JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1987, p. 1772–1773
0095-1137/87/091772-02$02.00/0

Vol. 25, No. 9

Detection of Antibodies to Human Immunodeficiency Virus by Latex Agglutination with Recombinant Antigen

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Received 19 February 1987/Accepted 28 May 1987

Recombinant human immunodeficiency virus (HIV) *env* antigen was attached to polystyrene particles, and these complexes were used to develop the first latex agglutination assay for antibodies to HIV. A total of 95 positive and 116 negative human serum samples were assayed for antibodies to HIV by latex agglutination, and results were compared with those of a commercial enzyme immunoassay. Latex agglutination was also compared with, and found to be completely concordant with, Western blot (immunoblot) analysis with virion antigens.

Human immunodeficiency virus (HIV) has been shown to be the etiologic agent for acquired immune deficiency syndrome (1, 2). An antibody response to HIV indicates exposure and infection. Current clinical assays for antibodies to HIV are viral-based enzyme immunoassays (EIAs). Viral-lysate EIAs offer the advantage of high sensitivity but the disadvantage of requiring several hours to complete a test and instrumentation which is not available in some laboratories. A latex agglutination assay based on purified recombinant HIV-specific antigen could offer the advantages of relatively high sensitivity, specificity, speed, and simplicity in situations in which the time and technology required for EIA were not available or appropriate. Latex agglutination, unlike EIA, is a direct assay for antibodies. This test is based upon cross-linking antigen which is attached to microbeads with antibodies to form visible aggregates. In this study, highly purified recombinant HIV *env* antigen was attached to microbeads to make a very sensitive and specific antibody test. We have previously developed for HIV *env* antibodies an EIA with this recombinant antigen derived from the gp120 and gp41 regions of the envelope gene which had sensitivity and specificity equivalent to those of radioimmunoprecipitation (4). Recently Burke et al. (1a) have also used this antigen in another EIA format to study 2,707 consecutive serum samples referred for confirmatory testing for antibodies to HIV. The results of that study indicate that all individuals infected with HIV have antibodies to the envelope protein and that no other antigen is required for highly sensitive and specific immunoassays.

Production and purification of the recombinant envelope antigen CBre3 have been described already by Thorn et al. (4). Briefly, the conserved and immunodominant regions of the gene for gp120 and gp41 were cloned and expressed in *Escherichia coli*. Recombinant CBre3 polypeptide was purified to homogeneity as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blots (immunoblots) by standard column chromatography methods and shown to be free of contamination with *E. coli* antigens (4). Purified CBre3 antigen was then attached to polystyrene beads (PolyScience Corp.) 0.5 μm in diameter, washed, and suspended in 1% bovine serum albumin in phosphate-buffered saline (pH 7.6). The agglutination assay was performed by spreading 25 μl of serum or a dilution thereof on an agglutination card in a 1.5-cm-diameter circle, adding 15 μl of a 0.6% suspension of CBre3-latex, and mixing by rotating the slide gently for 3 to 5 min. A positive reaction for antibodies to HIV was agglutination visible in bright light, and a negative reaction was no agglutination, i.e., a serum-and-latex suspension with a milky, smooth consistency.

The data shown in Table 1 are from 211 serum samples tested retrospectively for antibodies to HIV by latex agglutination and commercial EIA and by Western blot of those samples positive by EIA. Four samples which were positive for the *gag* antigen only by Western blot (data not shown) were positive by CBre3-latex agglutination even though no *gag* antigen was present on the latex. There was concordance between all samples tested by Western blot and CBre3-latex agglutination and only one discordant sample by commercial EIA. With Western blot as the reference method, the discordant sample was a false-positive by EIA.

Even though for some of the specimens the optical densities by commercial EIA were as low as 0.517, latex agglutination was still able to detect antibodies to HIV in these samples. In this group, samples for latex agglutination were tested both undiluted and diluted 1:10; this dilution did not affect whether a sample scored positive or negative. However, some of the positive samples with high optical densities by EIA had a more intense reaction by agglutination when they were diluted 1:10 than when they were undiluted. This means that a prozone effect is likely with high-titer positive samples.

TABLE 1. Comparison of HIV antibody determinations and confirmation of the EIA positive responses by Western blot of viral lysates[a]

Western blot response	No. of responses in:			
	Latex agglutination		Commercial EIA	
	Pos	Neg	Pos	Neg
Pos	95[b]	0	95	0
Neg	0	30	1	29
NT	0	86	0	86

[a] Optical density range for positive samples was 0.517 to >2.0; for negative samples, the range was 0.049 to 0.121. Pos, Positive; Neg, negative; NT, not tested by Western blot.

