

Normal Range of Serum Anti-Streptolysin-O Determined by Automated Turbidimetric Immunoassay

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Abstract

Normal range of serum anti-streptolysin-O (ASO) was determined by a new automated turbidimetric immunoassay, in 760 normal healthy persons which comprised of 300 blood donors, 260 traffic polices, 110 medical students and 90 post-graduated students. The study revealed that distribution of ASO antibody values was not like a bell shape pattern. The ASO value at 80th percentile was calculated to be 270 U/mL which could be used as an upper limit of normal range. The advantages of turbidimetric immunoassay are rapid, auto-mated applicable, reproducible and quantitative. It should replace all current methods in the near future. (*J Infect Dis Antimicrob Agents* 1997;14:61-5.)

INTRODUCTION

Serologic methods for the diagnosis of group A streptococcal (GAS) infection have proved to be of considerable importance to distinguish invasiveness from colonization when the organism is isolated from a mucosa or skin. Streptolysin-O (SLO) produced by group A streptococci during invasiveness interacts with membrane cholesterol and exerts cytolytic-cytotoxic effects on a broad spectrum of mammalian cells. It also stimulates antibody production known as anti-streptolysin-O (ASO) which is regarded as a sign of host response to invasiveness by the organism. The widely available and accepted diagnostic method is to determine and compare ASO titer obtained from paired sera. Up to now in Thailand, determination of the ASO titers has been made by semiquantitative measurements of the inhibition of SLO-induced hemolysis of erythrocytes by ASO (1-3). This Rantz-Randall method

has its disadvantages because hemolysis by SLO can be inhibited by serum cholesterol and depends on resistance of erythrocyte membrane which highly varies among animal species (4,5). In addition, erythrocyte suspension needs to be freshly prepared prior to use. Although latex particles are substituted for red blood cells to ease the preparation and storage, the final value for ASO is still semiquantitative as measured in term of interval (titer). Thus the discrimination power of ASO antibodies by this method is rendered impossible for those whose the antibody levels are different but fall in the same intervals (6).

A devised turbidimetric immunoassay method has been recently introduced to solve those problems. The procedure is simple, rapid and automated applicable for quantitative measurement of ASO (7). Since almost every step of the test is carried out by an automated machine, the result is more reliable because it is less subject to

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manual error or variation and eliminates bias in reading the final reaction as happened in conventional method. In addition, the result is reported as a continuous value and thus is more discriminative. This method is perceived to overcome the disadvantages of all currently conventional methods with the exception of the high cost of the machine. Our study was designed to use the automated method to determine a normal range of ASO antibodies in Thai population.

MATERIALS AND METHODS

Apparatus

We used an automated analyzer (Hitachi 717 autoanalyzer, Boehringer Mannheim Thai) which can measure degree of suspension turbidity at wavelength of 700 nm. in term of continuous variable as a unit. The volume of serum sample used in each test was only 3 microlitres (μ L).

Reagents

Two types of reagent were used in the test. Reagent 1 contained TRIS (hydroxymethyl) aminomethane at concentration of 170 mmol/L and pH of the solution was adjusted to 8.2. Reagent 2 (Streptolysin O-latex) contained borate buffer at concentration of 10 mmol/L at pH 8.2. The latex particles were coated with solution of streptolysin O at concentration of 2 mmol/L. Both reagents were obtained from Boehringer Mannheim Thai and were kept at 4-10° C for three weeks after reconstitution.

Specimens

Seven hundred and sixty sera were collected from 573 male and 187 female healthy adults whose ages ranged between 17-68 years old. They comprised of 300 blood donors, 260 traffic polices, 110 medical students and 90 post-graduated students.

Procedures

The turbidimetric immunoreactions were carried out at 37° C. Firstly, 200 μ L of reagent 1 was added to 3 μ L of the sample, and the mixture was preincubated at 37°C for 5 min. Then 200 μ L of reagents 2 was added. After a lag phase of 80 seconds, change in absorbance was recorded for the next 80 seconds at 700 nm. A reference serum of known ASO value was included in each assay for quality control. Comparison of ASO value among the four groups was performed using one factor analysis of variances (ANOVA, STATVIEW II on Macintosh).

