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Prozone-like phenomenon found in chemiluminescent enzyme immunoassay using magnetic particles for measurement of serum anti-single stranded DNA antibody titers: Definition and management

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ABSTRACT

Background: Serum anti-single stranded DNA antibody (anti-ssDNAab) is used as a marker for systemic lupus erythematosus. We found a 'prozone-like phenomenon,' which was different from an original prozone phenomenon, in chemiluminescent enzyme immunoassay using magnetic particles for the measurement of serum anti-ssDNAab titers. We investigated mechanisms of the prozone-like phenomenon and countermeasures to prevent it from being overlooked.
Methods: This study examined 679 samples from patients tested for anti-ssDNAab titer at our hospital. In addition, the BF photometry OD value 2 (OD2), an index of optical density, was monitored simultaneously.
Results: The undiluted samples with a prozone-like phenomenon showed extremely lower OD2. Those samples were able to be distinguished from other samples by setting OD2 criteria based on the 95% prediction interval. Significant differences ($p < 0.05$) were found in the titer ratios (ten-fold diluted against undiluted) between groups with > 1.5 and other groups with < 1.5 for the ratios of OD2.
Conclusions: We proposed two valuable methods to find a prozone-like phenomenon: one by setting OD2 criteria based on the 95% prediction interval and the other by analyzing the ratios both in titers and OD2 between undiluted and 10-fold diluted samples.

1. Introduction

An anti-DNA antibody, a serum autoantibody found frequently and specifically in systemic lupus erythematosus (SLE), is used as a marker for diagnosis and therapy [1, 2]. Antibodies to double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA) are generally known as anti-DNA antibodies [3, 4].

Generally, the IgG class antibody titer to ssDNA has been measured using enzyme-linked immunosorbent assay (ELISA) [2, 5–7]. Recently, the ELISA method is being replaced by chemiluminescent enzyme immunoassay (CLEIA) in some immunological test items. Because its analysis time is remarkably shorter, the CLEIA method enables test results to be returned to physicians before patient consultation.

At our laboratory, the CLEIA method was introduced for measuring anti-ssDNA antibodies, replacing ELISA in May 2014. After switching measurement methods, we have found some specimens that are suspected of showing a prozone phenomenon. The titer values in these specimens were beyond the upper limit of the ELISA method, but were within reference intervals in the CLEIA method. Generally, in immunological measurements, a prozone phenomenon or a hook effect is well known as a cause of false lower values [8, 9]. Because the measurement of diluted samples is shown to be effective to dissolve a prozone phenomenon [8, 9], we then measured the diluted samples using the CLEIA method. Results showing higher values over the upper limit of the CLEIA method suggest that the initial values were false lower values.

For the CLEIA method used in our laboratory, the magnetic particles are included in reagents. We distinguished the present phenomenon

Abbreviations: SLE, systemic lupus erythematosus; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; anti-ssDNAab, anti-ssDNA antibody; anti-dsDNAab, anti-dsDNA antibody; ELISA, enzyme-linked immunosorbent assay; CLEIA, chemiluminescent enzyme immunoassay; OD, optical density; BF, bound/free; OD2, BF photometry OD value 2; ENA, extractable nuclear antigen; Sm, smith; RNP, ribonucleoprotein; CCP, cyclic citrullinated peptide

