

# Comparative Evaluation of Western Blot and Geenius HIV 1/2 Confirmatory Assay in an Acute HIV Infection Case

CAS-115

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B A N C K O K

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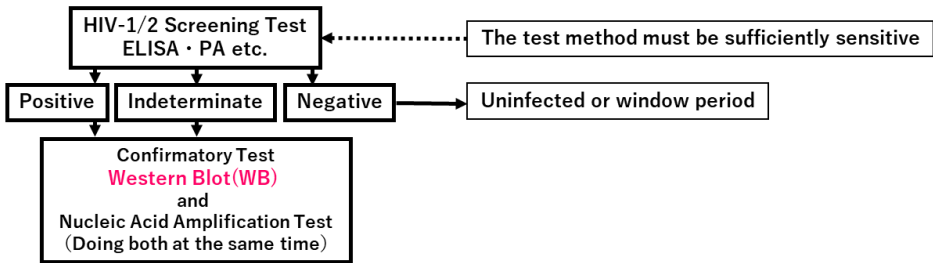
## Background

In 2020, Japan revised HIV diagnostic guidelines, introducing the Geenius HIV 1/2 Confirmatory Assay (ICA) as a replacement for conventional Western Blot (WB). Geenius has demonstrated improved sensitivity and specificity. This study compares antibody detection dynamics between WB and Geenius in acute HIV infection.

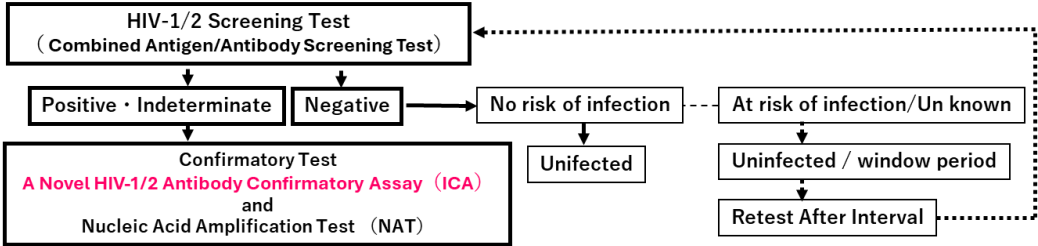
### Diagnostic guidelines for HIV-1/2 infection in clinical practice

( Standard Diagnostic Methods Recommended by The Japanese Society for AIDS Research and Japanese Society of Laboratory Medicine )

2008 Edition



2020 Edition



## Case Summary

Patient: Male, 20s

Sexual History:Heterosexual intercourse in early February 20XX; MSM (men who have sex with men) was denied.

Present Illness:The patient developed fever, sore throat, cough, and sputum production beginning on February 10, 20XX.

March 20XX at the previous hospital

March 11 : HIV screening positive, HIV-1 RNA  $9.8 \times 10^6$  copies/mL, WB negative, CD4+ T cells 136/ $\mu$ L

→ diagnosed with acute HIV infection

→ March 17 : ART initiated

March 23 (Day 1): Transferred to our hospital due to persistent fever, diarrhea, and fatigue

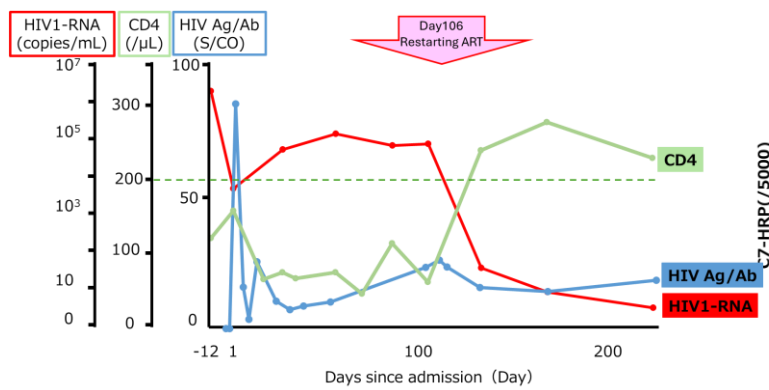
CMV co-infection diagnosed → ART discontinued, ganciclovir started

