

Stability and Antibiotic Activity Vancomycin Ophthalmic Solution Prepared from Vancomycin Dry Injection Against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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ABSTRACT

Vancomycin is Glycopeptide antibiotic relatively unstable in aqueous solution and is not commercially available as eyedrops, so measurement of pH and antibiotic activity are needed¹. This study aim to assess the stability and the in vitro antibacterial potency of vancomycin eyedrops against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively, under different solvent and storage temperatures Stock solutions of vancomycin 100 mg/ml was prepared by reconstituting vancomycin dry injection with water for injection (Aqua PI) or NaCl 0,9% and stored at cold and room temperature. The minimum inhibitor concentrations against *P. aeruginosa* and *S. aureus* were measured to evaluate the antimicrobial potency at concentration 30µg / mL, 45µg / mL and 60 µg/ mL. Changes in the pH values and physical characteristics of the solutions were recorded for evaluate stability of solution Vancomycin 60 µg/ mL has antibacterial effect against *S. aureus* The antibacterial potency of Vancomycin in NaCl 0,9% and in aqua PI solution decreased significantly from day 3 and day 4 respectively, storage temperature affected antibacterial potency. The pH value of vancomycin in NaCl 0.9% solution at a room and cold temperature are 4.68-6.66 and 5,41- 6.66 successively, while in aqua PI solution are 2,8-4.50 at room temperature and 3,51- 4.45 at cold temperatures.

Keywords: *Stabilitiy and antibiotic activity, Vancomycin, ophthalmic solution, Pseudomonas aeruginosa and Staphylococcus aureus*

INTRODUCTION

Eye infection is a red and swollen eye condition caused by microbiological agents such as viruses, fungi, parasites or bacteria. Some cases of eye infections caused by microbiological agents that keratitis, corneal ulcers, endophthalmitis, Anterior Uveitis, and Konjungtivis². Eye infections such as conjunctivitis, keratitis, endophthalmitis, dacryocystitis, blephritis, infection of the eyelids, scleritis microbes, canaliculitis, preseptal cellulitis, orbital cellulitis, endophthalmitis and panophthalmitis etc., lead to increased incidence of morbidity and blindness in the world³. Under normal conditions, eye impermeable to agents that come from the environment through the blinking reflex, ocular surface cleaning by mechanical, and prevention of the accumulation of microorganisms. Presence of lysozyme, lactoferrin, secretory immunoglobulin and defensins at high levels in tear can prevent bacterial colonization on the eye⁴. Bacteria and viruses can infect the eye and into the posterior part of the eye in several ways, that occurred after intraocular surgery, injury to the eyeball, the spread of bacteria from the site of infection in other places. Endophthalmitis can also be caused by infection of the cornea (keratitis) which may lead to complications³. The most common bacteria that cause keratitis is *Pseudomonas aeruginosa* (58.8% of cases) and *Staphylococcus aureus*

(11.8% of cases). Infections caused by these bacteria cause damage to the cornea progressively⁵. Majority of ocular infection is caused by gram positive organisms which were susceptible to vancomycin followed by gram negative organisms susceptible to amikacin, fluoroquinolone, gram negative coccobacilli to amikacin and tobramycin, and gatifloxacin effective against both type of organisms⁶. Eye infections, including bacterial keratitis requires antibiotics. Antibiotics used to treat infections of the eye must fulfill the following criteria to produce a sufficiently high concentration of drug in the cornea and is able to maintain the antibacterial effect in the long term. Under conditions of microbes causing the infection is not known, it is recommended to use empirical antibiotic while waiting the germs that cause the infection are identified⁷. There are several drug dosage forms on the eye, one of which is eye drops⁸. Eye drops is sterile dosage form of solution or suspension is used with a drop of drugs on the mucous membranes of the eye around the eyelid and eyeball. Eyedrops must meet the requirements, that a sterile, clear, tonicity, should be comparable with 0.9% NaCl, having pH 4,4 that similar with tear and free from foreign particles. Eye drops should not be used more than one month after the packaging is opened, because possibility of contamination⁹. Limitations of sterile ophthalmic antibiotic regimen that is often made ophthalmologist at

