

Title: GACPAT Assay for the Detection of Anti-HIV Antibody in Urine

Virology Division, Public Health Laboratory Services Branch

Centre for Health Protection

Department of Health

Hong Kong



GACPAT Assay for the Detection of Anti-HIV Antibody in Urine

1 Source reference

- (1) An IgG Capture Particle Adherence Test (GACPAT) for the detection of Anti-HIV. PHLS Virus Reference Laboratory. Central Public Health Laboratory. London. 1990.
- (2) John V. Parry and Philip P. Mortimer. An IgG Antibody Capture Particle Adherence Test (GACPAT) for antibody to HIV-1 and HTLV-1 that allows economical large-scale screening. AIDS Vol. 3. No.3. 1989.

2 Principle

The IgG antibody capture particle adherence test (GACPAT) is a simple, inexpensive and sensitive assay for the detection of HIV antibody in urine. Test urine specimens are placed into wells of microtitre plate previously coated with anti-gamma chains so that any human IgG present in the urine will be captured onto the wells of the plate. HIV antigen-coated particle from the Serodia HIV kit is then added so that particle adherence will occur if HIV antibody is present.

3 Testing Criteria

The GACPAT test is used for screening of HIV antibody in urine specimens, including those taken from methadone clinics, chest clinics, drug addict treatment centres and prisoners.

4 Limitations on use

This is a specially modified assay used for screening HIV antibody and which carries a significant false-positive rate. Reactive samples must be confirmed by Western blot.

5 Safety precautions

All human specimens and reagents should be handled with care. Staff performing test should follow Safety Manual. Essentially, these are standard precautions applicable to all specimens in the laboratory, which include appropriate protective clothing, steps taken to avoid potential percutaneous injuries, safe disposal of contaminated wastes, prompt and effective cleaning up of spillages.

6 Sample requirements

200 µl of neat urine

7 Equipment requirements

- (1) Nunc immuno-plate U96 maxisorp 4-49824
- (2) Micropipettes
- (3) Multi-channel micropipette
- (4) Dynatech washer
- (5) Roller mixer

8 Reagents

- (1) Coating buffer
- (2) Dakopatts Rabbit anti-human IgG (gamma chain)
- (3) Washing buffer
- (4) Diluent buffer
- (5) Fetal calf serum
- (6) Serodia HIV kit
- (7) In-house controls

9 Preparation of reagents

- 9.1 Coating buffer
 - (1) Dissolve 1.6g Na_2CO_3 anhydrous and 2.94g NaHCO_3 in 1 litre distilled water.
 - (2) Dispense 105ml into 100ml bottles and keep at 4°C.
- 9.2 Washing buffer (1 litre for every 4 plates)
 - (1) Dissolve 20 PBS tablets in 2 litres distilled water.
 - (2) Add 1ml Tween 20 and store at 4°C.
- 9.3 Diluent buffer
 - (1) Dissolve 5 PBS tablets in 500ml-distilled water.
 - (2) Add 250µl Tween 20.
 - (3) Dispense 96ml into 100ml bottles and keep at 4°C.
 - (4) Add 4ml inactivated fetal calf serum into 96ml buffer immediately before use.
- 9.4 Serodia gelatin particles
 - (1) Reconstitute sensitized particles with 0.6ml or 1.5ml reconstituting solution (see vial label). Reconstitute unsensitized particles with 1.0ml or 2.0ml reconstituting solution (see vial label).
 - (2) Roll for 30 minutes in a roller mixer.
 - (3) Add 140µl particles to 4.9ml diluent buffer immediately before use (1: 35 dilution).
 - (4) Store unused particles at 4°C and use within 1 week.

10 Test procedures

Read the kit insert carefully before taking up the test. Check any modifications in the new version of kit insert.

10.1 Coating of microtitre plate

- (1) Add 70µl Rabbit anti-human IgG to 105ml coating buffer.
- (2) Dispense 100µl diluted anti-human IgG to each well.
- (3) Seal plate with plate sealer and keep at 4°C until use (plates can be used 4 hours after coating if stored at room temperature. Use plates within 4 weeks when keep at 4°C).

10.2 Particle adherence test

- (1) Reconstitute sensitized and unsensitized gelatin particles.
- (2) Wash plates with washing buffer 4 times.
- (3) Add 100µl neat urine sample to each of 2 wells, starting from E1 and E2.
- (4) Add 100µl diluent buffer to wells A1 to D2.
- (5) Add 5µl of strong reactive control to each of 2 wells, A1 & A2.
- (6) Add 5µl of weak reactive control to each of 2 wells, B1 & B2.
- (7) Add 5µl of negative control to each of 2 wells, C1 & C2.
- (8) Wells D1 & D2 are diluent control.
- (9) Cover the plates and incubate at room temperature for at least 30 minutes.
- (10) Wash the plates with washing buffer 4 times.
- (11) Dilute reconstituted particles with diluent buffer (1: 35).
- (12) Add 100µl diluted unsensitized particles to columns 1, 3, 5, 7, 9 & 11.
- (13) Add 100µl diluted sensitized particles to columns 2, 4, 6, 8, 10 & 12.
- (14) Seal plates with transparent plate sealer.
- (15) Incubate at room temperature overnight.
- (16) Read plates for particle adherence giving grades of -, +, 2+, 3+ & 4+.

11 Reading and interpretation of results

11.1 Reading

The adherence patterns of each specimen with sensitized and unsensitized particles is read and recorded according to the criteria below.

Adherence pattern	Score
A tight button in the bottom of the well	-
A button with a narrow zone of adherence	+
A small disc of particles seen which does not fill the well	2+
Particles adherent to most of the well, but are just visible	3+
Particles adherent to the whole of the well	4+

11.2 Test validation

- (1) The strong reactive control should give a score of “4+” (complete agglutination) with sensitized particles and “-” with unsensitized particles.
- (2) The weak reactive control should give partial agglutination (“+” to “3+”), and “-” with unsensitized particles.
- (3) The negative and diluent controls should give a “-” score with both sensitized and unsensitized particles.

11.3 Interpretation of results

- (1) Specimens which gave a “-” adherence pattern with sensitized particles are considered to be negative.
- (2) Specimens which gave adherence pattern of between “+” and “4+” with sensitized particles are considered to be reactive. They should be followed up by further repeating the test. Those repeatedly reactive specimens are further confirmed by Western Blot.

12 Quality control procedures

A full set of controls must be put-up for each plate and their results are used to validate the test (see section 11.2). All results must be counter-checked by another experienced staff.

13. Potential problems

Mistakes can arise from failure to coat or wash the plates properly; therefore it is essential to use a properly maintained multichannel pipette and automatic platewasher.

14. Training requirements

The trainee must read the test manual and be familiar with the test procedure. He must then observe the whole assay once before he is allowed to carry out the test. He should be supervised for at least twice before being allowed to carry out the test on his own.

15. Contacts:

For further information related to testing please contact us at:

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