RESULTS

The ASO values of 760 healthy adults ranged from 2 to 814 U/mL with a mean and standard deviation of 177.4 \pm 130.2 U/mL as shown in Fig. 1. No substantial differences could be observed between both sexes, nor between different age groups. The 80th percentile of ASO values was at 270 U/mL which should be the upper limit of normal value in these population (Fig. 2). The means and standard deviations of ASO value of blood donor, traffic polices, medical students and post-graduated students were 189.1 \pm

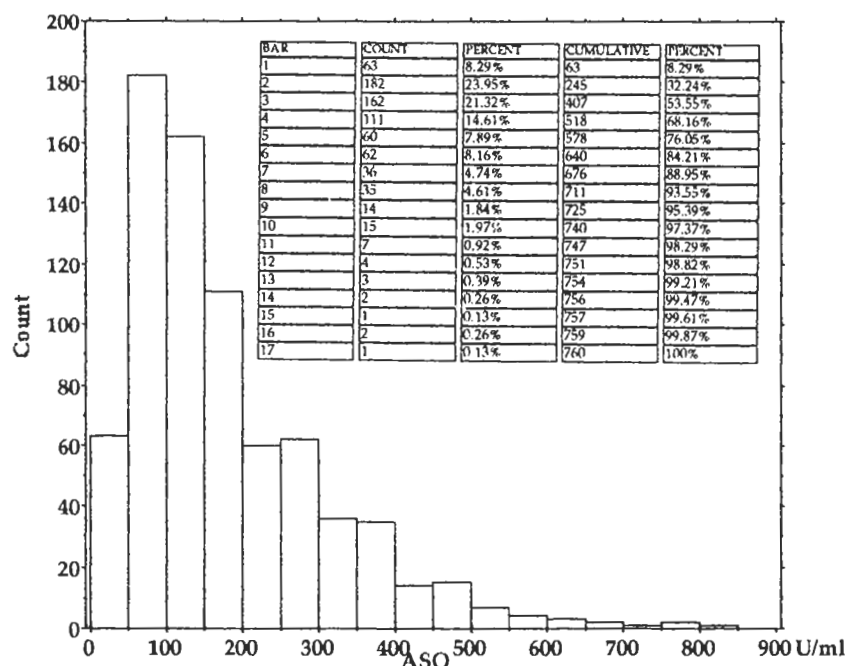


Fig. 1 Histogram of ASO values in 760 male and female individuals.

143.2, 186.4 ± 131.4 , 143.8 ± 96.4 and 153.1 ± 106.7 U/mL respectively (Fig. 3). One factor ANOVA showed that the variances of ASO values of the four groups were statistically different ($p = 0.0027$). When one factor ANOVA was used

again to compare ASO values between pairs among the four groups, statistical significance was obtained when ASO values of medical students were compared with either blood donors or traffic polices (Scheffe F-test, $p < 0.05$).

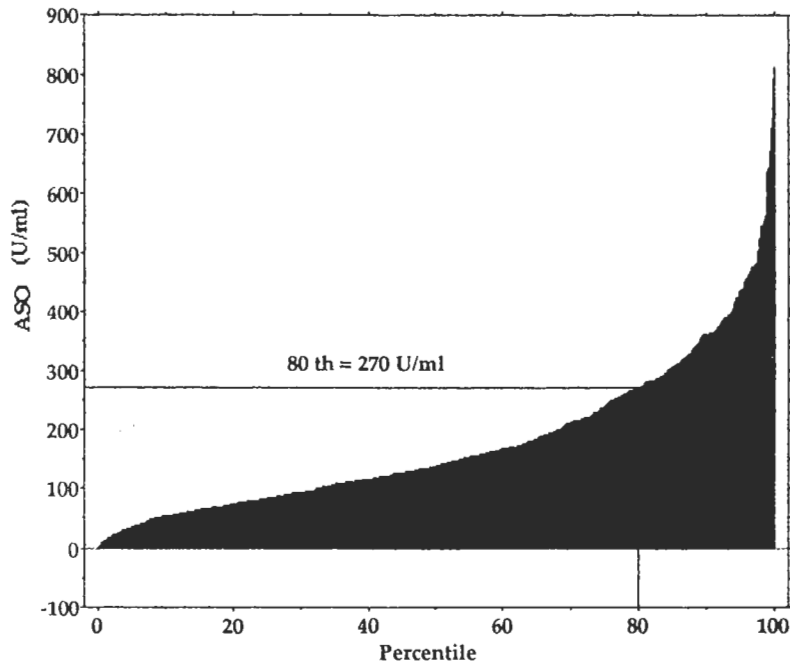


Fig. 2 Percentile plot of 760 ASO values showing the 80th percentile.