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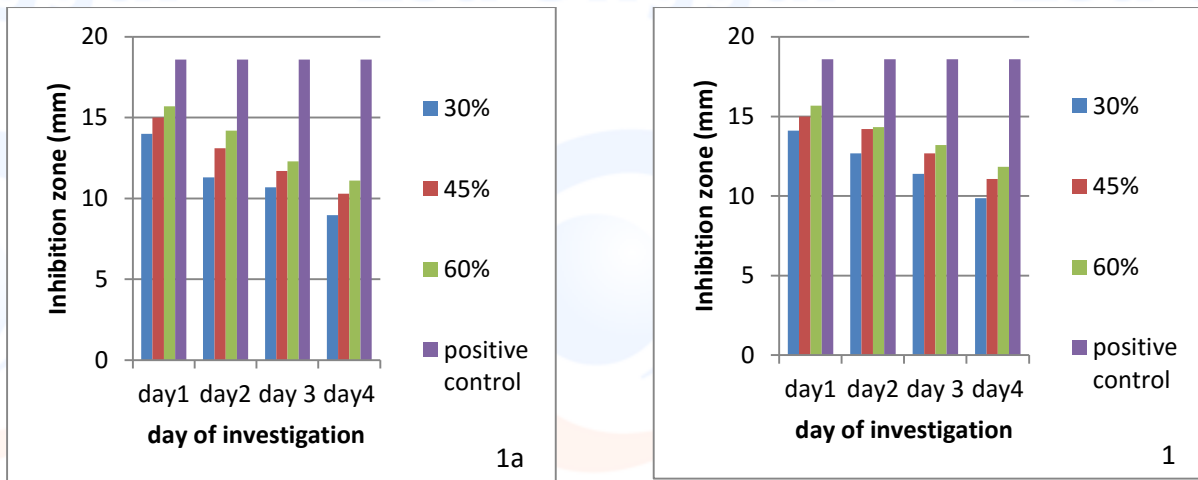


Figure 1: Results of antibacterial activity test (mean of inhibition zone) Vancomycin against *Staphylococcus aureus* in NaCl 0.9% that is stored at room temperature (1a) and cold temperature (1b)

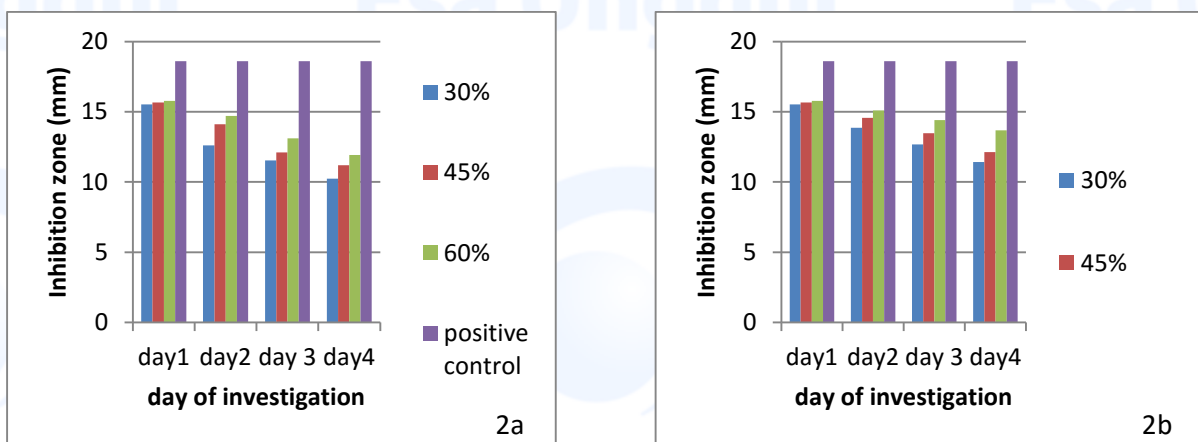


Figure 2: Results of antibacterial activity test (mean of inhibition zone) Vancomycin against *Staphylococcus aureus* in water for injection that is stored at room temperature (2a) and cold temperature (2b)