ART resumed on Day 106

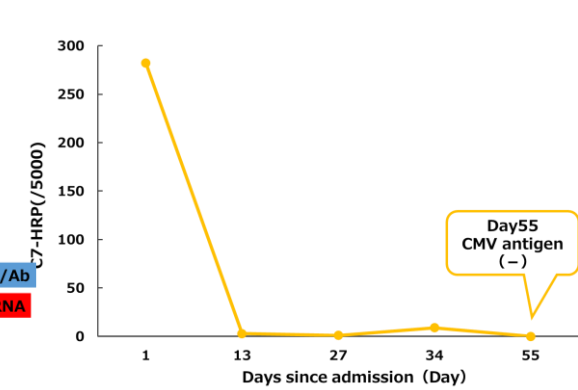
### Laboratory Results at Admission March 23 (Day 1)

| Hematology Tests                    |                       |           | Biochemical Tests        |       |       | Infectious Disease Tests                                 |                           |                  |
|-------------------------------------|-----------------------|-----------|--------------------------|-------|-------|--|---------------------------|------------------|
| WBC                                 | 3.9×10 <sup>9</sup>   | /L        | TP                       | 7.1   | g/dL  | RPR  | (-)                       |                  |
| RBC                                 | 4.14×10 <sup>12</sup> | /L        | Alb                      | 4.0   | g/dL  | <i>T.Pallidum</i> antibody                               | (-)                       |                  |
| Hb                                  | 122                   | g/L       | T-Bil                    | 0.6   | mg/dL | HBs antigen  | (-)                       |                  |
| PLT                                 | 104×10 <sup>9</sup>   | /L        | CH.E                     | 318   | U/L   | HBs antibody   | (-)                       |                  |
| Differential White Blood Cell Count |                       |           | AST                      | 76    | U/L   | HBc antibody   | (-)                       |                  |
| stab                                | 9.5                   | %         | ALT                      | 117   | U/L   | HCV antibody   | (-)                       |                  |
| seg                                 | 47.0                  | %         | LD                       | 655   | U/L   | HTLV- I antibody   | (-)                       |                  |
| lymp                                | 24.5                  | %         | ALP                      | 387   | U/L   | Influenze Virus antigen                                  | (-)                       |                  |
| mono                                | 7.0                   | %         | γ-GT                     | 59    | U/L   | <b>HIV antigen/antibody (-)</b>                          |                           |                  |
| eosino                              | 2.5                   | %         | CRP                      | 1.49  | mg/dL | <b>HIV1-RNA</b>  | <b>3.5×10<sup>4</sup></b> | <b>copies/mL</b> |
| baso                                | 1.0                   | %         | <b>Immunologic Tests</b> |       |       | CMV(C7-HRP)  | 282/5000                  |                  |
| atypical lymp                       | 8.5                   | %         | IgG                      | 1120  | mg/dL | CMV IgG  | (+)4.30                   | <b>Index</b>     |
| <b>Cell Surface Marker</b>          |                       |           | IgA                      | 259   | mg/dL | CMV IgM  | (+)4.11                   | <b>Index</b>     |
| CD4                                 | 18                    | %         | IgM                      | 147   | mg/dL | (1→3) β-D-glucan   | <6.0                      | pg/mL            |
| CD4 Cell Count                      | 172                   | / $\mu$ L | PCT                      | 0.174 | ng/mL | <b>Acute HIV Infection</b><br><b>Acute CMV Infection</b> |                           |                  |
| CD8                                 | 68                    | %         | ANA                      | (-)   |       |  |                           |                  |
| CD4/CD8 Ratio                       | 0.26                  |           | Ferritin                 | 5604  | ng/mL |  |                           |                  |
|                                     |                       |           | sIL2-R                   | 2031  | U/mL  |  |                           |                  |

### Clinical Course of HIV Infection



### Antigenemia Profile of CMV Infection



## Methods

Specimen Period: Day 1 to Day 223

Tests:

①New LAV Blot I/New LAV Blot II: WB( Bio-Rad Laboratories, Marnes-la-Coquette, France)

②Geenius HIV 1/2 :ICA( Bio-Rad Laboratories, Marnes-la-Coquette, France)

In addition,the results of the screening tests are also presented.