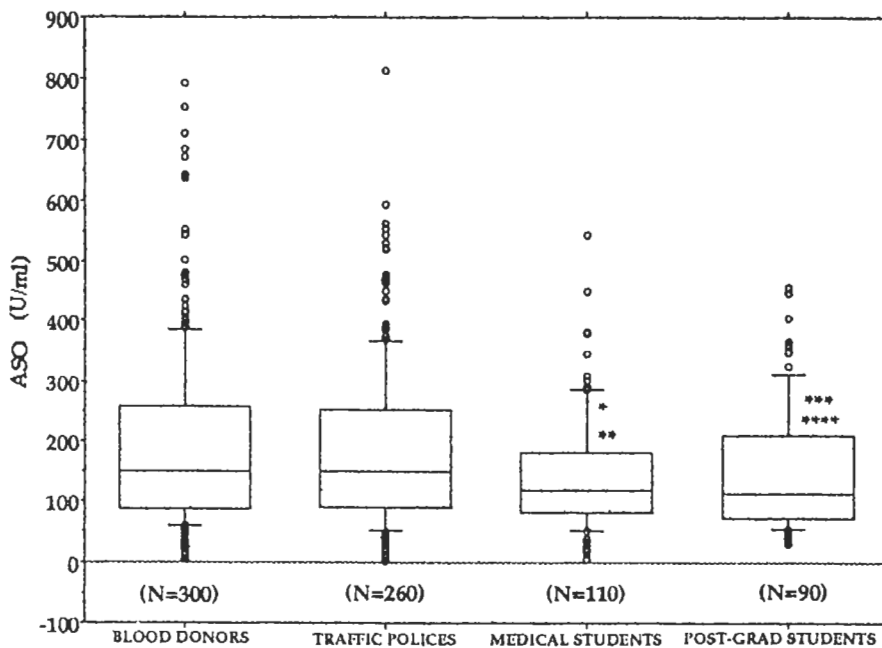


Fig. 3 Box and Whisker plot showing the median, interquartile range and range of ASO values by 4 group of people.

- * blood donors VS medical students ($p < 0.05$)
- ** traffic polices VS medical students ($p < 0.05$)
- *** blood donors VS post-grad students ($p < 0.05$)
- **** traffic polices VS post-grad students ($p < 0.05$)

DISCUSSION

This cross-sectional study is the first study in Thailand to use the automated machine to determine normal range of the ASO values. The result of the study revealed that distribution of ASO antibodies in our so called "normal" persons was in fact not like a bell shape as seen in Fig. 1. If the antigen is specific and only reacts to ASO antibody. The skewness of the distribution is best explained by variation of true ASO levels within the studied population. In fact, we can perhaps, classified our "normal" studied persons into four categories by time elapsed from group A streptococcal infection to blood collection. Since patient with acute apparent infection was excluded from the study, the remaining so called "normal" persons can be viewed as anyone who just recovered from group A streptococcal infection or who had recovered from the infection 3-5 or 6-8 months ago or who had recovered from the infection longer than 1 or 2 years. The ASO antibodies of the former will theoretically yield highest values as found in convalescent sera while the three latter yield lower to lowest values respectively. All the above-mentioned "normal" sera were included in our study and explained perfectly well why the distribution of ASO antibodies was not normal or parametric (8,9). This phenomenon occurs with any antibody determination in healthy population whose antibodies produced by a past infection, still remain in the circulation for months (10,11). A longitudinal study of a case with group A streptococcal bacteremia disclosed that the ASO antibody remained high for at least 1-2 months after the infection (12). The finding of ASO level in this case may support our explanation and emphasizes the necessity of obtaining convalescent antibody to discriminate acute infection from recovery phase or past infection (13). Although we do not know exactly the proportion of the four categories of population enrolled in our study, we arbitrarily choose the 80th percentile of ASO antibody value as the highest normal value. It means that a single convalescent antibody level below 270 U/mL (80th percentile value) can be comfortably treated as non-related to group A streptococcal infection.

Our study also revealed significant difference of the level of ASO antibodies among the blood donors, traffic polices, medical students and post-graduated students. The mean value of ASO antibody was noticed to be lowest in medical students which was significant different from those of the blood donors and traffic polices. Our data are insufficient to explore why the level of ASO antibody in this group is lowest. The average age of medical student

group may be lower than others but the analysis showed that age was not related to level of ASO value in our study. It could be that host defense of the blood donors and traffic polices were easily prone to stimulation of antibody production since the environment they had been working, was heavily polluted. However, this speculation needs to be proven in other study. The upper limit of ASO value in adult was chosen to be 270 U/mL and is in accordance with previous study in Thai population (9). Although the new machine is still expensive for many laboratories in Thailand, the advantages of new immunoturbidimetry are enormous. The result is reported in quantitative values; the assay could apply to automatic machine to eliminate manual error and variation and the reagents are not so expensive. The reproducibility of the test is within 2 percent of initial value. So in the near future we think that this new method will be employed to improve the laboratory diagnosis of group A streptococcal infection.

In conclusion, the ASO values of normal Thais vary among different groups of population studied. However, the upper normal limit of ASO value was chosen to be 270 U/mL. The immunoturbidimetry is a new method and yields high reproducibility when applied to automated machine.

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