one of the General hospital in Indonesia prescribe an antibiotic injection was prepared by reconstituting using aqua pro injection or Sodium Chloride 0.9% as ophthalmic preparations. One of antibiotics that is prescribed and used as eye drops is vancomycin for treating the infections due to *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Vancomycin is a glycopeptide class of antibiotics that effective on gram-positive bacteria, works by inhibiting the synthesis of cell wall in bacteria¹⁰. It has been used for its antibacterial activity against gram positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and resistant enterococci¹¹. Injection Vancomycin is often prescribed to be made in the form of eye drops for patients with endophthalmitis. The main problem of the preparation of drugs using water as a solvent is that the tendency interact molecules of drug with water through hydrolysis reaction that makes the dosage form becomes unstable. Therefore, in the manufacture of eye drops need to require special attention in terms of sterility, clarity, pH, and a tonicity value¹². Antibiotic activity is shown by the effect of inhibition against microorganisms. Decreasing antimicrobial activity could not be demonstrated by chemical methods, so that the microbiological or biological test which is a standard to

overcome doubts about the possibility of the loss of antibiotic activity¹³.

The way to establish the organism susceptibility to antibiotics by inoculating agar plates with culture and allowing mediated antibiotic diffuses order. The effectiveness of antibiotics will be indicated by inhibition zones¹³. Based on this background it is necessary to investigate whether Vancomycin injection that prepared by reconstituting using aqua pro injection or Sodium Chloride 0.9% as ophthalmic preparations has stability and high potency against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

From the results of the study is expected to provide information on the efficacy of vancomycin ophthalmic preparations that is made by diluting Vancomycin injection with water for injection or NaCl 0.9% against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Results of this study can be the basis of guidelines for the manufacturing and storing of ophthalmic preparations of Vancomycin.

MATERIAL AND METHODS

Material

Table 1: Stability test and pH value of Vancomycin ophthalmic solution in aqua PI or NaCl 0,9% stored at cold and room temperature

Solvents	Temperature	Day of testing	Concentration		
			30 µg	45 µg	60 µg
NaCl 0,9%	room	1	6,662	6,662	6,663
		2	6,234	6,234	6,237
		3	5,417	5,418	5,418
		4	4,675	4,677	4,677
	cold	1	6,662	6,662	6,663
		2	6,451	6,452	6,455
		3	5,911	5,913	5,913
		4	5,412	5,412	5,413
Water for injection	room	1	4,497	4,497	4,499
		2	3,825	3,825	3,828
		3	3,135	3,211	3,213
		4	2,810	2,811	2,813
	cold	1	4,497	4,497	4,499
		2	4,076	4,078	4,080
		3	3,903	3,906	3,909
		4	3,511	3,513	3,515

Vancomycin / Vancep® dry injection (0.5 gram Vancomisin), Aqua pro injection, NaCl 0.9%, Nutrient Agar (OXOID) and Nutrient Broth (OXOID), *Water for Injection*(WFI) 25 mL, Natrium Klorida 0,9 % 500 mL, Mueller Hinton (MH) Agar, Cetrimate Agar, Mc Farland standard 0,5, gliserol, analytical grade paper disks (diameter 6 m), Vankomisin 30 µg disc standard, *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853), obtained from the Laboratory of Microbiology, Indonesia University.

Methods

Sterilization of Equipment and Materials

All tools and materials that is used for microbiological testing sterilized using an autoclave at a temperature of 121°C for 15 minutes. However, for materials made of rubber was sterilized by soaking in 70% alcohol.

Preparation of disks.

Blank, analytical grade paper disks (diameter 6 m) were impregnated with the antibiotic solutions by pipette delivery method. The sterile discs were placed in petri-dishes approximately 5mm apart. Using a mechanical pipettor with a fixed volume delivery of 0.02 ml, the disks were loaded with antibiotic solutions. The disks were allowed to dry in a clean incubator at 35°C for 1-2 hours.

Preparation of Inoculum

Three well-isolated colonies selected from agar plate culture. The top of colony is touched with a loop, and the growth is transferred into a tube containing 5 mL nutrient broth for *Staphylococcus aureus* and Cetrimide for *Pseudomonas aeruginosa* and following incubated at 35°C¹⁴. the number of bacteria was adjusted to standard Mc Farland 0.5 (according to the number of bacteria 10⁷-10⁸ / mL¹⁵).

Preparation of vancomycin concentration

Vancomycin injection vials (500 mg) was obtained from Eli Lilly was dissolved in NaCl 0,9% and aqua PI using aseptic techniques to give concentrations of 100 mg/ml for stock solution.

Then the stock solution was diluted with respective solvents to obtain a concentration of 30µg / mL, 45µg / mL and 60µg / mL.

