph. Coag.neg.*, 8 cfu *Micrococcus spp.* 1cfu *Corynebacterium spp.*
26-Nov-07	NG	NG	NG	NG	4 cfu *Micrococcus spp.* 2cfu *Corynebacterium spp.*	NG	1 cfu *Bacillus spp.*, 1 cfu *Staph. Coag.neg.*, 2 cfu *Micrococcus spp.* 1cfu *Corynebacterium spp.*

Abbreviations: Oct=October, Nov=November, Dec=December, 07=2007, NG=No microorganism growth cfu= colony forming unit
Staph. Coag. Neg.=Staphylococcus coagulase negative, Hyaline fungi=Hyaline fungi(non-spore),*Asp=Aspergillus, K = Klebsiella*

Table B-1 Result of microorganism culture on TSA plates in the separated room (continued)

	Laminar Airflow Hood						Preparation room
Plate No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
28-Nov-07	NG	NG	NG	3 cfu Staph. Coag.neg., 7 cfu *Micrococcus spp.* 4 cfu *Corynebacterium spp.*	NG	NG	4 cfu Staph. Coag.neg., 3 cfu *Micrococcus spp.* 1 cfu *Acremonium spp.,*
30-Nov-07	NG	1 cfu *Bacillus spp.*	NG	NG	NG	NG	1 cfu *Corynebacterium* 1 cfu Staph. Coag.neg.,
3-Dec-07	NG	NG	NG	1 cfu Staph. Coag.neg., 1 cfu *Corynebacterium spp.*	NG	NG	2 cfu *Bacillus spp.* 2 cfu Staph. Coag.neg., 1 cfu *Micrococcus spp.* 1 cfu *Acremonium spp.,* 1 cfu *Asp. Glaucus*
5-Dec-07	NG	NG	NG	2 cfu *Bacillus spp.* 2 cfu Staph. Coag.neg., 3 cfu *Micrococcus spp.* 1 cfu *Corynebacterium spp.*	1 cfu *Micrococcus spp.*	NG	2 cfu Staph. Coag.neg., 2 cfu *Micrococcus spp.*
7-Dec-07	NG	NG	NG	1 cfu Staph. Coag.neg., 1 cfu *Micrococcus spp.*	NG	NG	3 cfu Staph. Coag.neg., 3 cfu *Micrococcus spp.* 2 cfu *Corynebacterium spp.* 1 cfu *Serratia spp.*
10-Dec-07	NG	NG	NG	1 cfu *Micrococcus spp.*	1 cfu *Micrococcus spp.*	NG	3 cfu *Bacillus spp.* 3 cfu Staph. Coag.neg., 3 cfu *Micrococcus spp.* 1 cfu *Acremonium spp.,*

Abbreviations: Oct=October, Nov=November, Dec=December, 07=2007, NG=No microorganism growth cfu= colony forming unit
Staph. Coag. Neg.=Staphylococcus coagulase negative , Hyaline fungi=Hyaline fungi(non-spore),*Asp=Aspergillus, K = Klebsiella*

Table B-1 Result of microorganism culture on TSA plates in the separated room (continued)

	Laminar Airflow Hood						Preparation room
Plate No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
12-Dec-07	NG	NG	NG	3 cfu *Corynebacterium spp.* 3 cfu *Staph. Coag.neg.*, 3 cfu *Micrococcus spp.*	1 cfu *Micrococcus spp.*	NG	3 cfu *Corynebacterium spp.* 3 cfu *Staph. Coag.neg.*, 6 cfu *Micrococcus spp.* 3 cfu *Bacillus spp.*
14-Dec-07	2 cfu *Pseudomonas spp.*	1 cfu *Bacillus spp.*	NG	2 cfu *Bacillus spp.* 3 cfu *Staph. Coag.neg.*, 4 cfu *Micrococcus spp.* 3 cfu *Corynebacterium spp.*	NG	NG	3 cfu *Corynebacterium spp.* 3 cfu *Staph. Coag.neg.*, 4 cfu *Micrococcus spp.* 3 cfu *Bacillus spp.*

Abbreviations: Oct=October, Nov=November, Dec=December, 07=2007, NG=No microorganism growth cfu= colony forming unit
Staph. Coag. Neg.=Staphylococcus coagulase negative , Hyaline fungi=Hyaline fungi(non-spore),*Asp=Aspergillus, K = Klebsiella*

