INTRODUCTION

Currently, the demand of total parenteral nutrition (TPN) compounding in hospital increases.[1] Preterm infants are born during the growth of their body and brain; therefore, preterm birth may lead to high stress or catabolism.[2] In addition, the gastrointestinal system is not ready yet to suck, swallow, and obtain the nutrition required.[3] This brings the importance of parenteral nutrition support until the infants are able to suffice the nutrition from the breast milk or enteral nutrition.[4] Therefore, premature infants, especially with very low birth weight should receive parenteral nutrition (PN) to obtain optimum growth rate.[5]

Provision of TPN containing glucose, amino acid, electrolyte as well as lipid is paramount significance for preterm babies to fulfill the nutrition including essential fatty acids and fat-soluble vitamins. However, those compositions of TPN are manufactured as single or double components. Each administration of parenteral nutrition through a different single line will be a burden since venous access is limited. Furthermore, a very young baby has to get restricted quantity. Therefore,

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establishment of a high concentrated solution in a low volume formulation is often preferred. In order to obtain those total components of parenteral nutrition in the minimum volume, hospital pharmacy must mix all the nutrition as all-in-one parenteral nutrition (AIO-PN).

An issue may arise with intravenous admixtures or AIO-PN associated with incompatibility or instability. Physical instability is a significant matter that often causes vascular microemboli and induces deadly effect. Lipid added in the parenteral nutrition along with other components may form a two-phase medication as creaming, coalescence, or cracking that cause larger particle size. Additionally, the interaction of molecule among the cation–anion and acid–base of each ingredient may induce instability. High temperature may increase the reaction faster. In fact, lipid added in one chamber and stored at room temperature is common in a local hospital. However, relevant studies reporting this situation are limited.

**Materials and Methods**

The nutrition was obtained from hospital stock; those are 40% Dextrose (Otsuka), 5% Dextrose (Otsuka), Aminosteril® Infant 6% (Fresenius Kabi Combiphar), NaCl 3% (Otsuka), Lipofundin® 20% (Braun), Potassium Chloride Injection 7.46% (Otsuka), Calcium Gluconate Injection (Ethica Pharmacy Industry), Magnesium Sulphate injection 20% (Otsuka), Nutrient Pad Set Standard TTC Media, Membrane Filter (Sartorius), and Peptone Water (OXOID).

**Study design**

This study has been done to fulfill the quality control of production of AIO-PN standard in hospitals. This research was conducted in vitro including visual detection, pH, particle size, and sterility. The formula has been provided by mimicking the production of parenteral nutrition in hospital under ambient temperature in a sterile room and Laminar Air Flow. To reduce any contamination during sampling, a 10-mL sample was taken once for pH, sterility, and also particle measurement every day. In addition, the sterility test has been done each day to confirm the sterility shown with no microbial growth on microbial culture.

**Formula**

This study investigated the standardized parenteral nutrition for premature infant weighing 1000mg. The composition of nutrients was based on a guidance of first, second, and third day of a very low weight premature baby birth as seen in Table 1. The study of stability was carried out on three formulas (F1, F2, and F3).

**Experimental design**

Three ready formulas (F1, F2, and F3) were prepared in triplicate, then were stored in two conditions: room temperature (25.43°C ± 0.535) and cold temperature (6.2°C ± 2.038), using climatic chamber. Therefore, there were six formulas in the triplicate that were evaluated. The formulas were stored for 7 days and were controlled every day, by which the assays are as follows:

1. **Visual detection**
   
   The clarity of formulas in the spike container was detected under light and dark background. The changes in color, turbidity, gas formation, or emulsion changes (creaming, cracking, and coalescence) were justified as instability.

2. **pH testing**
   
   The pH of 5 mL aliquot samples of each formula was detected by using a calibrated-pH meter with specification ISFET pH electrode (Horiba, Germany) each day for 7 days. The pH changes >0.5 from the pH baseline were indicated as instability.

