

Evaluation of Passive Particle Agglutination Test for Antibody to Human Immunodeficiency Virus

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A gelatin particle agglutination assay was compared with indirect immunofluorescence by using 663 serum samples from acquired immunodeficiency syndrome and acquired immunodeficiency syndrome-related complex patients and asymptomatic male homosexuals in the United States and from hemophiliacs and healthy adult controls in Japan. The results showed that all 104 samples which were positive by indirect immunofluorescence were also positive by particle agglutination, while 5 additional samples were positive by particle agglutination only. The coincidence rate for antibody-positive and antibody-negative specimens was 99% (658 of 663) between particle agglutination and immunofluorescence. Four of the five samples which were positive by particle agglutination only were found by radioimmunoprecipitation to contain anti-*env* gene products of human immunodeficiency virus. Antibody titers of samples giving a positive reaction by particular agglutination varied from low (titer, 256) to remarkably high (256×10^5). All specimens having particle agglutination titers of more than 10^5 were positive by immunofluorescence. A high correlation ($r = 0.66$) was observed between the titers of antibodies determined by particle agglutination and those determined by immunofluorescence. After fractionation of a serum sample from an individual at high risk by using high-performance liquid chromatography, it was shown that immunoglobulin M as well as immunoglobulin G human immunodeficiency virus antibody was detected by particle agglutination. Additional serum samples with a potential risk of giving false-positive results, such as heat-treated specimens, specimens containing antibodies to HLA, specimens containing auto-antibodies, and serum samples from individuals with a history of multiple blood transfusions, were shown to be clearly negative by particle agglutination.

Human immunodeficiency virus (HIV) is the etiologic agent of the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC) (1, 13, 16). HIV is transmitted by transfusion and the administration of blood products (3), although other means, such as sexual contact (11, 17), are also important. HIV has consistently been found in HIV antibody-positive individuals (4, 5). Accordingly, to prevent the spread of the virus, it is most important to screen for HIV antibody. Very recently we developed a simple gelatin particle agglutination test based on the principle of passive agglutination for HIV antibody (7, 20). The results of the particle agglutination test can be read with the naked eye without any special apparatus. In addition, the procedure is performed at room temperature. Therefore the particle agglutination test for HIV antibody seems to be especially suitable for use in developing nations.

A particle agglutination test (10), which is based on the same principle as that for HIV antibody, has already been developed for the detection of antibody to the human T-cell lymphotropic virus type I (HTLV-I), a possible causative agent of adult T-cell leukemia (9, 19), which is endemic in southwest Japan (8, 14). To prevent the spread of this virus by blood transfusion, serum samples from blood donors have been tested for HTLV-I antibody by the particle agglutination test with the Serodia-ATLA kit (Fujirebio Inc., Tokyo, Japan) in Japanese Red Cross Blood Centers since February 1986. It has been shown that the particle agglutination test for anti-HTLV-I antibody is effective in screening blood donors in Japan.

In this report we present further detailed data on the detection of HIV antibody by using this particle agglutination test and several groups of serum samples from high-risk

individuals. The particle agglutination test was compared with an indirect immunofluorescence test, and specimens giving discrepant results were investigated by the radioimmunoprecipitation technique.

MATERIALS AND METHODS

Preparation of viral antigen-coated particles. Viral antigen-coated gelatin particles were prepared as described by Yoshida et al. (20). Briefly, the culture supernatant of MOLT-4/HTLV-III cells was concentrated and purified by centrifugation through a 15 to 50% (wt/vol) sucrose density gradient at $85,000 \times g$ for 16 h. The fractions (about 1.16 g/cm^3) corresponding to the highest reverse transcriptase activity of the virus were collected. The virus pellet was disrupted with 0.5% (vol/vol) Nonidet P-40. The viral antigen ($150 \mu\text{g}$ of protein per ml) was mixed with the same volume of 5% (vol/vol) gelatin particle suspension activated with $5 \mu\text{g}$ of tannic acid per ml in 0.15 M phosphate buffer. The antigen-sensitized particles were washed with phosphate-buffered saline, and then suspended in phosphate-buffered saline and lyophilized.