[b] Includes four specimens which were positive for the *gag* antigen only and one specimen which was p24 negative.

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TABLE 2. Latex agglutination by CBre3-EIA with diluted sera[a]

Dilution	Latex agglutination response (OD by CBre3-EIA) of serum sample:		
	1	2	3
1:9	4+ (1.10)	4+ (1.10)	4+ (0.70)
1:27	4+ (0.93)	4+ (0.93)	3+ (0.47)
1:81	3+ (0.41)	3+ (0.49)	N (0.16)
1:243	N (0.16)	N (0.18)	N (0.08)
1:2,400	N (0.08)	N (0.08)	N (0.02)

[a] N, No agglutination or a negative reaction; OD, optical density. An optical density of <0.20 indicates a negative reaction by CBre3-EIA. Positive serum samples were diluted with negative serum to the dilution ratios indicated.

An experiment was performed to compare agglutination and CBre3-EIA (4) with respect to endpoint dilutions of positive sera. Negative serum was used to dilute three positive serum samples. Each diluted sample was then assayed by latex agglutination and CBre3-EIA. The results (Table 2) show similar reactivities by CBre3-EIA and latex agglutination with these three diluted positive serum samples. This means that latex agglutination with recombinant *env* antigen has sensitivities similar to those of EIA with these serum samples. It should be pointed out that the specimen can be tested undiluted by latex agglutination, while EIA generally requires that the specimen be diluted at least 1:20 during the procedure.

In Fig. 1 are shown the relative intensities of the reactivities of 95 positive serum samples by latex agglutination. A 4+ reaction is the strongest and most obvious, and a 1+ reaction is the least discernible above the lack of reactivity with a negative control (3). These data indicate that most of the 95 positive serum samples are strongly reactive by latex agglutination and are therefore easily read.

The thermal stability of CBre3 antigen on latex was determined by incubating a preparation at 37°C and testing

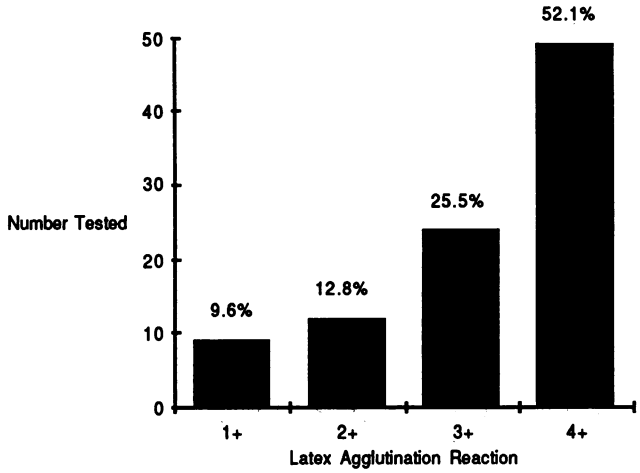

FIG. 1. Plot of reactivity of positive serum samples versus number of samples tested.

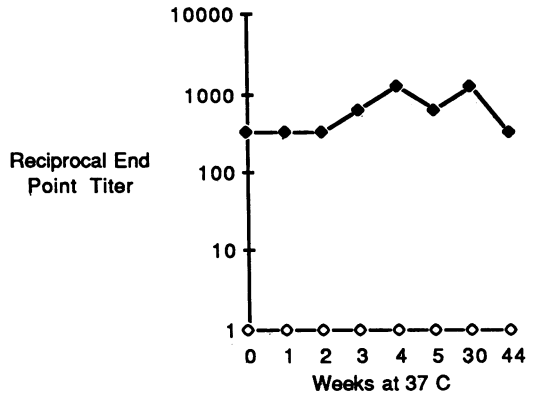

FIG. 2. Thermal stability of CBre3 on latex. Endpoint titers by agglutination of serum samples positive (◆) and negative (◇) for antibodies to HIV versus weeks at 37°C for the latex-antigen complex are shown.

samples at various times with positive and negative serum samples. Negative serum samples were diluted 1:10; positive serum samples were diluted serially from 1:10 until there was no agglutination. Endpoint titer, the last dilution which had a 1+ agglutination, was plotted versus time at 37°C. The results of this experiment are shown in Fig. 2. These results indicate that CBre3-latex has reactivity with positive serum after 44 weeks at 37°C and no reactivity with negative serum during the same period.

The results presented here show that CBre3 could be used in a latex agglutination format to provide a rapid, very sensitive, and specific alternative to EIA for antibody testing for HIV. This test could be used in an emergency clinical setting where there would be a need for a rapid test, requiring 5 min or less, for antibodies to HIV and in areas lacking EIA instrumentation.

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