Table B-2 Result of microorganism growth on TSA plates in the cleanroom

Plate No.	Laminar Airflow Hood						Preparation room
	1	2	3	4	5	6	7
3-Nov-08	NG	NG	1 cfu *Bacillus spp.*, 4 cfu *Micrococcus spp.*	NG	NG	NG	1 cfu *Bacillus spp.*, 1 cfu *Fusarium spp.*
5-Nov-08	NG	NG	NG	NG	NG	NG	1 cfu *Micrococcus spp.*, 1 cfu *Nocardia spp.*
7-Nov-08	NG	NG	NG	NG	NG	NG	NG
10-Nov-08	NG	NG	NG	NG	NG	NG	NG
12-Nov-08	NG	NG	NG	NG	NG	NG	NG
14-Nov-08	NG	NG	NG	NG	NG	NG	NG
17-Nov-08	NG	NG	1 cfu *Micrococcus spp.*	NG	NG	NG	NG
19-Nov-08	NG	NG	NG	NG	NG	NG	NG
21-Nov-08	NG	NG	NG	NG	NG	NG	NG
24-Nov-08	NG	1 cfu *Micrococcus spp.*	NG	NG	NG	NG	1 cfu *Micrococcus spp.*
26-Nov-08	NG	NG	NG	NG	NG	NG	1 cfu *Micrococcus spp.*
28-Nov-08	NG	NG	NG	NG	NG	NG	NG

Abbreviations: Nov=November, Dec=December, 08=2008, NG=No microorganism growth, cfu=colony forming unit

Table B-2 Result of microorganism growth on TSA plates in the cleanroom (continued)

Plate No.	Laminar Airflow Hood						Preparation room
	1	2	3	4	5	6	7
1-Dec-08	1 cfu *Micrococcus spp.*	NG	NG	NG	NG	NG	2 cfu *Micrococcus spp.*
3-Dec-08	NG	NG	NG	NG	NG	NG	2 cfu *Bacillus spp.*
5-Dec-08	NG	NG	NG	NG	NG	NG	NG
8-Dec-08	NG	NG	NG	NG	NG	NG	NG
10-Dec-08	NG	NG	NG	NG	NG	NG	1 cfu *Micrococcus spp.*
12-Dec-08	NG	NG	NG	NG	NG	NG	NG
15-Dec-08	NG	NG	NG	NG	NG	NG	1 cfu *Bacillus spp.*
17-Dec-08	NG	NG	1 cfu *Bacillus spp.*	NG	NG	NG	NG
19-Dec-08	*Micrococcus spp.*	NG	NG	NG	NG	NG	NG
22-Dec-08	NG	NG	NG	NG	NG	NG	NG
24-Dec-08	NG	NG	NG	NG	NG	NG	NG
26-Dec-08	NG	NG	NG	NG	NG	NG	NG

Abbreviations: Nov=November, Dec=December, 08=2008, NG=No microorganism growth, cfu=colony forming unit

Appendix C

Cost analysis

Cost analysis of PN prepared from the separated room during 22 October 2007-16 December 2007

Table C-1 Labor cost of PN prapared in the separated room

Position	Wages (Baht/week) ❶	PN work hours (hours/week) ❷	Overall work hours (hours/week) ❸	PN wages (Baht/week) ❷/❸x❶	PN wages (Baht/8 week) ❷/❸x❶x8
Pharmacist	6,300.00	49	56	5,512.50	44,100.00
Pharmacist	6,300.00	49	56	5,512.50	44,100.00
Pharmacist Assistant	2,800.00	56	56	2,800.00	22,400.00
Pharmacist Assistant	2,800.00	40	56	2,000.00	16,000.00
Pharmacist Assistant	2,800.00	20	56	1,000.00	8,000.00
Worker	1,680.00	5	56	150.00	1,200.00
Messenger	2,240.00	5	56	200.00	1,600.00
				Labor cost	**137,400.00**