3. **Particle size analyses**

   Particles were measured using LA-960 (Horiba, Germany) with Mie and Fraunhofer principles.

| Nutrients     | Volume | F1 (cc/day) | F2 (cc/day) | F3 (cc/day) |
|--------------|--------|-------------|-------------|-------------|
| Amino acid 6%| 25     | 33,33       | 33,33       |
| Lipid 20%    | 5      | 7,5         | 7,5         |
| NaCl 3%      | —      | 6           | 6           |
| KCl 7.4%     | —      | 2           | 2           |
| Ca gluconate 10% | 10 | 10         | 10          |
| MgSO4 20%    | 0.36   | 0.36        | 0.36        |
| Dextrose 5%  | 28,846 | 30,182      | 53,04       |
| Dextrose 40% | 10,794 | 10,63       | 7,77        |

F1 = the first day standard formulation in one chamber to fulfill water body volume (80 mL), F2 = the second day standard formulation in one chamber to fulfill water body volume (100 mL) and maximum electrolyte dose, F3 = the third day standard formulation in one chamber to fulfill water body volume (120 mL) and maximum electrolyte dose
Two different wavelengths were used inside the instrument: Solid state laser diode 650 nm (Red) and Solid State LED 405 nm (Blue). Smaller particles would be covered by shorter wavelength and larger particles would be covered by longer wavelength of laser. Solid state laser would be better than Helium Neon Laser (gas) in terms of brighter/intensity and energy efficiency. We used an external validation with NIST Traceable Standard Particles (PSL) 0.1, 1, and 10 μm. The 1 mL aliquot samples were put on the flow cell with ultrasonic probe attachment in order to homogenize and prevent agglomeration. Optimal speed was used to adjust the particle circulation leading to conclusion that larger particle than 500 nm was considered as instability.

4. Sterility
The sterility was measured using a validated 0.45μm membrane filtration method and then transferred to nutrient pad set standard TTC media. No microbial growth specifies as sterile condition

RESULTS
Table 2 shows that the formula of AIO-PN was physically stable and sterile for 4 days and 7 days under room and cold temperatures, respectively. AIO-PN under both temperatures showed creaming process, but it was reversible after shaking. Instability of AIO-PN under room temperature was performed with discoloration since day 4. Meanwhile, AIO-PN under cold temperature was able to maintain the physical stability until day 7. There is no significant change in the particle and pH that showed instability. Furthermore, there is no microbial growth observed on the three formulas in either cold or room temperature.

DISCUSSION
This study found that the three formulas of AIO-PN are stable under cold temperature up to 7 days. Even by using different formulas, this study confirmed the findings of Bouchoud that AIO-PN is stable until more than 7 days under cold temperature. Moreover, parenteral nutrition for preterm babies has a higher concentration than other parenteral nutrition; however, this AIO-PN maintains its physical stability. Even though the creaming process was seen, it is commonly not a problem; it will be reversible by shaking and maintaining the particle size consistency. Parenteral emulsion will be unstable when there are coalescence and cracking, following the particle globule that grows larger. Meanwhile, in this study the smaller particle size is in a good range (<500 nm). Emulsion droplet size less than 500 nm will not be dangerous as it is smaller than the smallest vasculature (400 nm). This is possibly because the particles circulate freely in a tiny capillary; therefore, it will not trap and induce emboli in a blood vessel. A recent study stated that high concentration of amino acid in this formula might increase buffer effect; therefore, the formula will be more stable. This is also confirmed with the value of pH in a range 5–6. Degradation of AIO-PN is commonly realized from the decrease in pH. Lipid added to AIO-PN may degrade by forming of fatty acid that decrease the pH into more acid. Therefore, the pH value of PN in a range 5–6 indicates the stability; in contrast, the pH that is less than 5 specifies free fatty acid formation being followed with larger globule.

This study also identified that the AIO-PN under room temperature was less stable and able to maintain its stability only for 3 days storage. Emulsion might be adhesive and adsorbed into the container during storage. Instability was justified from the visual changes as yellowish colour start from day 4. To the best of our knowledge, no study was done under room temperature. However, this study is relevant for hospitals that have no enough storage for TPN. Moreover, the administration of TPN in the ward is also under room temperature, so this finding will show how long the TPN will be stable during administration. This finding is different from the guideline that stated AIO-PN should not be used after 24 h. The temperature theoretically increases chemical reaction and hydrolysis that will cause instability. In terms of this situation, the different humidity may influence the stability.

According to the sterility test, this study acknowledged that AIO-PN mixture was sterile according to the sterility test of aliquot sample taken in each day up to 7 days. However, in practice, the sterility of intravenous admixture depended on the quality of management system in production unit. However, aseptic preparation may still preserve the contamination. Meanwhile, in practice, it is troublesome to test sterility in every single bag. Therefore, it is critical to check the visible sign of contamination in every single aseptic product prior to use.

