Federation of Islamic Medical Associations
Volume 2022
http://dx.doi.org/10.11594/nstp.2022.2204

NST PROCEEDINGS

OPEN ACCESS

Conference Paper

Effect of Varied Concentrations of Ethylhene Glycol as Pooled Serum Preservative on the Stability of Alkaline Phosphatase (ALP) Ezyme

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ABSTRACT

Quality controls aim to ensure the quality, accuracy, and thoroughness of an examination. Internal quality assurance is carried out by the laboratory itself using control materials, both commercial control materials and homemade control materials. This controlled material is then used to ensure the quality of an inspection. The stability of the control material is strongly influenced by the storage temperature where the recommended temperature is -20℃ which will be stable for up to 6 months and 2-8℃ which is stable for 6 days. In the manufacture of homemade control materials, a stable preservative, antifreeze, and antibacterial is needed but still maintains the integrity of the group serum. The preservative used is ethylene glycol. This study aims to determine the optimal concentration of ethylene glycol in stabilizing alkaline phosphatase levels in refrigerator temperature storage (4-8ºC) for 60 days. In this study, tests were conducted on pooled sera preserved with ethylene glycol in varying concentrations, namely 7.5%; 10%; 12.5%; 15%; and 17.5% and observed the stability of the Alkaline Phosphatase enzyme in pooled sera for 60 days with an interval of 10 days with a storage temperature of 4-8℃. The study was conducted in July-August 2021 in the BLUD laboratory of RSUD dr. Ben Mboi, Manggarai. The results showed that the stability of alkaline phosphatase persisted until day 30, began to decrease on day 40, and continued to decrease until day 60 in each different concentration variation. different, namely 7.5%; 10%; 12.5%; 15%; and 17.5% had the same stability up to 30 days. The recommendation from the author is to observe at different temperatures so that the stability of Alkaline phosphatase can be known.

Keywords: Ethylene glycol, pooled serum, alkali phosphatase, enzyme

Introduction

Three important stages in laboratory examination are pre-analytical, analytical, and post-analytical. All three are factors that greatly affect the results of the examination. Errors in the pre-analytic process accounted for about 61% of the total laboratory errors in addition to analytical errors (25%) and post-analytic errors (14%) (Cornes et al., 2016; Guder, 2014). Internal Quality Assurance (IQA) is a prevention and control activity carried out by all laboratories to reduce the occurrence of irregularities so that accurate results are obtained. Control materials are used to assess correctness, especially precision and accuracy in monitoring analysis performance (Plebani, 2010; Plebani et al., 2019).

IQA is carried out by the laboratory every day to reduce or prevent deviations or errors in the examination results. IQA determines the type of control material in monitoring the quality of daily inspection results contained in the inspection tool. These control materials can be obtained from

How to cite:
Fadhilah, F., Riyadi, S. A., & Yuliani, E. (2022). Antimicrobial production by an actinomycetes isolated from the termite nest. *Federation of Islamic Medical Associations.* NST Proceedings. pages 25-30. doi: 10.11594/ nstp.2022.2204

commercial control materials or control materials made by themselves using a collection of serum (Tuna & Widyaningsih, 2016). Control material made from serum is also called pooled serum or pooled sera. Pooled sera are a mixture of residual patient serum which is sent daily for examination in the laboratory. Pooled sera can be used as a control agent for all clinical chemistry analysis analytes (Lima-Oliveira, 2018). The World Health Organization (WHO) recommends adding ethylene glycol as a preservative in pooled sera because of the antifreeze and antibacterial properties of ethylene glycol (Yao et al., 2017).

In contrast, to control materials in laboratory tests whose stability values are known, pooled sera do not have a known stability value, so it is necessary to study the stability of pooled sera. Ethylene glycol in pooled sera can stabilize some analytes in serum. It can lower or suppress the freezing point by allowing the serum to melt at a temperature of -15^{o}C to -20^{o}C and can minimize the growth of microorganisms because it has anti-microbial properties (Nalawade et al., 2015).