Table C-2 Capital cost of PN prepared in the separated room

Item	quantity	unit price (Baht/item)	Lifetime (year)	Depreciation rate (Baht/year)	Depreciation rate (Baht/8 weeks)	Maintenance cost (Baht/year)	Maintenance cost (Baht/8 weeks)	total cost (Baht/8 weeks)
	❶	❷	❸	❶×❷/❸=❹	❹x56/365=❺	❻	❻/365x56=❼	❺+❼
LAF	1	499,750.00	5	99,950.00	15,334.79	50,000.00	7,671.23	23,006.02
Refrigerator	1	25,669.30	5	5,133.86	787.66	1,000.00	153.42	941.08
Computer&Printer	1	54,680.00	3	18,226.67	2,796.42	-	-	2,796.42
Office furnitures (1 desk+4 chairs)	1	12,208.00	8	1,526.00	234.13	-	-	234.13
Office cabinet	1	166,000.00	8	20,750.00	3,183.56	-	-	3,183.56
Air conditioner	2	28,500.00	5	11,400.00	1,749.04	4,800.00	736.44	2,485.48
Telephone	1	2,250.00	1	2,250.00	345.21	-	-	345.21
Stainless stool	2	1,615.00	8	403.75	61.95	-	-	61.95
Stainless shelf	1	9,050.00	8	1,131.25	173.56	-	-	173.56
Stainless cart	2	4,000.00	8	1,000.00	153.42	-	-	153.42
Sterile gown	4	350.00	1	1,400.00	214.79	-	-	214.79
Work area development	-	140,000.00	5	28,000.00	4,295.89	-	-	4,295.89
							Capital cost	37,891.51

Table C-3 Material cost: Nutrient cost and volume of PN prepared in the separated room

Nutrients and Electrolytes	unit price (Baht/ml)	Pediatrics PN		Adult PPN		Adult TPN	
		Volume (ml)	Cost (Baht)	Volume (ml)	Cost (Baht)	Volume (ml)	Cost (Baht)
10% Amiparen®	0.55	104,975.00	57,736.25	312,150.00	171,682.50	315,040.00	173,272.00
10%Aminoven infant®	2.46	13,509.50	33,233.37				
15%Aminoplasmal®	0.90	27,007.00	24,306.30			84,766.00	76,289.40
7.2%Kidmin®	0.57			2,800.00	1,596.00	17,125.50	9,761.54
8%Aminoleban®	0.59	600.00	354.00	1,625.00	958.75	2,000.00	1,180.00
Dextrose	0.12	174,358.20	20,922.98	252,680.00	30,321.60	394,470.00	47,336.40
3% NaCl	0.06	84,407.40	5,064.45	126,980.00	7,618.80	127,894.00	7,673.64
15%KCl	0.49	5,632.70	2,760.03	502.00	245.98	1,884.00	923.16
8.71%K_2HPO_4	2.89	8,738.60	25,254.56	17,582.00	50,811.98	14,421.00	41,676.69
KAc	1.55	600.50	930.78	6,736.00	10,440.80	6,947.30	10,768.32
NaAc	1.20	11.50	13.80	1,360.70	1,632.84	1,718.70	2,062.44
50%$MgSO_4$	3.21	1,912.00	6,137.52	25,405.00	8,155.01	2,391.00	7,675.11
10%Calcium gluconate	0.65	13,280.10	8,632.07	12,033.00	7,821.45	11,197.00	7,278.05
Zinc sulfate	2.40	2,174.40	5,218.56				
Heparin	17.98	122.29	2,198.78				
SWFI	0.04	223,998.00	8,959.92	514,970.00	20,598.80	83,028.00	3,321.12
Total		**661,327.19**	**201,723.37**	**1,274,823.70**	**311,884.51**	**1,062,882.50**	**389,217.87**
Preparation ratio		**0.2205**		**0.4251**		**0.3544**	

Table C-4 Material cost: Chemical cost of PN prepared in the separated room

Chemical	unit price (Baht/ml)	Volume (ml)	Cost (Baht)
Povidone Iodine	0.25	30.00	7.50
70% Alcohol	0.07	56,000.00	3,920.00
Hibiscrub	0.27	2,000.00	540.00
Liquid soap	0.07	1,867.00	130.69
Sterile water	0.03	1,867.00	56.01
		Total	**4,654.20**

Table C-5 Material cost: Medical devices cost of PN prepared in the separated room