This shows that the production of parenteral nutrition under Laminar Air Flow and HEPA filter in a local hospital is acceptable. As a previous study, mixing the AIO-PN in a hospital maintained the stability either in two or one chamber formulation. An issue may arise regarding the safe administration of AIO-PN. The provision of AIO-PN may reduce the necessity of intravenous line. Moreover, the result of the osmometer showed that osmolarity of this AIO-PN is lower than 850 mOsm (F1: 700–800 mOsm, F2: 600–700 mOsm, and F3: 500–600mOsm). According to the
Table 2: Result of control quality of AIO-PN in the specific storage temperature

| Testing period | Temperature | Visual detection | Particle size | pH | Microbial growth |
|----------------|-------------|------------------|---------------|----|------------------|
|                |             |                  | F1        | F2        | F3        | F1        | F2        | F3        | F1        | F2        | F3        | No | No | No |
| Day 1          | Room        | White, milky     | White, milky | 344.97 ± 3.700 | 314.60 ± 2.307 | 341.70 ± 3.915 | 5.86 ± 0.012 | 6.06 ± 0.087 | 5.90 ± 0.026 | No | No | No |
|                | Cold        | Slight and reversible creaming | White, milky | 351.30 ± 7.227 | 313.23 ± 1.159 | 338.57 ± 2.228 | 6.06 ± 0.087 | 5.75 ± 0.038 | 5.84 ± 0.012 | No | No | No |
| Day 2          | Room        | Slight and reversible creaming | Slight and reversible creaming | 317.73 ± 0.777 | 322.63 ± 2.593 | 356.20 ± 4.244 | 5.91 ± 0.015 | 5.76 ± 0.015 | 6.02 ± 0.036 | No | No | No |
|                | Cold        | Slight and reversible creaming | Slight and reversible creaming | 344.90 ± 0.800 | 356.63 ± 2.122 | 475.57 ± 2.442 | 5.63 ± 0.049 | 6.04 ± 0.030 | 6.03 ± 0.036 | No | No | No |
| Day 3          | Room        | Slight and reversible creaming | Slight and reversible creaming | 292.37 ± 1.656 | 334.40 ± 1.353 | 351.27 ± 6.704 | 6.03 ± 0.036 | 5.94 ± 0.068 | 6.16 ± 0.0416 | No | No | No |
|                | Cold        | Slight and reversible creaming | Slight and reversible creaming | 329.90 ± 3.951 | 300.03 ± 6.058 | 307.37 ± 1.955 | 5.52 ± 0.098 | 6.04 ± 0.030 | 6.03 ± 0.036 | No | No | No |
| Day 4          | Room        | Yellowish, oily in the bottle | Yellowish, oily in the bottle | 585.23 ± 2.970 | 334.37 ± 2.984 | 453.23 ± 2.579 | 5.71 ± 0.053 | 5.66 ± 0.065 | 6.12 ± 0.015 | No | No | No |
|                | Cold        | Slight and reversible creaming | Slight and reversible creaming | 349.00 ± 0.436 | 342.43 ± 0.929 | 319.67 ± 0.643 | 6.12 ± 0.015 | 6.04 ± 0.026 | 6.10 ± 0.031 | No | No | No |
| Day 5          | Room        | Yellowish, oily in the bottle | Yellowish, oily in the bottle | 587.53 ± 12.266 | 352.27 ± 8.693 | 766.20 ± 2.227 | 5.46 ± 0.091 | 5.68 ± 0.047 | 5.76 ± 0.040 | No | No | No |
|                | Cold        | Slight and reversible creaming | Slight and reversible creaming | 335.87 ± 2.829 | 361.13 ± 1.007 | 324.87 ± 4.010 | 6.13 ± 0.035 | 6.15 ± 0.101 | 6.16 ± 0.040 | No | No | No |
| Day 6          | Room        | Yellowish, oily in the bottle | Yellowish, oily in the bottle | 317.83 ± 1.069 | 318.77 ± 0.635 | 305.93 ± 2.485 | 5.80 ± 0.015 | 5.70 ± 0.068 | 5.98 ± 0.064 | No | No | No |
|                | Cold        | Slight and reversible creaming | Slight and reversible creaming | 352.10 ± 3.727 | 362.20 ± 0.283 | 323.03 ± 1.193 | 5.90 ± 0.015 | 5.98 ± 0.064 | 6.08 ± 0.040 | No | No | No |
| Day 7          | Room        | Yellowish, oily in the bottle | Yellowish, oily in the bottle | 332.67 ± 2.055 | 766.57 ± 12.169 | 291.37 ± 3.179 | 5.4 ± 0.075 | 5.85 ± 0.040 | 5.78 ± 0.090 | No | No | No |
|                | Cold        | Slight and reversible creaming | Slight and reversible creaming | 369.20 ± 1.136 | 303.63 ± 0.603 | 325.70 ± 1.153 | 5.82 ± 0.006 | 6.22 ± 0.062 | 6.15 ± 0.062 | No | No | No |