Procedure for the particle agglutination test. The test serum sample was serially diluted in volumes of $25 \mu\text{l}$ per well in a U-bottom microtiter plate. Portions ($25 \mu\text{l}$) of 1% (vol/vol) antigen-sensitized particles and unsensitized particles were dropped into the wells containing 1:16 (final dilution, 1:32) and 1:8 (final dilution, 1:16) diluted serum, respectively. The contents of the wells were mixed with a tray mixer and then allowed to stand at room temperature for 2 h. A result was considered positive when unsensitized particles were not agglutinated and sensitized particles gave a definite agglutination pattern. The particle agglutination titer was expressed as the reciprocal of the highest dilution of serum giving a positive reaction.

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TABLE 1. Prevalence of HIV antibody by particle agglutination and immunofluorescence according to clinical status

Origin and clinical status	No.	No. (%) positive by ^a :		
		PA	IF	RIP ^b
United States				
AIDS patients	48	48 (100)	46 (96)	2/2 (100)
ARC patients	21	21 (100)	21 (100)	
AMH ^c	29	7 (24)	6 (21)	0/1 (0)
Healthy individuals	10	0 (0)	0 (0)	
Japan				
Hemophiliacs	105	33 (31)	31 (30)	2/2 (100)
HTLV-I positive individuals	50	0 (0)	0 (0)	
Healthy individuals	400	0 (0)	0 (0)	

^a PA, Particle agglutination; IF, immunofluorescence; RIP, radioimmunoprecipitation.

^b Radioimmunoprecipitation was carried out on serum samples that were particle agglutination positive but immunofluorescence negative. Results are expressed as number positive/number tested.

^c AMH, asymptomatic male homosexuals.

Indirect immunofluorescence test. The immunofluorescence test was carried out with H9/HTLV-III cells as described by Popovic et al. (16). Methanol-fixed H9/HTLV-III cell smears were incubated for 30 min at 37°C with a 1:10 dilution of each serum sample for a screening. The preparation was then washed for 15 min with phosphate-buffered saline. Fluorescein isothiocyanate-conjugated anti-human immunoglobulin G (IgG) (Dakopatts A/S, Copenhagen, Denmark) was applied, and the mixture was incubated for 30 min at 37°C and washed with phosphate-buffered saline. The fluorescent cells were examined under a fluorescence microscope (SF-EFA; Nikon, Tokyo, Japan). The immunofluorescence titer was expressed in the same manner as described for the particle agglutination test.

Radioimmunoprecipitation. Viral antigens were labeled by incubating MOLT-4/HTLV-III cells at 37°C for 16 h in leucine-free RPMI 1640 medium supplemented with 10% fetal calf serum, 10⁶ cells per ml, and 50 µCi of L-[³H]leucine (Amersham International plc, Amersham, United Kingdom) per ml. The labeled viral particles were concentrated and lysed with extraction buffer containing 10 mM Tris hydrochloride (pH 8.0), 0.14 M NaCl, 3 mM MgCl₂, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 0.5% Nonidet P-40. The lysate was cleared by centrifugation at 14,000 × g for 10 min. Immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis were performed as described by Koyanagi et al. (12).

Determination of immunoglobulin class. Serum samples were fractionated by high-performance liquid chromatography (chromatograph model 6000A; Waters Associates, Inc., Milford, Mass.) with a TSK gel G3000 SW column (Toyo Soda, Tokyo, Japan). The column was eluted at 1 ml/min with 0.2 M sodium phosphate buffer (pH 7.0). The A₂₈₀ of the eluate was monitored. The antibody fractions were titrated by particle agglutination.

RESULTS

Antibodies detected by particle agglutination and immunofluorescence tests. Serum samples (*n* = 663) from AIDS and ARC patients, asymptomatic male homosexuals, and other healthy individuals in the United States and from hemophiliacs, HTLV-I antibody-positive individuals, and healthy individuals in Japan were tested for the presence of the

TABLE 2. Comparison of particle agglutination and immunofluorescence^a

PA result	IF result		Total
	Positive ^b	Negative	
Positive ^c	104	5	109
Negative	0	554	554
Total	104	559	

^a The 663 specimens in Table 1 were examined by particle agglutination and immunofluorescence simultaneously. PA, Particle agglutination; IF, immunofluorescence.