This study aims to determine the optimal concentration of ethylene glycol in stabilizing alkaline phosphatase levels in refrigerator temperature storage (4-8ºC) for 60 days. The storage temperature was chosen according to conditions that often occur in the field where not all laboratories have special freezers for storage of control materials and in the event of delivery of control materials to other areas where only use iceboxes (Fadhilah et al., 2021; Fadhilah et al., 2019). Alkaline phosphatase examination is an examination of enzyme activity that must be carried out carefully so that the measured enzyme activity is directly proportional to the amount of enzyme present in the sample (Fadhilah et al., 2021).

The alkaline phosphatase test should be performed as soon as possible after collection because the activity of the ALP enzyme in serum can increase by about 3% to 10% at 25ºC or 40ºC for several hours. Stability of ALP enzyme activity in the literature based on the DEA method (diethanolamine). The temperature interference value for ALP enzyme activity was 2-8^{0} for 2-3 days while at room temperature 18-22ºC for 5 days (Zhou et al., 2020).

Material and Methods

The research is a laboratory experiment, which aims to find out a symptom or effect that arises as a result of certain treatments of the object under study with the addition of Ethylene Glycol with a certain concentration and different measurement times on the sample and storage at refrigeration temperature erator (2-8ºC).

The study was conducted in June-August 2021. The subjects of this study were serum collections that had the following inclusion criteria; Male, 25-35 years old, meets donor screening criteria, namely non-reactive HIV, HCV negative, HBsAg negative, normal blood pressure, normal hb, Not currently taking drugs. The number of samples required for this study was 250 ml of serum obtained from 5 patients.

In this study, alkaline phosphatase was examined in serum pools derived from normal human serum. The resulting serum was then added with ethylene glycol with concentrations of 7.5%, 10%, 12.5%, 15%, and 17.5%. This concentration variation was obtained based on CLSI EP 6 where the measurement concentration range is 20-30% and it is recommended that the concentration between pooled sera and ethylene glycol is diluted as small as possible (< 10%). Each concentration was then divided into 7 cups for each day of observation alkaline.

Preparation of pooled sera with various concentrations of ethylene glycol

Ethylene glycol with a concentration of 99.8% diluted to a concentration of 7.5%, 10%, 12.5%, 15%, and 17.5, respectively, with pooled sera added and homogenized. Store in a refrigerator at a temperature of 4-80C.

ALP examination procedure

A continuous-monitoring technique based on a method devised by Bowers and McComb allows calculation of ALP activity based on the molar absorptivity of p-nitrophenol. p-

Nitrophenylphosphate (colorless) is hydrolyzed to p-nitrophenol (yellow), and the increase in absorbance at 405 nm, which is directly proportional to ALP activity, is measured.

Results and Discussion

The purpose of this study was to determine the optimal concentration of ethylene glycol as a preservative for pooled sera in ALP (U/L) examination. Preservatives added to pooled sera in this study varied in concentrations ranging from 7.5%; 10%; 12.5%; 15%; 17.5% as control were pooled sera samples that were not treated with ethylene glycol as a preservative. The stability test of preservation was measured by the variation of day 1; 10th day; 20th day; day 30; 40th day; day 50; and day 60. This study uses commercial quality control as a control on the quality of the tools and reagents where the treatment is to do quality control before checking the sample.

The results of ALP measurements based on variations in ethylene glycol concentration and test days are available in Figure 1 to Figure 6.