Stability Test

Vancomycin that has been diluted with NaCl 0,9% or aqua PI divided into two treatments, which were stored at room temperature (24°C) and stored at cold temperatures (20-80°C). pH measurements were taken from each treatment everyday.

Antibiotic potency test by microbiological assay

Vancomycin antibiotic potency test performed using bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* using Disc diffusion methods. Medium Mueller Hinton (MH) Agar to both bacteria that are still liquid is poured into each sterile petri dish as much as 20 mL and allowed to solidify. Furthermore if the medium has become solid, then made of four zones, each of the two zones to solvent using aqua pro Injection and Sodium Chloride 0.9%. Sterile 6 mm paper disks (Becton Dickinson and Company, USA) with the vancomycin solution in aqua PI or in NaCl 0,9% (30µg / mL, 45µg / mL and 60µg / mL) or positive control (vankomycin) were then placed on the inoculated plates. The plates were incubated at (35 ± 2) °C for 16–24 h. Antibacterial activities were evaluated by measuring the diameters of zones of inhibition in mm against the test organism.

Data analysis

Stability of vancomycin observed by measurement of pH, and the data obtained by antibiotic potency test performed by calculating the diameter of inhibition zone. Data was analyzed with Anova using SPSS 20 for windows..

RESULTS AND DISCUSSION

Results of stability test and pH value of Vancomycin ophthalmic solution was prepared by reconstituting vancomycin dry injection with water for injection (Aqua PI) or NaCl 0,9% stored at cold and room temperature can be seen in Table I.

Ophthalmic solution is sterile solution, especially free from foreign particles, suitably compounded and packaged for instillation into the eye¹⁶. Preparation of an ophthalmic solution requires careful consideration of such factors as inherent toxicity and isotonicity value, buffering agent, preservative, sterilization and proper packaging were needed to make good ophthalmic preparation¹. Stability testing ophthalmic preparations began with the number of requests preparation of eye drops from dry injection of vancomycin to aseptic dispensing division. Based on screening of prescription obtained from the aseptic dispensing at a general hospital in Jakarta during January to December 2014, Vancomycin was prescribed as many as 136 sheets. Solvent choice may also important because, as well as influencing antibiotic stability, it can affect patient tolerance according to the level of irritation induced by non-favourable physicochemical properties, including pH. pH is one of the most important factors in the stability of a product. Many pH:stability profiles are published or can be obtained and can be used to determine the pH of maximum stability of a drug. After the pH range is determined, buffers can be prepared to maintain the pH for the expected shelf-life or duration of therapy of the product¹⁶. pH value of vancomycin in aqua PI solution at room and cold temperature was 2,81 - 4,50 and 3,51 - 4,50 respectively, while in NaCl 0,9% was 4,68-6,66 at room temperature and 3,51-4,5 at cold temperature. Study was done by McLellan et al, 2008 showed that pH Vancomycin ophthalmic solution was prepared by reconstituting vancomycin injection with NaCl 0,9% in room temperature was 3,49 at day 0 and 3,71 at day 60¹⁷. The pH vancomycin ophthalmic solution with benzalkonium chloride stored at room temperature was 3,59 at day 0 and 3,79 at day 60. Vancomycin is stable over the pH range 3-5¹⁸. the maximum stability region of vancomycin is pH 3.0-5.7¹⁹. Ophthalmic solutions are generally formulated in the range of pH 4-8²⁰. while the pH value of normal tears is about 7.4. Eye irritation may occur outside the physiological pH range¹¹. Results antibacterial activity Vancomycin against *Staphylococcus aureus* in NaCl 0.9% and aqua PI were stored at room and cold temperature can be seen in Figure I and Figure 2, vancomycin did not have antibacterial activity against *Pseudomonas aeruginosa*. Observations formation inhibition zone was made up until the inhibitory zone is not formed again. The inhibition zone occurred only until the fourth day, although on fifth day inhibition zone was formed but very weak potential for antibiotics activity. A good inhibition zone of Vancomycin for antibiotic activity is greater than 12 mm (sensitive)¹³. Figure 1 show the diameter inhibition zones Vancomycin solution in NaCl 0.9% stored at room temperature and cold temperatures, inhibition zones that were formed on the fourth day at the