^b Titer > 1:10.

^c Titer > 1:32.

antibody to HIV by particle agglutination and immunofluorescence tests (Table 1).

Comparison of particle agglutination and immunofluorescence tests. All 104 serum samples showing positive results by immunofluorescence were positive by particle agglutination (Table 2). No samples that were negative by particle agglutination were positive by immunofluorescence; five (0.8%) were positive by particle agglutination but negative by immunofluorescence. Hence the particle agglutination results agreed well with those of immunofluorescence, with a consistency of 99% (658 of 663).

To study whether the five serum samples that were positive by particle agglutination but negative by immunofluorescence were indeed positive for anti-HIV antibody, we used radioimmunoprecipitation. Four of the five serum samples (two from AIDS patients and two from hemophiliacs) were positive for the antibodies to the *env* gene-related HIV proteins gp120 and gp160 but were not positive, or were only weakly positive, to the *gag* gene-related proteins p19 and p25 (Fig. 1, lanes 1 to 4). One specimen from an asymptomatic

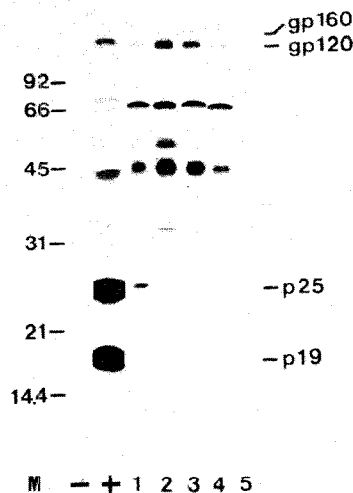


FIG. 1. Electrophoretic analysis of HIV polypeptides immunoprecipitated with five particle agglutination-positive, immunofluorescence-negative sera. Immunoprecipitation of labeled HIV extracts with serum samples from two AIDS patients (lanes 1 and 2) two hemophiliacs (lanes 3 and 4), and one asymptomatic male homosexual (lane 5) was carried out. Lanes - and + contain negative and positive control sera, respectively. The marker proteins (lane M) were phospholipase B (*M_r* 92,500), bovine serum albumin (*M_r* 66,200), ovalbumin (*M_r* 45,000), carbonic anhydrase (*M_r* 31,000), soybean trypsin inhibitor (*M_r* 21,500), and lysozyme (*M_r* 14,400).

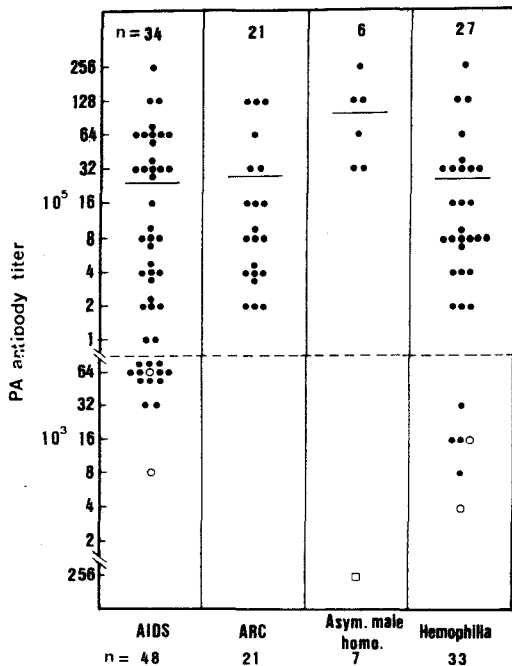


FIG. 2. Particle agglutination (PA) antibody titers in the 10^5 serum samples from patients with AIDS and ARC, asymptomatic male home sexuals, and hemophiliacs. Symbols: ●, immunofluorescence positive; ○, immunofluorescence negative, radioimmunoprecipitation positive; □, immunofluorescence negative, radioimmunoprecipitation negative. The bar in each column shows the mean titer of different groups of specimens.

asymptomatic male homosexual (particle agglutination titer, 256) did not react with any viral proteins (lane 5).

