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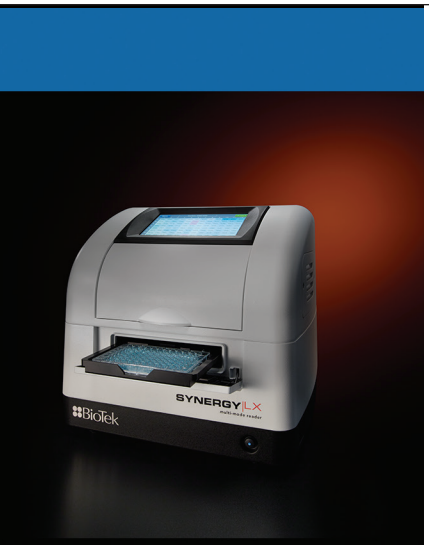
Application Bulletin

Detection of HTLV I and II Antibodies using Absorbance-based ELISA



Introduction

The human T-lymphotropic virus (HTLV) are a family of retroviruses that are known to cause adult T-cell leukemia/lymphoma and a demyelinating disease called HTLV-1 associated myelopathy[1]. HTLVs-are lentiviruses that infect a number of different cell types and integrate into the cellular genome as part of its growth cycle[2]. HTLV testing is routinely performed in blood transfusion, tissue transplantation and clinical diagnosis and is based on the detection of HTLV-1 and/or -2 antibodies present in serum[3]. These tests are normally done using an enzyme-linked immunosorbent assay (ELISA). Specimens with a non-reactive result from the initial ELISA are considered HTLV-negative; while specimens with a reactive ELISA result are retested in duplicate and confirmed by Western blot.

ELISA is one the most utilized assay formats in biomedical research. Numerous clinical, veterinary, and research assays use the specificity of antibodies to identify a diverse array of analytes from any number of different matrices. Despite the diversity of analytes, the general process of ELISA is constant. With a typical ELISA protocol, several repeat cycles of microplate washing, reagent addition and incubation are executed to elicit the chemistries and remove unbound material before data collection. In many instances, the data derived from an ELISA reaction is color absorbance. Here we describe the use of the low cost Synergy™ LX Multi-Mode Reader to perform detection of a human HTLV I/II ELISA assay.

Materials and Methods

A multi-plate HTLV I/II microelisa kit was obtained from Avioq (P/N 500576). The assay was performed as described in the assay kit instructions. Briefly, controls (negative serum and positive HTLV I and HTLV II serum) were diluted 1:5 with sample diluent and 100 µL pipetted into wells of the precoated ELISA plate. Samples were made by further dilution of the HTLV I and II controls with sample buffer. The plate was incubated at 37 °C for 1 hour, after which the plate was washed 4 times with 300 µL of wash buffer with a 30-second soak between wash cycles using an EL406™ Washer Dispenser (BioTek Instruments). After washing 100 µL of EnzAbody working solution was added to all the wells. Working solution was made daily by diluting EnzAbody concentrate 1:251 with EnzAbody diluent. After EnzAbody conjugate addition the plate was incubated for 1 hour at 37 °C and washed again as previously described. After washing, 100 µL of TMB substrate (1:1 mixture of TMB and peroxide solutions) was added to all wells and the plate incubated in the dark at room temperature for 30 minutes. Reactions were stopped by the addition of 100 µL of $2NH_2SO_4$. The absorbance at 450 nm and 630 nm was determined using a Synergy LX Multi-Mode Reader. Data analysis was performed using the delta OD values (630 nm reference).

Results

A comparison of absorbance for positive and negative control specimens from a clinical HTLV I & II kits show good repeatability. As demonstrated in Figure 1, separate controls for HTLV-I or HTLV-II prepared together, but run on separate assay plates, shows a very good correlation. All of the negative and positive controls passed the assay kit's validation criteria (data not shown).

