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Immunoglobulin G Antibody Capture Enzyme-Linked Immunosorbent Assay: a Versatile Assay for Detection of Anti-Human Immunodeficiency Virus Type 1 and 2 Antibodies in Body Fluids

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In tests on specimens of dried blood, saliva, and urine from 55 human immunodeficiency virus (HIV)-seropositive and 55 HIV-seronegative patients, an immunoglobulin G capture enzyme immunoassay for the detection of antibodies to HIV types 1 and 2, GACELISA, gave 109 of 110, 109 of 109, and 109 of 110 correct results, respectively. This performance, achieved in a laboratory previously unfamiliar with the assay, suggests that GACELISA is a useful new epidemiological tool for the study of HIV infection, equally applicable to all three kinds of specimen.

Immunoglobulin G (IgG) antibody capture (GAC) immunoassay has been developed to detect antibody to human immunodeficiency virus (HIV) types 1 and 2 in body fluids with immunoglobulin concentrations lower than those in serum. During initial development and evaluation at the Public Health Laboratory Service Virus Reference Laboratory the assay was used in the form of a radioimmunoassay to investigate saliva specimens (3, 4) and in the form of an enzyme-linked immunoassay (GACELISA HIV 1+2) to investigate urine (2) and saliva specimens. To assess the accuracy of GACELISA in an independent laboratory, a prototype kit was sent to the Virology Division, Siriraj Hospital, Bangkok, Thailand. There, after a brief self-familiarization, staff used it to test the serum, dried blood, saliva, and urine specimens of 55 HIV-seropositive and 55 HIV-seronegative patients whose serostatus had already been established. The HIV-seropositive group comprised 12 in patients with HIV-related disease and 43 asymptomatic users of injected drugs. The HIV-seronegative group comprised 9 healthy volunteers and 46 patients who were users of injected drugs. All the patients were drawn from three hospitals in Thailand.

The patients consented to give finger prick blood specimens, which were dried onto filter paper; saliva specimens, which were dribbled into a pot; and urine specimens. After the paper was dried, 5-mm discs were cut from the paper and eluted into phosphate-buffered saline so that the blood was diluted approximately 1 in 20. Ten microliters of each eluate was then diluted in 90 μl of assay diluent for testing by GACELISA HIV 1+2. The saliva and the urine specimens (50-μl volume), undiluted and untreated, were similarly tested. Previously collected serum specimens from the same patients were also tested (10 μl in 90 μl of assay diluent).

Microtiter plates (Nunc Maxisorp 4-75078A) were supplied precoated and dried. For the GACELISA procedure (2) the microplate wells had been coated by the addition of a dilution in carbonate buffer, pH 9.4, of a rabbit antiserum (5 mg/liter) to gamma chains of human IgG (Dakopatts A424). After an interval of at least 18 h the anti-IgG solution was removed and the wells were blocked by the addition of 0.5% gelatin. The following day the gelatin was removed and the plates were dried overnight at 37°C and then packaged with desiccant.

Specimens and controls (three negative and one HIV-1 positive) were added to individual wells. After 30 min of incubation at 37°C the specimens and controls were washed off and 50 μl of a solution of HIV-1 and HIV-2 antigens, both conjugated to alkaline phosphatase (Wellcome Diagnostics, Dartford, United Kingdom), were added. After 60 min at 37°C and another wash, a substrate was added, followed, after 20 min, by an amplifier (5). Ten minutes later, the reactions were stopped with 50 μl of 2 M sulfuric acid and the density of color generated was read at 492 nm. The cutoff value was calculated as three times the mean optical density of the negative controls. Routine HIV antibody testing of the serum specimens from the patients was by Wellcozyme HIV 1+2 (VK 54/55) enzyme immunoassay (EIA) done according to the manufacturer's instructions and confirmed where necessary by Western blot (immunoblot). For the purpose of assessing the GACELISA findings on the blood, saliva, and urine specimens, these serological results were taken as correct.

For 107 of the 110 patients, the routine EIA result on serum and the GACELISA results on the same serum and corresponding capillary blood eluate, saliva, and urine specimens were concordant. The distributions of the assay reactions of seropositive and seronegative patients, expressed as test optical density/cutoff (OD/CO) ratios, are shown in Table 1. Discrepant results are shown, underscored, in Table 2.

Our findings indicate that GACELISA would be a useful epidemiological tool in any context where it would be impractical to collect venous blood. Out of 110 samples examined there was one weak false-positive result on a finger prick specimen and one false-negative result on a urine

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TABLE 1. Median and range of OD/CO ratios by routine EIA and GACELISA for specimens from HIV-seropositive and -seronegative patients

Patient group	OD/CO [median (range)] for sample and test				
	Serum		Blood eluate GACELISA	Saliva GACELISA[a]	Urine GACELISA
	Routine EIA	GACELISA			
Seropositive (n = 55)	11.7 (4.6–12.6)	9.2 (4.2–10.0)	9.0 (3.9–10.3)	8.5 (1.3–10.4)	8.6 (0.9–10.0)
Seronegative (n = 55)	0.4 (0.1–0.5)	0.5 (0.3–2.0)	0.6 (0.4–1.3)	0.3 (0.3–0.6)	0.4 (0.3–0.9)

[a] A saliva specimen was not available from 1 HIV-seropositive subject (A55).

specimen; otherwise the results on noninvasive specimens were correct. Serum from two subjects gave rise to weak false-positive GACELISA reactions, including the one from the subject whose finger prick sample was falsely reactive. While it is possible that seroconversion was occurring in this case, we have found GACELISA to have a 0.65% false-positive rate when testing serum specimens. These false-positive reactions are usually weak, as in patients B2 and B26. A recent modification of the assay to incorporate a peroxidase conjugate has lowered the false-positive rate (unpublished results).

To avoid false-negative results in GACELISA, we suggest that attention be paid to ensuring that the saliva specimens collected are rich in crevicular fluid (1). This can be done by swabbing the gum surfaces extensively, using the same routine as for toothbrushing. As long as the patient or attendant is instructed beforehand how to collect these specimens, the swabs will yield an eluate with a higher immunoglobulin content than is found in dribbled saliva (unpublished observations). It may then be assumed, at least for epidemiological purposes (2), that both these eluates and urine specimens have an IgG concentration sufficient for GACELISA testing. For clinical purposes, however, a measurement of total IgG may still be necessary to exclude false-negative findings which may arise as a result of unusually low total IgG content of the specimen. Even without these precautions, the accuracy of GACELISA tests on the finger prick blood, saliva, and urine specimens collected in this study was close to that to be expected when serum specimens are tested by conventional screening EIAs.

TABLE 2. OD/CO ratios for tests on the three patients whose specimens gave discrepant results[a]

Patient	OD/CO for sample and test				
	Serum		Blood spot GACELISA	Saliva GACELISA	Urine GACELISA
	Routine EIA	GACELISA			
A55	6.7	9.3	4.8	NA[b]	0.9[c]
B2	0.4	2.0	1.3	0.3	0.5
B26	0.1	1.3	0.9	0.4	0.9

[a] All results were concordant for the other 107 patients.
[b] NA, specimen not available.
[c] Discordant results are underscored.

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