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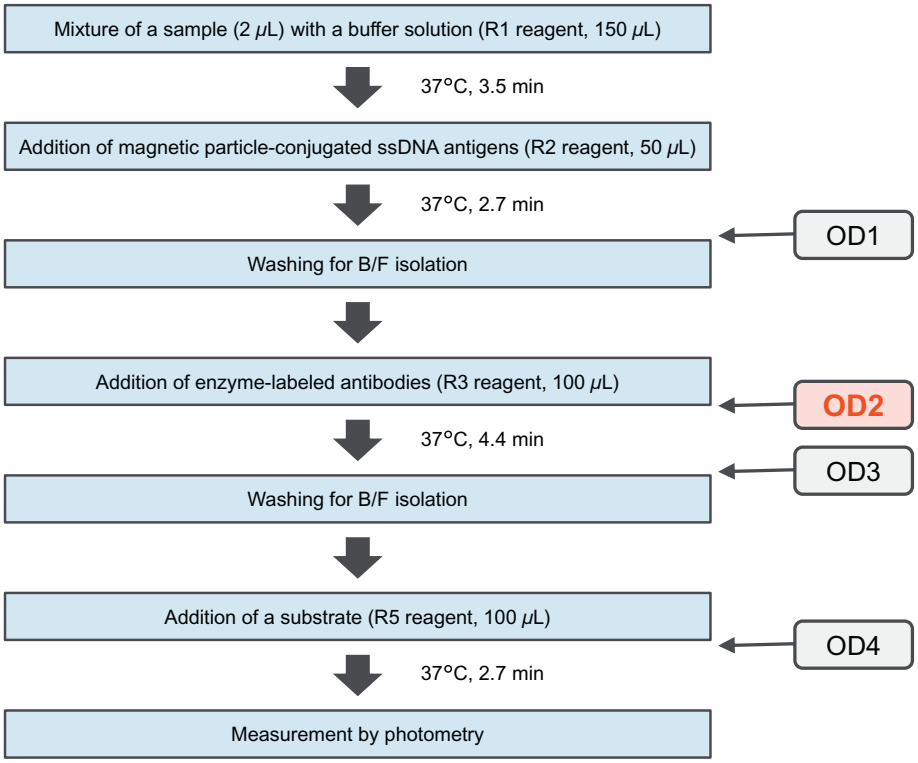

Fig. 1. Assay principle based on the CLEIA method.

from a common prozone phenomenon and designate it in this report as a 'prozone-like phenomenon,' which was found using the CLEIA method with magnetic particles in the measurement of serum anti-ssDNAab titers. This prozone-like phenomenon was defined as fulfilling all three of the following conditions.

i). True titer of the undiluted sample is higher than the measurement upper limit.
ii). Titer of its undiluted sample is falsely lower within a measurement range.
iii). The false lower value presumably results from the aggregation of magnetic particles.

We speculated that falsely lower titers shown in a prozone-like phenomenon might derive from strong aggregation of magnetic particles that are conjugated with the antigens, by means of the antibodies in an extremely high density. To confirm our speculation, we used an index of optical density (OD), designated as BF photometry OD value 2 (OD2), to ascertain whether a reagent was added properly before and after washing processes for bound/free (BF) isolation in the CLEIA method. For the present CLEIA method, the BF photometry OD values are numbered serially as 1–4 because they are measured four times during the total washing process for BF isolation. OD2 is the second among these indexes, which confirms the aggregation states of magnetic particles after an antigen–antibody reaction. Therefore, OD2 was expected to be an index confirming the aggregation states of magnetic particles in a sample showing a prozone-like phenomenon. It is extremely important to prevent a prozone-like phenomenon from being overlooked because falsely lower values derived from a prozone-like phenomenon might have serious influences on diagnosis and treatment. In this paper, we propose some countermeasures using OD2 to prevent a prozone-like phenomenon from being overlooked.

2. Materials and methods

2.1. Subjects

Subjects examined for this study were 679 consecutive samples from outpatients and inpatients tested for the anti-ssDNAab titer at Iwate Medical University Hospital during April 2014 through July 2015. Samples were divided into three groups depending on the period of each study (n = 366, n = 172, n = 141). This study approved by the ethics committee of Iwate Medical University School of Medicine (approval number: H29-13). Although the informed consent was not provided from each subject, the content of this study was disclosed through the internet and the rights of rejection from the corresponding individuals were guaranteed.

2.2. Analyser and reagent

The anti-ssDNAab titer was measured using an automated immunoanalyser (STACIA; LSI Medience Corp., Tokyo, Japan) with its specific reagent (STACIA MEBLux Test ssDNA; Medical & Biological Laboratories Co., Ltd., Nagoya, Japan). Analyte diluent II for STACIA (LSI Medience Corp.) was used to dilute the samples. In this paper, all the titers of the diluted samples are shown as the titers calculated by multiplying the measured values with their dilution rates. Other reagents were processed based on the manufacturer's instructions for STACIA MEBLux Test ssDNA.

The reagent lots used in each study were the following: Lot 009 was used for determining the re-measurement criteria using their diluted samples based on OD2. Lots 010 and 011 were used to verify the re-measurement criteria. Lot 101 was used for analyzing the ratios of the titers and OD2 in high-titered samples.

2.3. Measurement principle

The assay principle is based on the CLEIA method (Fig. 1). As a first-