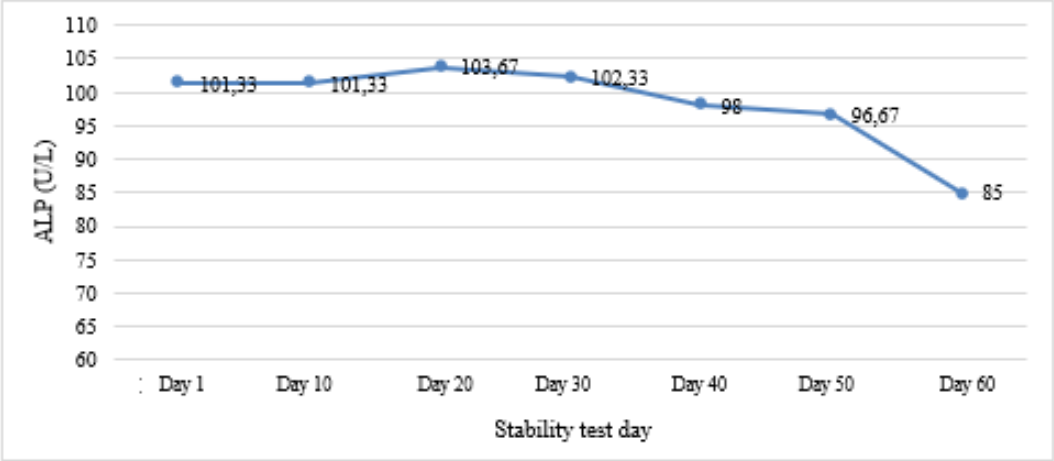

Figure 1. Decrease in ALP on Pooled sera + ethylene glycol 7.5%

It can be seen on the 40th day the decrease in the ALP value began to occur and continued to decline until the 60th day.

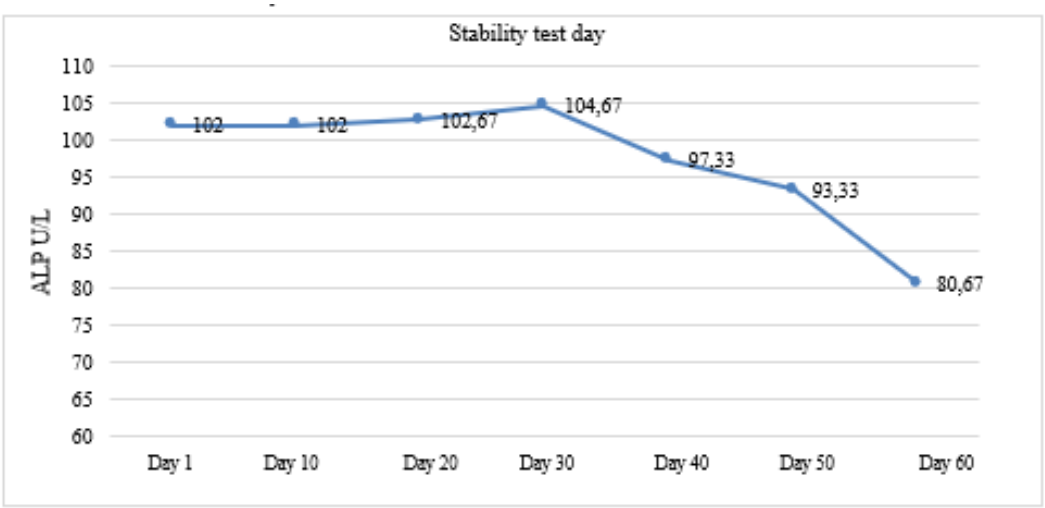

Figure 2. ALP reduction in Pooled sera + 10% ethylene glycol

It can be seen on the 40th day the decrease in the ALP value began to occur and continued to decline until the 60th day.

