Newborn calf serum supplemented by tellurite as alternative transport medium for *Corynebacterium diphtheriae*

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**Abstract.** Indonesia is one of the five countries with highest diphtheria cases in the world. Laboratory confirmation by culture method as a gold standard requires bacterial survival. Indonesia's geographical condition as an archipelagic country and difficulties in transporting clinical samples are often obstacles in maintaining bacterial survival. This study aims to evaluate the ability of several transport mediums to maintain the survival of *Corynebacterium diphtheriae*. A total of 90 isolates were divided into nine groups of transport mediums. Samples were divided into 2 treatment groups, namely room temperature and temperature 2-8 ºC. On day 2, 4, 8, 16, and 32, 1 isolate from each group with 2 different incubation temperatures was cultured on blood agar medium and incubated for 24 hours at 37 ºC. Bacterial survival was indicated by the growth of suspect colonies which were identified by microscopic and biochemical tests. Results show serum with tellurite can be used with viability lower than silica gel, but higher than other media. Meanwhile at a temperature of 2-8 ºC, there are 2 types of the best transport medium, namely serum with tellurite and open silica gel in aluminum foil. Newborn Calf Serum supplemented with Tellurite can be used as an alternative transport medium for *Corynebacterium diphtheriae*, both at room temperature and at 2-8 ºC.

1. Introduction

Diphtheria is still a health problem, especially in developing countries with relatively low immunization coverage, including Indonesia. Based on data from WHO and the Indonesian Ministry of Health, Indonesia is in the 3rd to 2nd position in the country with the most diphtheria cases in the world from 2013 to 2017. Data from 2013 shows that Indonesia is ranked 2nd after India with 775 cases. In 2017, Indonesia was again ranked 2nd after India with 954 cases [1]

Diphtheria laboratory examination begins with taking specimens until the laboratory examination is complete. Taking specimens in the form of swabs taken from several body locations such as the throat and nasopharynx as well as skin lesions if diphtheria is suspected. After the specimen is obtained, the sample should be sent to the examining laboratory as soon as possible [2.3.4]

Laboratory confirmation by culture method as a gold standard requires bacterial survival. The ability to survive diphtheria-causing bacteria outside the host body is very limited, thus, samples taken must be examined as soon as possible. Indonesia's geographical condition as an archipelagic country and difficulties in transporting clinical samples are often obstacles in maintaining bacterial survival. Therefore, this study aims to evaluate the ability of several transport mediums to maintain the survival of *Corynebacterium diphtheriae*. 

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[1] WHO and Indonesian Ministry of Health data.

[2] Further details on specimen collection and transport can be found in WHO guidelines.

[3] Immediate processing of samples is crucial to maintain bacterial viability.

[4] Additional information on the importance of timely examination and laboratory processes can be found in the literature review section of this study. 

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2. Method
This study tried to test the viability of bacteria on several types of transport medium. The medium tested was Medium Amies which is a control medium, sterile tube without medium, NaCl 0.9% in a sterile tube, serum + tellurite in a sterile tube, and silica gel which is the second control medium. The research sample was 10 l x 0.5 Mc Farland isolate Corynebacterium diphtheriae in 0.9 % NaCl solution. A total of 90 isolates were divided into 9 groups so that each group consisted of 10 isolates. The isolates were dropped into a dacron swab and put into 9 different transport mediums. Each transport medium was given two treatment groups, namely placed at room temperature for group A and in a refrigerator with a temperature of 2-8 ºC for group B. On day 2, 4, 8, 16, and 32, 1 isolate from each group with 2 different incubation temperatures was cultured on blood agar medium and incubated for 24 hours at 37 ºC. Bacterial survival was indicated by the growth of suspect colonies which were identified by microscopic and biochemical tests. This research was conducted at the Bacteriology Laboratory, Infectious Disease Research Laboratory, Dr. Sri Oemijati, Research and Development Center for Biomedical and Basic Health Technology.

3. Results
The results of the evaluation of the viability of bacteria placed in various transport media can be seen in table 1.

Table 1. The results of the evaluation of the viability of C. diphtheriae bacteria on various transport media.

| Type of Medium Transport | Room temperature (Viability) | Cold temperature (2-8 ºC) |
|--------------------------|-----------------------------|----------------------------|
| Amies                    | < 4                         | ≥ 32                       |
| Steril tube              | < 4                         | < 4                        |
| Vacuum steril tube       | < 2                         | < 16                       |
| NaCl 0.9%                | < 2                         | < 2                        |
| Serum + tellurite        | < 32                        | ≥ 32                       |
| Silica gel               | < 32                        | ≥ 32                       |

Table 1 shows that at room temperature, the best transport medium for C. diphtheriae is serum + tellurite which has the same viability as silica gel and is higher than other media including Amies transport medium which is the standard medium for transporting diphtheria samples. Meanwhile at a temperature of 2-8 ºC, there are 2 types of the best transport medium, namely serum with tellurite and open silica gel in aluminum foil with a viability of more than 32 days, comparable to the Amies transport medium. In this study, Amies medium and silica gel were used as controls because these two mediums are the medium recommended by WHO and CDC.

4. Discussions
Diphtheria is an acute infectious disease of the upper respiratory tract, characterized by an inflammatory response at the site of infection and the formation of a pseudomembrane followed by systemic symptoms due to the spread of diphtheria toxin such as myocarditis and neuropathy [5,6,7]. Diphtheria is transmitted through droplets and direct contact with sufferers or carriers as well as through sexual intercourse.[8] Indirect transmission of diphtheria is mediated by eating utensils and contaminated food, especially milk and dairy products [9]. Diphtheria laboratory examination begins with sampling until the laboratory examination is complete. The ability to survive diphtheria-causing bacteria outside the host body is very limited, thus, samples taken must be examined as soon as possible. In areas that are difficult to reach by transportation, or other obstacles that are common in the field, sending samples to the examining laboratory usually
takes a long time. In such cases, a transport medium is needed to maintain bacterial viability. The transport medium commonly used is the Amies or Stuart medium. These two mediums are the ones recommended by WHO. Amies medium is a non-nutritive medium containing NaCl and Na₂HPO₄. Sodium thioglycolate, KCL, CaCl₂-2H₂O, MgCl₂-6H₂O KH₂PO₄ and 0.4% agar [10,11]. The results of the study are in table 3.1. In the table, it can be seen that Amies medium is the right medium to use at cold temperatures (2-8 °C) but is not optimal at room temperature. Besides Amies medium, serum + tellurite and silica gel can be used as alternative media, especially at room temperature. Silica gel is a bacterial storage medium that has been known for a long time, but its use as a medium for transporting and storing bacteria in the field is rarely found [12]. This is constrained by the limited literature regarding the technical use of silica gel. While the use of serum + tellurite in the form of broth as a storage medium for bacteria is a very new thing. However, it is common practice to add bovine serum and tellurite to other media such as Tinsdale's medium [13]. From this study, it can be seen that serum + tellurite is a good transport medium for storing diphtheria samples at room temperature and at 2-8 °C.

5. Conclusions
The best transport medium for C. diphtheriae to be used at room temperature and equivalent to silica gel is serum + tellurite. This medium is also suitable for use as a transportation medium for cold temperatures (2-8 °C) which is equivalent to amies as commercial media and silica gel.

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