Evaluation:Visual comparison of band detection and interpretation

### HIV Screening Test

| Generation      | Reagent Name                     | Manufacturer | Assay Method | Instrument Name   |
|-----------------|----------------------------------|--------------|--------------|-------------------|
| 4 <sup>th</sup> | ARCHITECT® HIV Ag/Ab Combo Assay | Abbott       | CLIA         | ARCHITECT i2000SR |
|                 | LUMIPULSE HIV Ag/Ab              | FUJII REBIO  | CLEIA        | LUMIPULSE G1200   |
|                 | DinaScreen® HIV Combo            | Abbott       | ICA          | Manual Assay      |
| 3 <sup>th</sup> | Genedia HIV -1/2 Mix PA          | FUJII REBIO  | PA           | Manual Assay      |
| 2 <sup>nd</sup> | LUMIPULSE HIV -1/2               | FUJII REBIO  | CLEIA        | LUMIPULSE G1200   |
| Other           | LUMIPULSE I HIV1-p24             | FUJII REBIO  | CLEIA        | LUMIPULSE G1200   |

## Results

### Results – HIV-1

WB:Multiple bands from Day 1 → indeterminate

Day 55: gp120 and gp41 detected → HIV-1 positive

Geenius:Day 6: p31 only → indeterminate

Day 27: p31, gp160, gp41 → HIV-1 positive→ 28 days earlier than WB

### Results – HIV-2

WB:Persistent p26 from Day 1 → indeterminate throughout

Geenius:Day 6: gp140 detected → transient indeterminate

Day 17: HIV-2 negative

### ① Temporal Changes in Results

| Day                      | 4 <sup>th</sup>  |                       |                  |     | 3 <sup>th</sup> | 2 <sup>nd</sup>         | Other               | NAT                        | Antibody Confirmatory Test |            |                 |                 |
|--------------------------|------------------|-----------------------|------------------|-----|-----------------|-------------------------|---------------------|----------------------------|----------------------------|------------|-----------------|-----------------|
|                          | ARCHITECT (S/CO) | LUMIPULSE Ag/Ab (COI) | Dinascreen Ag Ab |     | Genedia (titer) | LUMIPULSE HIV-1/2 (COI) | LUMIPULSE p24 (COI) | HIV1-RNA HIV-1 (copies/mL) | WB (HIV-1)                 | WB (HIV-2) | Genieus (HIV-1) | Genieus (HIV-2) |
| -12 (Referring Hospital) | Pos              | NT                    | Neg              | Neg | NT              | NT                      | NT                  | 9.8 x 10 <sup>6</sup>      | Neg                        | Neg        | NT              | NT              |
| 1                        | 0.2              | 0.6                   | Neg              | Neg | Neg             | 0.1                     | 0.6                 | 3.5 x 10 <sup>4</sup>      | Ind                        | Ind        | Neg             | Neg             |
| 3                        | 0.2              | 0.4                   | Neg              | Neg | Neg             | NT                      | 0.7                 | NT                         | NT                         | NT         | NT              | NT              |
| 6                        | 84.9             | >15                   | Pos              | Neg | Neg             | 0.1                     | >100                | NT                         | NT                         | NT         | Ind             | Ind             |
| 10                       | 15.8             | >15                   | Pos              | Neg | Neg             | 0.1                     | 24.8                | NT                         | Ind                        | Ind        | Ind             | Neg             |
| 13                       | 3.7              | 6.3                   | Neg              | Neg | Neg             | 0.2                     | 6.4                 | NT                         | NT                         | NT         | Ind             | Ind             |
| 17                       | 25.4             | >15                   | Pos              | Neg | Neg             | 0.2                     | 27.4                | NT                         | Ind                        | Ind        | Ind             | Neg             |
| 27                       | 10.6             | 12.1                  | Neg              | Neg | Ind             | 0.8                     | 11.1                | 3.3 x 10 <sup>5</sup>      | Ind                        | NT         | Pos             | Neg             |
| 34                       | 7.4              | 9.4                   | Neg              | Neg | Ind             | 1.1                     | 7.4                 | NT                         | Ind                        | NT         | Pos             | Neg             |
| 41                       | 8.8              | 9.8                   | Neg              | Pos | 640             | 2.7                     | 6.9                 | NT                         | Ind                        | NT         | Pos             | Neg             |
| 55                       | 10.2             | 10.2                  | Neg              | Pos | 256             | 9.3                     | 5.4                 | NT                         | Pos                        | Ind        | Pos             | Neg             |
| 85                       | NT               | NT                    | NT               | NT  | NT              | NT                      | NT                  | 4.2 x 10 <sup>5</sup>      | NT                         | NT         | NT              | NT              |
| 104                      | 23.3             | >15                   | NT               | NT  | 2048            | 22.2                    | 4.1                 | 4.6 x 10 <sup>5</sup>      | Pos                        | Ind        | Pos             | Neg             |
| 111                      | 26.1             | >15                   | NT               | NT  | 4096            | 22.3                    | 0.9                 | NT                         | NT                         | NT         | NT              | NT              |
| 167                      | 14.2             | >15                   | NT               | NT  | 512             | 10.9                    | 0.6                 | 8.6 x 10 <sup>1</sup>      | NT                         | NT         | NT              | NT              |
| 223                      | 18.5             | >15                   | NT               | NT  | 2048            | 13.4                    | 0.7                 | 3.5 x 10 <sup>1</sup>      | Pos                        | Ind        | Pos             | Neg             |