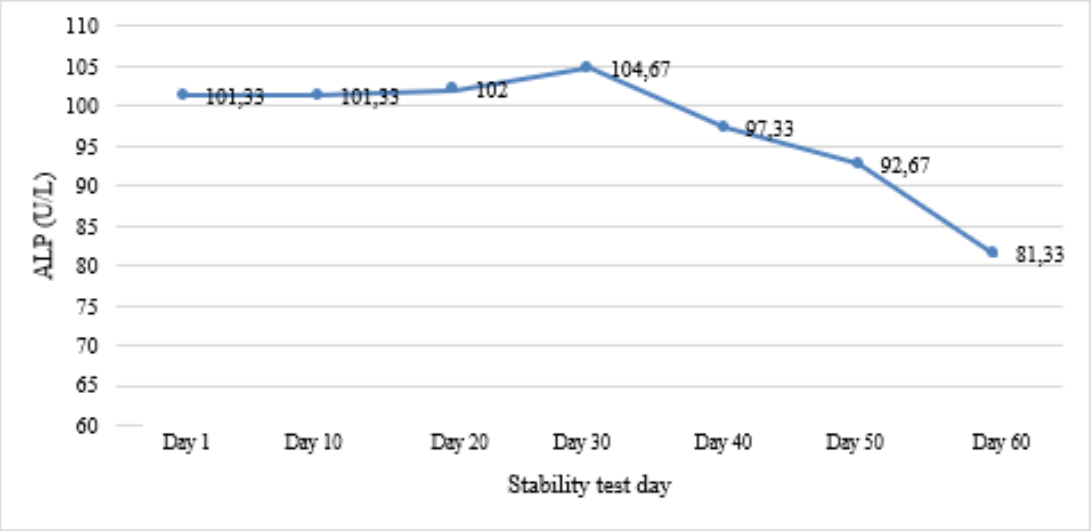

Figure 3. Decrease in ALP on Pooled Sera + ethylene glycol 12.5%

It can be seen on the 40th day the decrease in the ALP value began to occur and continued to decline until the 60th day.

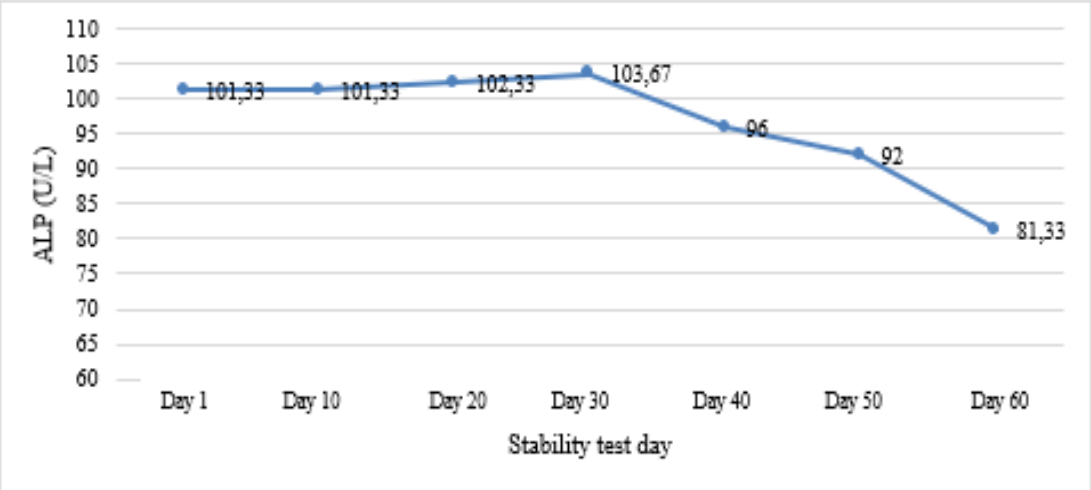

Figure 4. Decrease in ALP on Pooled Sera + 15% ethylene glycol

It can be seen on the 40th day the decrease in the ALP value began to occur and continued to decline until the 60th day.