Medical devices	unit price (Baht/pcs)	Item used (pcs/8weeks)	Cost (Baht)
Gauze	1.00	16,800.00	16,800.00
Face mask	2.00	280.00	560.00
Hair cover	1.00	280.00	280.00
Soluset	115.00	280.00	32,200.00
Needle No.18	1.00	5,600.00	5,600.00
Needle No.20	1.00	5,600.00	5,600.00
Needle No.25	1.00	56.00	56.00
Syringe 1 ml	4.00	56.00	224.00
Syringe 3 ml	2.00	112.00	224.00
Syringe 10 ml	4.00	112.00	448.00
Syringe 20 ml	7.00	560.00	3,920.00
Syringe 50 ml	20.00	448.00	8,960.00
Sterile gloves	25.00	280.00	7,000.00
Stopper	13.00	112.00	1,456.00
Parafilm	1,875.00	0.50	937.50
Plaster	1.00	56.00	56.00
		Total	**84,321.50**

Table C-6 Material cost: Office supplies cost of PN prepared in the separated room

Items	Unit prices (Baht/unit)	Item used (unit/8weeks)	cost (Baht)
Printer cartridge	2,815.85	0.47	1,323.45
A4 paper (70 grams)	73.48	2.67	196.20
A5 paper (70 grams)	42.69	1.87	79.84
Liquid glue	4.80	1.87	8.98
Punch	21.00	0.16	3.36
Stapler	181.35	0.16	29.02
Staples	3.68	3.74	13.77
Paper clip	5.45	3.74	20.39
Date stamp	17.00	0.16	2.72
Pencil sharpener	128.08	0.16	20.50
Adhesive tape	23.76	3.74	88.87
Ink	3.72	0.63	2.35
Scissor	26.60	0.63	16.76
Adhesive tape stand	20.38	0.16	3.27
Plastic folder	0.66	3.15	2.08
Pencil	3.20	5.60	17.92
Marker pen	19.76	5.60	110.66
Paper basket	150.00	0.32	48.00
Hard folder	27.42	0.48	0.48
Eraser	2.30	1.87	4.31
Ruler	1.20	0.63	0.76
Correcting pen	58.00	1.87	108.46
A5 Sticker	1,283.33	1.12	1,437.30
Notebook	100.50	1.87	187.94
Plastic box No.830	67.00	3.07	205.69
Waste bag	1.02	112.00	114.24
6cmX8cm Zipper bag	0.09	5,600.00	504.00
16cmX22cm Zipper bag	0.82	1,120.00	918.40
5X11 Plastic bag	0.25	560.00	140.00
8X16 Plastic bag	0.23	1,120.00	257.60
12X20 Plastic bag	0.45	1,120.00	504.00
Slipper	22.84	3.74	85.43
Plastic bin	258.98	0.47	121.73
Bloom	18.00	0.94	16.92
Mop	50.00	0.63	31.50
Rubber band	50.15	9.34	468.41
Plastic basket	37.45	3.07	114.98
36W Fluorescent lamp	29.96	1.87	56.03
		Total	**7,266.32**

Table C-7 Comparison of material cost of PN prepared in the separated room and cleanroom

Material cost	Separated room			Cleanroom		
	Formula (baht)			Formula (baht)		
	Pediatric	Adult PPN	Adult TPN	Pediatric	Adult PPN	Adult TPN
Nutrient cost	201723.37	311884.51	389217.87	235727.03	247782.14	569868.69
Chemical cost	4654.20			10254.20		
Medical devices cost	84321.50			95201.50		
Office supplies cost	7266.32			7946.84		
Water supply cost	42.84			42.84		
Electricity cost	7973.28			9315.60		

Cost analysis of PN prepared from the cleanroom during 3 November 2008-28 December 2008

Table C-8 Labor cost of PN prepared in the cleanroom

Position	Wages (Baht/week) ❶	PN work hours (hours/week) ❷	Overall work hours (hours/week) ❸	PN wages (Baht/week) ❷/❸x❶	PN wages (Baht/8 week) ❷/❸x❶x8
Pharmacist	6,300.00	49	56	5,512.50	44,100.00
Pharmacist	6,300.00	49	56	5,512.50	44,100.00
Pharmacist assistant	2,800.00	56	56	2,800.00	22,400.00
Pharmacist assistant	2,800.00	40	56	2,000.00	16,000.00
worker	1,680.00	5	56	150.00	1,200.00
messenger	2,240.00	5	56	200.00	1,600.00
				Labor cost	129,400.00