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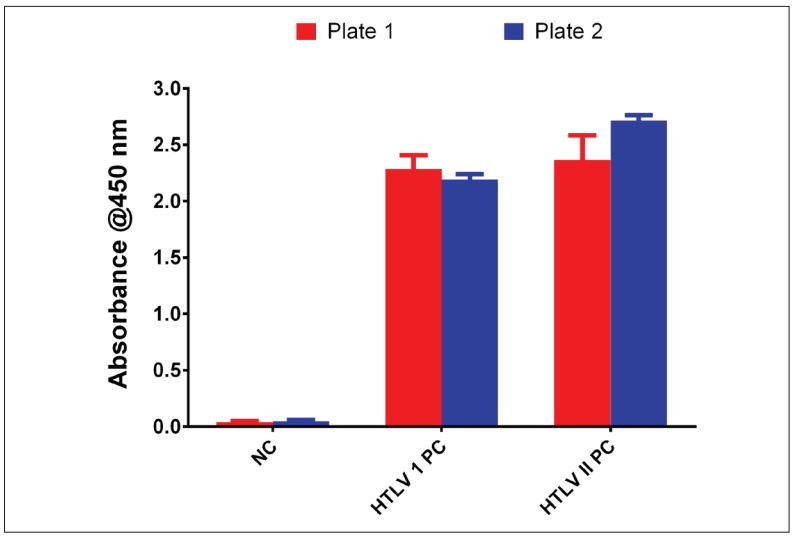

Figure 1. Intraplate comparison of control well absorbance values. The HTLV-I and HTLV-II positive and negative serum kit controls absorbance values from two separate plates are compared. Data for the negative controls represents the mean and standard deviation of 8 wells from each plate, while the positive controls (HTLV I & II) represent 4 wells on each plate

Serial dilution of the HTLV-I and HTLV-II positive control serum samples demonstrates the ability of the Synergy™ LX to make quantitative determinations using ELISA. Measurements made on separate plates are linear with respect to dilution and repeatable (Figure 2). Note that positive controls supplied by the assay kit can be diluted approximately 30 fold before the returned absorbance values are no longer considered to be positive.

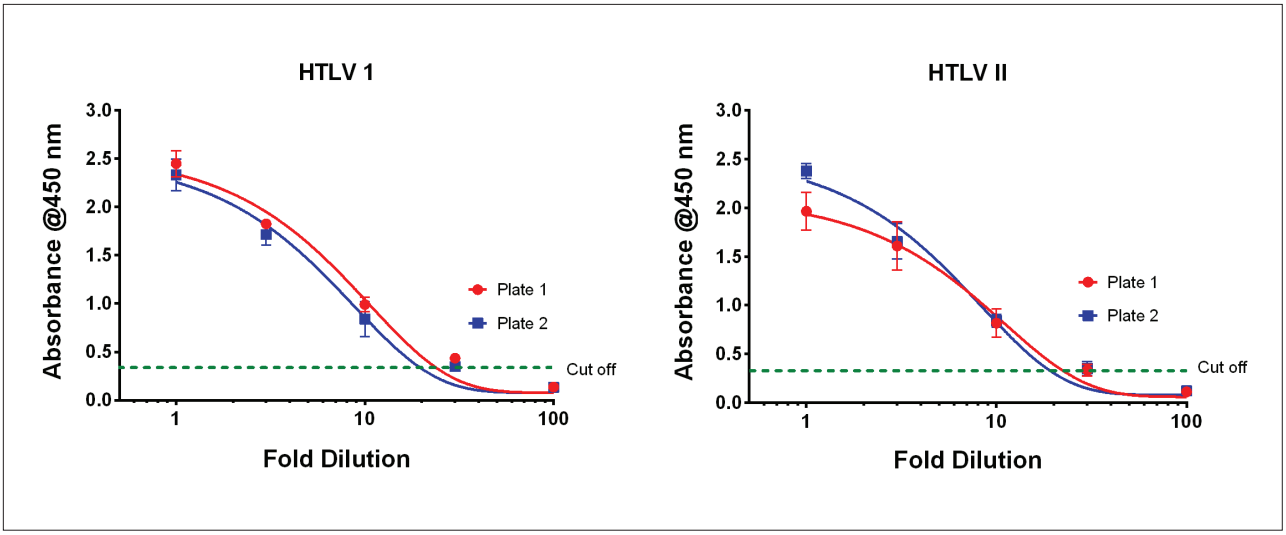

Figure 2. Comparison of HTLV1 and HTLV II Positive serum sample dilutions. Dilutions of positive control samples were assayed on separate plates and the absorbance results compared. A dashed green line indicates the assay positive sample cut off. Data points represent the mean and standard deviation of 4 determinations.

Discussion

These data indicate that the Synergy™ LX is capable of measuring the absorbance of routine ELISA reactions. Dilutions of known positive samples demonstrate the sensitivity of both the assay as well as the multimode reader. While many ELISA assays return a quantitative, positive/negative, determination based on a comparison between experimental and control samples, this technology is can also be used for quantitate unknown determinations. The optics of the reader produce a linear absorbance response from 0 to 2 optical density (OD) units.

References

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