Distribution of antibody titer of particle agglutination. The particle agglutination-positive specimens were titrated for the antibody to HIV (Fig. 2). Various samples including those from 48 AIDS and 21 ARC patients, 7 asymptomatic male homosexuals, and 33 hemophiliacs were studied. Particle agglutination titers ranged from 256 to 256×10^5 . All specimens with a particle agglutination titer of 1×10^5 or above were unequivocally positive by immunofluorescence. The frequency of such a high titer (10^5 or above) was 71, 100, 86, and 82% among serum samples from AIDS patients, ARC patients, asymptomatic male homosexuals, and hemophiliacs, respectively. All five immunofluorescence-negative specimens (from two AIDS patients [particle agglutination titers of 8×10^3 and 64×10^3], from one asymptomatic male homosexual [particle agglutination titer of 256], and from two hemophiliacs [particle agglutination titers of 4×10^3 and 16×10^3]) showed particle agglutination titers lower than 1×10^5 .

Correlation of antibody titer determined by particle agglutination and immunofluorescence. The antibody titer determined by particle agglutination was compared with that determined by immunofluorescence. Titers of antibodies obtained by particle agglutination and immunofluorescence showed a good correlation ($r = 0.66$) (Fig. 3).

Determination of immunoglobulin class. A serum sample from an individual in a high risk group was fractionated by high-performance liquid chromatography. The fractions corresponding to IgG and IgM antibody classes were titrated by particle agglutination. The result showed that the IgM as

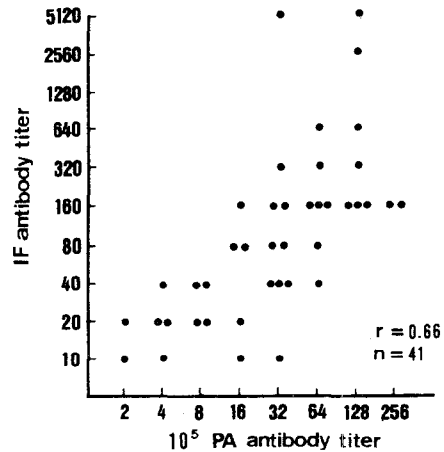


FIG. 3. Correlation of antibody titers determined by particle agglutination (PA) and immunofluorescence (IF).

well as the IgG antibody class was detected by particle agglutination (Fig. 4).

Particle agglutination test on samples with the potential for producing false-positive results. Various Japanese serum samples with the potential for causing false-positive results were examined by particle agglutination. They included 50 heat-treated samples from healthy adults, 46 HLA antibody-positive samples (10, A locus; 24, B locus; 4, C locus; 8, DR locus), and samples from 50 pregnant women, 36 systemic lupus erythematosus patients, 50 patients with a history of multiple blood transfusions, 156 rheumatoid factor-positive patients, 41 anti-thyroglobulin antibody-positive patients, and 36 patients with the anti-microsome antibody. All sam-