Table C-9 Capital cost of PN prepared in the cleanroom

Item	quantity	unit price (Baht/item)	Lifetime (year)	Depreciation rate (Baht/year)	Depreciation rate (Baht/8 weeks)	Maintenance cost (Baht/year)	Maintenance cost (Baht/8 weeks)	total cost (Baht/8 weeks)
	❶	❷	❸	❶×❷/❸=❹	❹x56/365=❺	❻	❻/365x56=❼	❺+❼
LAF	1	499,750.00	5	99,950.00	15,334.79	50,000.00	7,671.23	23,006.02
Refrigerator	1	55,000.00	5	11,000.00	1,684.67	1,000.00	153.42	1,838.09
Computer&Printer	2	54,680.00	3	36,453.33	5,592.84	-	-	5,592.84
Office chair	3	4,300.00	8	1,612.50	247.40	-	-	247.40
Intercom	1	2,950.00	1	2,950.00	452.61	-	-	452.61
Telephone	1	2,990.00	1	2,990.00	458.74	-	-	458.74
Fax	1	5,990.00	1	5,990.00	919.01	-	-	919.01
Stainless stool	2	3,300.00	8	825.00	126.58	-	-	126.58
Stainless shelf	1	18,000.00	8	2,250.00	345.21	-	-	345.21
Stainless table	1	16,700.00	8	2,087.50	320.27	-	-	320.27
Stainless cart	2	5,000.00	8	1,250.00	191.78	-	-	191.78
Stainless cart	1	7,300.00	8	912.50	140.00	-	-	140.00
Stainless cart	1	4,000.00	8	500.00	76.71	-	-	76.71
Construction cost	-	695,200.00	5	139,040.00	21,332.16	-	-	21,332.16
							Capital cost	55,047.42

Table C-10 Material cost: Nutrient cost and volume of PN in the cleanroom

Nutrients and Electrolytes	unit price (Baht/ml)	Pediatric PN		Adult PPN		Adult TPN	
		Volume (ml)	Cost (Baht)	Volume (ml)	Cost (Baht)	Volume (ml)	Cost (Baht)
10% Amiparen®	0.55	107,833.00	59,308.15	242,650.00	133,457.50	336,550.00	185,102.50
10%Aminoven infant®	2.46	26,278.50	64,645.11				
15%Aminoplasmal®	0.9	29,330.00	26,397.00			189,803.00	170,822.70
7.2%Kidmin®	0.57			4,539.00	2,587.23	5,221.00	2,975.97
8%Aminoleban®	0.59	462.50	272.88	3,720.00	2,194.80		
Dextrose	0.12	193,478.00	23,217.36	196,950.00	23,634.00	523,430.00	62,811.60
3% NaCl	0.06	67,764.40	4,065.86	101,160.00	6,069.60	145,050.00	8,703.00
15%KCl	0.49	4,263.50	2,089.12	140.00	68.60	1,905.50	933.70
8.71%K_2HPO_4	2.89	8,436.60	24,381.77	14,306.00	41,344.34	19,293.00	55,756.77
KAc	1.55	1,298.40	2,012.52	5,485.70	8,502.85	7,288.70	11,297.33
NaAc	1.2	7.70	9.24	1,395.70	1,674.70	2,691.00	3,229.20
50%$MgSO_4$	3.21	1,981.70	6,361.26	1,987.00	6,378.27	2,801.00	8,991.21
10%Calcium gluconate	0.65	11,757.40	7,642.31	9,429.00	6,128.85	12,082.00	7,853.30
Zinc sulfate	2.4	2,756.00	6,614.44				
Heparin	17.98	122.22	2,197.52				
SWFI	0.04	162,812.25	6,512.49	393,535.00	15,741.40	38,670.00	51,391.41
Total		**618,582.17**	**235,727.03**	**975,297.40**	**247,782.14**	**1,284,785.20**	**569,868.69**
Preparation ratio		**0.2149**		**0.3388**		**0.4463**	