dose 30 µg are 8.90 mm and 9.80 mm, respectively. Diameter inhibition zones 9 mm or less indicates resistance. While at the dose 45 mg and 60 µg inhibition inhibition zones were formed in the middle / intermediate¹³. The zones of inhibition for Vancomycin in aqua PI greater than 10 mm either stored in cold temperature or room temperature, its showed antibacterial activity at dose 30 µg/mL, 45 µg/mL and 60 µg/mL (Figure 2). Vancomycin has antibacterial effect if inhibition zone exhibited greater than 10 mm¹³ and according to the Clinical and Laboratory Standards Institute, 2015²¹. vancomycin sensitive against *Staphylococcus aureus* when diameter inhibition zone greater than 15 mm. Vancomycin sensitive to *Staphylococcus aureus* if the diameter of inhibition zone greater than 15 mm. Thereby Vancomycin that is reconstituting with NaCl 0.9% either stored at room temperature or cold temperatures has efficacy as antibiotics only until fourth day, and can not be used as eye drop again²¹. Vancomycin does not have effect on *Pseudomonas aeruginosa* growth either reconstituting with aqua PI or NaCl 9%, this can be seen from the absence of inhibition zone formed for these bacteria. *Pseudomonas aeruginosa* is a gram-negative bacteria, while vancomycin is only active against gram-positive bacteria²². *P. aeruginosa* is a ubiquitous organism present in many diverse environmental settings, and it can be isolated from various living sources, including plants, animals, and humans. The ability of *P. aeruginosa* to survive on minimal nutritional requirements and to tolerate a variety of physical conditions has allowed this organism to persist in both community and hospital settings²³. Majority of ocular infections are associated with bacterial etiology, which was more due to gram-positive organisms than Gram negative organism. Most of the Gram-positive organisms were susceptible to vancomycin and cefazolin, whereas Gram-negative organisms were susceptible to amikacin and gatifloxacin³. According to Riviera and Boucher, 2011 Vancomycin is an antibiotic with activity on Gram-positive spectrum is effective for the treatment of *Staphylococcus aureus* and *Enterococcus infections*²⁴. Vancomycin is a glycopeptide; it inhibits early stages in cell wall mucopeptide synthesis and it exhibited greatest potency against ocular Gram-positive isolates³. Whereas in the study done by Khosravi A D et al., vancomycin had good coverage 95% against Gram-positive. *Staphylococcus aureus* had 100% susceptibility to vancomycin but in the study done by Khosravi et al, 2007 the isolates of *S. aureus* were resistant to vancomycin. *Coagulase negative Staphylococci* was mostly susceptible (93%) to vancomycin and gatifloxacin²⁵. Vancomycin is Glycopeptide antibiotic - originally identified in the 1950, but now widely used due to the increasing incidence of infections due to Gram-positive organisms which are resistant to β-lactam antibiotics. Vancomycin interferes with lipid phosphodisaccharide-pentapeptide complex; no competition between penicillin and vancomycin for binding sites and no cross-resistance. In this study, vancomycin doesn't have antibiotic activity for *Pseudomonas aeruginosa* that can be seen from no inhibition zone was formed. *P. aeruginosa*, which

constitutes 43.7% of the Gram-negative bacteria were highly sensitive towards amikacin (30; 96.8%), ciprofloxacin (26; 83.9%), ceftriaxone (21; 67.7%), doxycycline (17; 54.8%), and chloramphenicol (16; 51.6%)²⁶. Vancomycin has antibacterial effect if inhibition zone greater than 10 mm¹³. and according to the Clinical and Laboratory Standards Institute 2015, Vancomycin sensitive against *Staphylococcus aureus* when inhibition zone greater than 15 mm²¹. Results from statistical test showed that no significant difference antibacterial potency between positive control and vancomycin ophthalmic solution at dose 60 µg/mL reconstituting with NaCl 0,9% either stored at room or cold temperatures until day 3 investigation and no significant difference between positive control and vancomycin ophthalmic solution at dose 60 µg/mL reconstituting with aqua PI that stored at cold temperature until day 4 investigation.

CONCLUSIONS

Vancomycin ophthalmic solution has antibacterial effect against *S. aureus* The antibacterial potency of Vancomycin 60 µg/mL in NaCl 0,9% and in aqua PI solution decreased significantly from day 3 and day 4 respectively, storage temperature affected stability of pH and antibacterial potency.

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