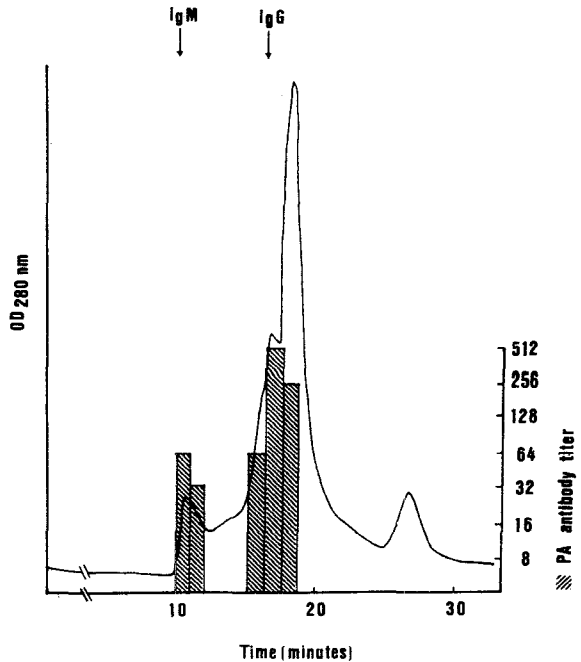


FIG. 4. Detection of HIV IgM antibody by particle agglutination (PA). A serum sample from an individual at high risk for AIDS was fractionated by high-performance liquid chromatography, and IgG and IgM fractions were subjected to the particle agglutination test.

ples were unequivocally negative by particle agglutination (data not shown).

DISCUSSION

In immunological assay systems for the detection of antigens or antibodies, hydrophobic plastic carriers such as polystyrene and polyvinyl have usually been used as the solid phase. Accordingly, the potential for false-positive results may arise from the nonspecific binding of immunoglobulins to the hydrophobic solid phase. To overcome nonspecific reactions, we used a newly developed artificial gelatin carrier for the particle agglutination test. The particles are made of gelatin and gum arabic and are hydrophilic. By this particle agglutination test, serum samples from 400 healthy individuals were all negative. Studies with a number of serum samples with the potential for causing false-positive results revealed that they were completely negative for HIV antibodies. Thus the use of gelatin particles greatly reduced nonspecific reactions. This is consistent with the data obtained with the particle agglutination assay kit for HTLV-I (10).

In the present study we applied the immunofluorescence test, in parallel, as a confirmatory test for HIV antibody. Immunofluorescence is superior in specificity because it is possible to discriminate between specific and nonspecific reactions through interpretation of the fluorescent image. The particle agglutination test did not give false-negative results when compared with the results for immunofluorescence-positive samples. However, the immunofluorescence test failed to detect the HIV antibody in 4 of 663 specimens. It is likely that this discrepancy is related to the different sensitivities of the tests. It is consistent with the fact that particle agglutination antibody titers of all positive specimens were much higher than immunofluorescence titers. All the specimens giving a particle agglutination titer of 10^5 or above were positive by immunofluorescence. Since all immunofluorescence-positive samples were radioimmunoprecipitation positive without exception, although not vice versa (6, 20), we studied only five serum samples which were positive by particle agglutination but negative by immunofluorescence. Four of the five specimens with a particle agglutination-positive but immunofluorescence-negative reaction were shown to contain the antibodies to *env* gene products gp120 and gp160 but not *gag* polypeptides p24 and p19 of the virus by radioimmunoprecipitation. Only one sample studied by radioimmunoprecipitation failed to show a positive reaction, and we do not know whether this is indeed negative, since the sensitivity of particle agglutination may be higher than that of radioimmunoprecipitation.

In the present study it was revealed that HIV antibody titers are much higher than HTLV-I antibody titers in comparison with the results reported by Ikeda et al. (10) with an identical particle agglutination assay system. However, it is surprising that an apparent prozone phenomenon was never encountered, even under the particle agglutination assay conditions used for HIV antibody. The reasons for the extremely high HIV antibody titers compared with those of HTLV-I antibody are as follows. HIV is considered to destroy T lymphocytes *in vivo*, inducing immune deficiency (16), whereas HTLV-I causes T-cell transformation (18), resulting in leukemia or lymphoma (9) after a long latency period. Thus the difference in host immunity to the viruses for antibody production is considered to cause the difference in antibody titers.

Some reports suggest that HIV-IgM antibody may be the only serological sign of primary infection in some instances

(2, 15). Since particle agglutination can simultaneously detect both IgG and IgM HIV antibody classes, the test may also be suited for such situations.

Since the particle agglutination test is very simple to perform, owing to the one-step antigen-antibody reaction, and requires only 2 h for completion, a large number of samples can be handled. The test can be performed at room temperature, and heat inactivation of the serum or plasma does not affect the results significantly. The results can be read with the naked eye without any special apparatus. For these reasons, we believe that the particle agglutination test is very useful for the mass screening of HIV antibody in blood centers.

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