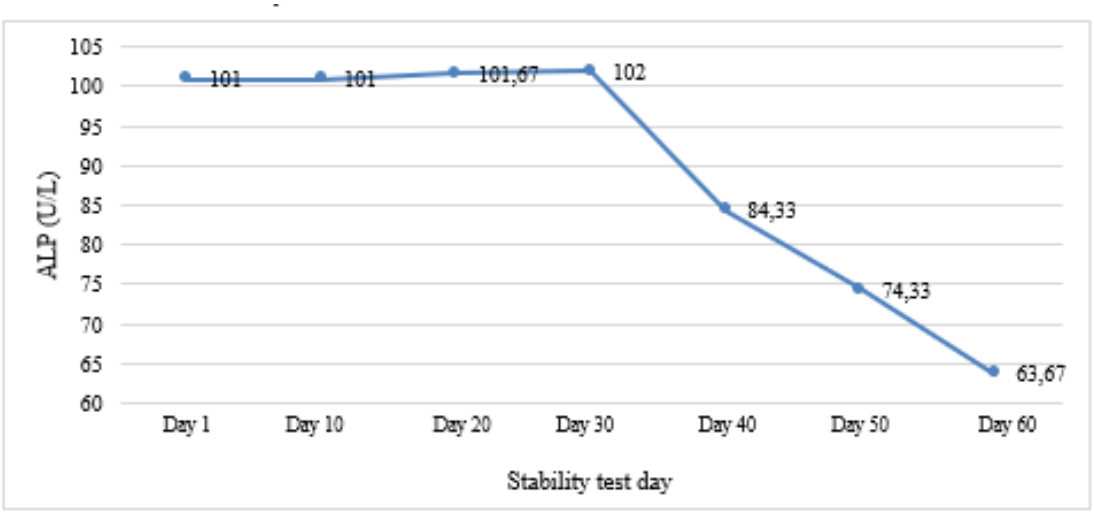

Figure 5. ALP reduction in Pooled sera + ethylene glycol 17.5%

It can be seen on the 30th day the decrease in the ALP value began to occur and continued to decline until the 60th day.

Figure 6. Decreased ALP in Pooled Sera without ethylene glycol

It can be seen on the 20th day the decrease in ALP values began to occur and was not detected until the 60th day. From day 20 ALP was not detected so it was assumed that the value was < 5 U/L.

From the results of the measured ALP observations, in general, from each variation in the concentration of ethylene glycol addition, ALP levels began to decrease to < 100 U/L on day 40. Meanwhile, pooled sera that were not added with ethylene glycol was stable until day 10 only. However, to determine whether the decrease was significant or not, a significant difference test was carried out.

The results of the Kruskal Wallis test showed that there were variations in the day which gave a significant difference. Based on the results of the post hoc test for each variation of the concentration of ethylene glycol preservation, the Asymp.Sig value < 0.05 was significantly different between day 1 and day 40, while in the treatment group without using ethylene glycol, the value of Asymp.Sig < 0.05 was significantly different between day 1 to day 20. So, it can be concluded that the variation of ethylene glycol from 7.5% to 17.5% provides pooled sera stability for up to 30 days. Meanwhile, pooled sera without ethylene glycol were only stable for up to 10 days on ALP measurement.

Conclusion

In this study, the type of preservative used was ethylene glycol with variations of 7.5%, 10%, 12.5%, 15%, and 17.5%. The results of data analysis on pooled sera that have added a variation of ethylene glycol to ALP measurements showed a significant difference on the 40th day (Asyimp. Sig < 0.05), then the pooled sera were only stable until the 30th day. Meanwhile, the pooled sera that were not treated with ethylene glycol were only stable until the 10th day.

Other studies have revealed that the type of examination can also affect the time difference between the stability of an analyte's examination. Clinical laboratories should freeze their samples as soon as possible to keep the stability of serum when there is a need to repeat analysis, verify a result, or add another laboratory testing (Flores et al., 2020; Sureda-Vives et al., 2017). The stability of the control material is also affected by the storage temperature. Improper storage temperature can affect the results of the inspection. Control materials must be stored at the specified temperature (Fadhilah et al., 2019).

Acknowledgment

The authors would like to express my appreciation and gratitude to the research team and the Padjajaran University as well as the School of Analyst Bakti Asih for the opportunity to publish this scientific paper.

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