F1 = standard formula for preterm baby weighing 1000 g in the first day, F2 = standard formula for preterm baby weighing 1000 g in the second day, F3 = standard formula for preterm baby weighing 1000 g in the third day
European Society for Clinical Nutrition and Metabolism (ESPEN), injection formula which has osmolarity less than 850 mOsm may be delivered through peripheral routes, although, the central routes is another choice for parenteral nutrition and being longer used.[14] This finding had validated what hospital had done related to the AIO-PN production, in which it will possibly administer within days through peripheral or central route access.

CONCLUSION
This study concluded that one chamber of AIO-PN maintained the original formulation for 3 days under room temperature and 7 days under cold temperature. This was confirmed visually and by the particle size and pH.

Limitations and future directions
Quality control of this AIO-PN was performed on physical matter; chemical assay was limited on pH. In the future, there is a need to assay chemical changes including the concentration and degradation.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Hanifah S, Ball P, Kennedy R. Medication incompatibility in intravenous lines in a paediatric intensive care unit (PICU) of Indonesian hospital. Crit Care Shock 2018;21:114-23
2. Sauer PJ. Can extrauterine approximate intrauterine growth? Should it? Am J Clin Nutr 2007;85:608S–13S.
3. Goldberg DL, Becker PJ, Brigham K, Carlson S, Fleck L, Gollins L, et al. Identifying malnutrition in preterm and neonatal populations: recommended indicators. J Acad Nutr Diet 2018;118:1571-82.
4. Olsen SL, Park ND, Tracy K, Younger D, Anderson B. Implementing standardized feeding guidelines, challenges, and results. Neonatal Netw 2018;37:218-23.
5. Wandita S. Nutrisi pada Bayi Prematur. Kumpulan Makalah Pertemuan Ilmiah Tahunan Ilmu Kesehatan Anak VIII; 2016; p. 180-6.
6. Bouchoud L, Fonzo-Christe C, Klingmüller M, Bonnabry P. Compatibility of intravenous medications with parenteral nutrition: in vitro evaluation. JPEN J Parenter Enteral Nutr 2013;37:416-24.
7. Slattery E, Rumore MM, Douglas JS, Seres DS. 3-in-1 vs 2-in-1 parenteral nutrition in adults: a review. Nutr Clin Pract 2014;29:631-5.
8. Lobo BW, da Veiga VF, Cabral LM, Michel RC, Volpato NM, de Sousa VP. Influence of the relative composition of trace elements and vitamins in physicochemical stability of total parenteral nutrition formulations for neonatal use. Nutr J 2012;11:26.
9. Skouroliakou M, Kountouri AM, Hatziantoniou S, Koutri K, Chiou A. Physicochemical stability assessment of all-in-one parenteral emulsion for neonates containing SMOFlipid. Eur J Hosp Pharm 2012;19:514-8.
10. Staven V, Wang S, Gronlie I, Tho I. Physical stability of an all-in-one parenteral nutrition admixture for preterm infants upon mixing with micronutrients and drugs. Eur J Hosp Pharm 2020;27:36-42.
11. Athanasiou C, Hatziantoniou S, Skouroliakou M, Markantonis-Kyroudis S. Assessment of the physicochemical stability of all-in-one parenteral emulsions for neonates according to USP specifications. JPEN J Parenter Enteral Nutr 2014;38:867-72.
12. Kis S. Preparation and examination of TPN systems for the individual clinical therapy. Thesis. Judit Balogh Kovács Semmelweis University Doctoral School of Pharmaceutical and Pharmacological Sciences. 2007. [cited March 19, 2019]. Available from: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.419.482&rep=rep1&type=pdf.
13. Turmezei J, Jávorszky E, Szabó E, Dredán J, Kállai-Szabó B, Zelkó R. Effect of storage temperature on the stability of total parenteral nutrition admixtures prepared for infants. Acta Pol Pharm 2015;72:843-9.
14. Pittiruti M, Hamilton H, Biffi R, MacFie J, Pertkiewicz M. ESPEN guidelines on parenteral nutrition: central venous catheters (access, care, diagnosis and therapy of complications). Clin Nutr 2009;28:365-77.
15. Hanifah S, Maulidani Y, Nugroho BH, Sari CP. All-in-one versus lipid-free parenteral nutrition for premature infants: visual, pH, and particle size analyses. Nutr Hosp 2019;36(6):1-4.