□ The observed negativity may be attributable to immune complex formation between antigen and antibody.

### ② Days to positivity by Each Assay

| Generation      | Reagent Name                     | Conversion to Positive (day) | Reagent Name    | Conversion to Positive (day) |
|-----------------|----------------------------------|------------------------------|-----------------|------------------------------|
| 4 <sup>th</sup> | ARCHITECT® HIV Ag/Ab Combo Assay | 6                            | New LAV Blot I  | 55                           |
|                 | LUMIPULSE HIV Ag/Ab              | 6                            | Geenius HIV 1/2 | 27                           |
|                 | DinaScreen® HIV Combo            | 6                            |                 |                              |
| 3 <sup>th</sup> | Genedia HIV -1/2 Mix PA          | 41                           |                 |                              |
| 2 <sup>nd</sup> | LUMIPULSE HIV -1/2               | 34                           |                 |                              |
| Other           | LUMIPULSE I HIV1-p24             | 6                            |                 |                              |

The Geenius assay yielded a positive result earlier than the Western blot.

### ③ Detailed Results of Western Blot and Geenius Assays

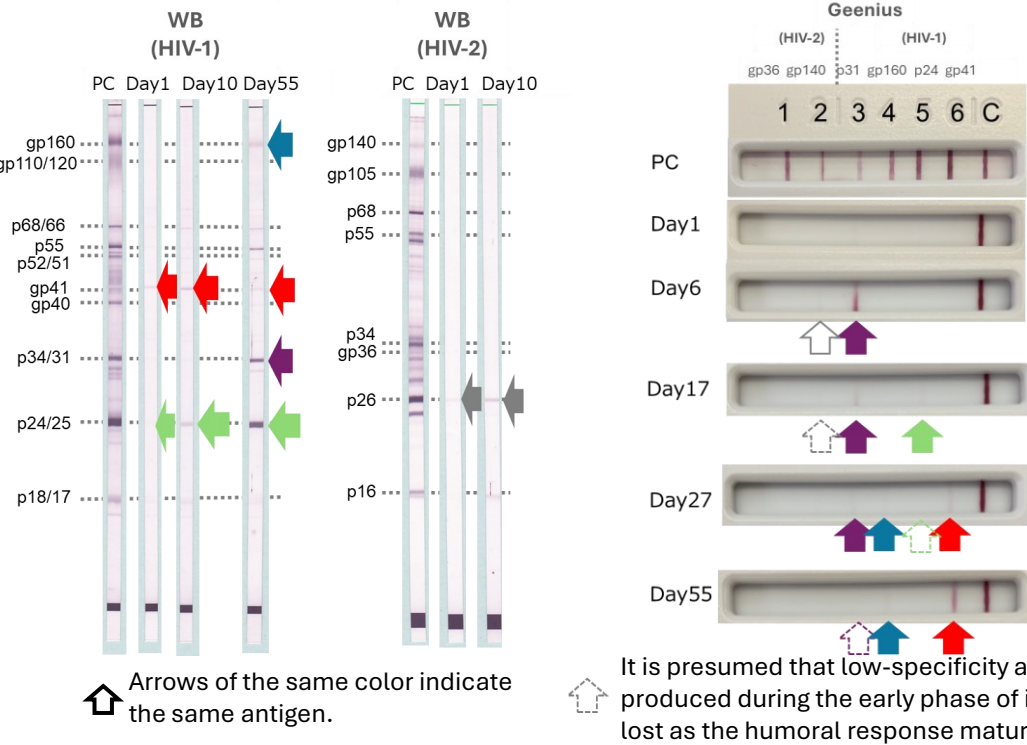
| Day | WB (HIV-1)                                | WB (HIV-2)   | Geenius (HIV-1)      | Geenius (HIV-2) |
|-----|---|--------------|----------------------|-----------------|
| 1   | Ind(p68,p55,gp41,p24,p18)                 | Ind(p26)     | Neg                  | Neg             |
| 6   | NT  | NT           | Ind(p31)             | Ind(gp140)      |