Table C-11 Material cost: Chemical cost of PN prepared in the cleanroom

Chemical	unit price (Baht/ml)	Volume (ml)	Cost (Baht)
Povidone Iodine	0.25	30.00	7.50
70% Alcohol	0.07	56,000.00	3,920.00
Benzalkonium chloride	0.05	112,000.00	5,600.00
Hibiscrub	0.27	2,000.00	540.00
Liquid soap	0.07	1,867.00	130.69
Sterile water	0.03	1,867.00	56.01
		Total	**10,254.20**

Table C-12 Material cost: Medical devices cost of PN prepared in the cleanroom

Medical devices	unit price (Baht/pcs)	Item used (pcs/8weeks)	Cost (Baht)
Gauze	1.00	22,400.00	22,400.00
Face mask	2.00	280.00	560.00
Hair cover	1.00	280.00	280.00
Soluset	115.00	280.00	32,200.00
Needle No.18	1.00	5,600.00	5,600.00
Needle No.20	1.00	5,600.00	5,600.00
Needle No.25	1.00	56.00	56.00
Syringe 1 ml	4.00	56.00	224.00
Syringe 3 ml	2.00	112.00	224.00
Syringe 10 ml	4.00	112.00	448.00
Syringe 20 ml	7.00	560.00	3,920.00
Syringe 50 ml	20.00	448.00	8,960.00
Sterile gloves	25.00	280.00	7,000.00
Stopper	13.00	112.00	1,456.00
Parafilm	1,875.00	0.50	937.50
Plaster	1.00	56.00	56.00
Lint free gown	220.00	24.00	5,280.00
		Total	**95,201.50**

Table C-13 Material cost: Office supplies cost of PN prepared in the cleanroom

Items	Unit prices (Baht/unit)	Item used (unit/8weeks)	cost (Baht)
Printer cartridge	2,815.85	0.47	1,323.45
A4 paper (70 grams)	73.48	2.67	196.20
A5 paper (70 grams)	42.69	1.87	79.84
Liquid glue	4.80	1.87	8.98
Punch	21.00	0.16	3.36
Stapler	181.35	0.16	29.02
Staples	3.68	3.74	13.77
Paper clip	5.45	3.74	20.39
Date stamp	17.00	0.16	2.72
Pencil sharpener	128.08	0.16	20.50
Adhesive tape	23.76	3.74	88.87
Ink	3.72	0.63	2.35
Scissor	26.60	0.63	16.76
Adhesive tape stand	20.38	0.16	3.27
Plastic folder	0.66	3.15	2.08
Pencil	3.20	5.60	17.92
Marker pen	19.76	5.60	110.66
Paper basket	150.00	0.32	48.00
Hard folder	27.42	0.48	0.48
Eraser	2.30	1.87	4.31
Ruler	1.20	0.63	0.76
Correcting pen	58.00	1.87	108.46
A5 Sticker	1,283.33	1.12	1,437.30
Notebook	100.50	1.87	187.94
Plastic box No.830	67.00	3.07	205.69
Waste bag	1.02	112.00	114.24
6cmX8cm Zipper bag	0.09	5,600.00	504.00
16cmX22cm Zipper bag	0.82	1,120.00	918.40
5X11 Plastic bag	0.25	560.00	140.00
8X16 Plastic bag	0.23	1,120.00	257.60
12X20 Plastic bag	0.45	1,120.00	504.00
Slipper	22.84	3.74	85.43
Plastic bin	258.98	0.47	121.73
Bloom	18.00	0.94	16.92
Mop	495.00	1.24	613.80
Rubber band	50.15	9.34	468.41
Plastic basket	37.45	3.07	114.98
18W Fluorescent lamp	22.56	1.87	42.19
36W Fluorescent lamp	29.96	3.74	112.06
		Total	**7,946.84**

BIOGRAPHY

NAME	Miss Wanida Watbamrungsakul
DATE OF BIRTH	April 3, 1980
PLACE OF BIRTH	Bangkok, Thailand
INSTITUTIONS ATTENDED	Srinakarinwirot University, 1998-2002; Bachelor of Science in Pharmacy Chulalongkorn University, 2006-2008; Master of Science in Pharmacy (Food Chemistry and Medical Nutrition)
OCCUPATIONS	Pharmacist at Ramathibodi Hospital 2003-present.