| 10  | Ind(p68,p55,p51,gp41,p24,p18)             | Ind(p26,p16) | Ind(p31)             | Neg             |
| 13  | NT  | NT           | Ind(p31)             | Ind(gp140)      |
| 17  | Ind(p68,p55,p51,gp41,p24,p18)             | Ind(p26,p16) | Ind(p31,p24)         | Neg             |
| 27  | NT  | NT           | Pos(p31,gp160,gp41)  | Neg             |
| 34  | Ind(p68,p55,p51,gp41,p24,p18)             | NT           | Pos(p31,gp160, gp41) | Neg             |
| 41  | Ind(p68,p55,p51,gp41,p24)                 | NT           | Pos(gp160, gp41)     | Neg             |
| 55  | Pos(gp120,p68,p55,p51,gp41,p24,p18)       | Ind(p26)     | Pos(gp160, gp41)     | Neg             |
| 85  | Pos(gp160,gp120,p68,p55,p51,gp41,p24)     | NT           | NT                   | NT              |
| 104 | Pos(gp160,gp120,p68,p55,p51,gp41,p24,p18) | Ind(p26)     | Pos(gp160, gp41)     | Neg             |
| 223 | Pos(gp160,gp120,p68,p55,p51,gp41,p24,p18) | Ind(p26)     | Pos(gp160, gp41)     | Neg             |

The band patterns detected by the two assays did not match.

One possible explanation for the discordant band profiles is antigen–antibody complex–mediated masking, as previously observed with DinaScreen.

Band pattern differences may reflect the use of native viral antigens in WB versus synthetic/recombinant antigens in Geenius.

Two trained staff members performed visual interpretation of the Geenius assay. NT : Not Tested



## Conclusions

Geenius enabled earlier HIV-1 detection and reduced HIV-2 cross-reactivity. Differences in antigen composition between reagents and antigen-antibody complexes in the specimen may have influenced band patterns. Additionally, altered immune responses due to CMV co-infection may have affected antibody production, warranting further investigation.