Interpretation algorithms for 4th generation HIV serology tests

Interpretation algorithm for 4th generation format serological tests (antigen/antibody combined detection) or “Advanced” 4th generation tests (orientation of positivity origin with differentiated antibody/antigen signals)

- **Negative result**
  - Retest twice using the same reagent on the same sample
  - Results in duplicate: 2 negative results
  - **No HIV Infection** (1)

- **Positive result**
  - Results in duplicate: 2 positive results or 1 negative result and 1 positive result
  - **It is necessary to perform a complementary analysis using the same sample and using a “fresh” sample collection to confirm HIV infection.** (See specific algorithm overleaf)

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(1) If clinical symptoms or risk factors are present, a new sample should be collected 2 weeks later. To give patients a more rapid diagnosis, viral load can be determined using a 2nd sample collected immediately.

This algorithm, which is intended for reference purposes only, is valid for the majority of cases. Country specific recommendations should be taken into consideration.
Interpretation algorithm in cases of POSITIVE results using 4th Generation assays

This algorithm, which is intended for reference purposes only, is valid for the majority of cases. Country specific recommendations should be taken into consideration.

1. All 4th generation positive results should be retested (see package insert).
2. It is recommended to perform a Western Blot distinguishing HIV1 and HIV2 (see Western Blot interpretation).
3. All HIV Ag positive results or repeatable equivocal results should be confirmed by neutralization (see package insert).
4. If clinical signs or risk factors or if HIV Ag sensitivity is lower than ELISA 4th generation assay, require a new bleeding two weeks after; it is also possible to ask for a viral load on a 2nd immediate bleeding to provide the patient with a more rapid diagnosis orientation.
5. Usually confirmed showing a seroconversion profile by WB.

Note: in some countries, HIV viral load is not considered as a diagnostic assay due to the risk of false positive reactions.
Interpretation algorithm in cases of POSITIVE results using “Advanced” 4th Generation assays

- **Repeatable “Advanced” 4th generation tests (+)** (1)
  - Depending on test values printed

- **If Test Value (TV) indicative of HIV Antigen presence only**
  - HIV Ag research
    - If HIV Ag (+) and confirmed by neutralization
      - Further testing on a 2nd bleeding 2 weeks after: Anti HIV ELISA
    - If HIV Ag (+/-) retested twice, perform a neutralization test
      - If there is a neutralization
    - If HIV Ag (-) or not confirmed by neutralization
      - If NO neutralization

- **If Test Value (TV) indicative of HIV Antibody presence**
  - Western Blot (2)
  - If Western Blot (+)
  - If Western Blot (-) or indeterminate
    - Further testing on a 2nd immediate bleeding to avoid sample identification error: Anti HIV ELISA

This algorithm which is intended for reference purposes only, is valid for the majority of cases. Country specific recommendations should be taken into consideration.

1. All “Advanced” 4th generation positive results should be retested (see package insert).
2. It is recommended to perform a Western Blot distinguishing HIV1 and HIV2 (see Western Blot interpretation).
3. All HIV Ag positive results or repeatable equivocal results should be confirmed by neutralization (see package insert).
4. If clinical signs or risk factors or if HIV Ag sensitivity is lower than ELISA 4th generation assay, require a new bleeding two weeks after; it is also possible to ask for a viral load on a 2nd immediate bleeding to provide the patient with a more rapid diagnosis orientation.
5. Usually confirmed showing a seroconversion profile by WB. Note: in some countries, HIV viral load is not considered as a diagnostic assay due to the risk of false positive reactions.

HIV Early Infection (5)

Follow-up:
- CD4 count
- Viral load

Likely false + HIV result (4)

To be confirmed on a 2nd bleeding to avoid sample identification error

HIV Infection confirmed

Follow-up:
- CD4 count
- Viral load

Likely false + HIV result if HIV Ag is also negative

To be confirmed on a 2nd bleeding to avoid sample identification error
## Actions to be taken and interpretation of HIV-1 Western Blot

<table>
<thead>
<tr>
<th>Confirmed positivity</th>
<th>Actions to be taken and interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least 2 anti-“env” Ab (anti-gp120 and anti-gp160) AND 1 Ac anti-“gag” ou anti-“pol”</td>
<td>• Second sample requested immediately to ensure that there has been no collection error or no contamination of the 1st sample.</td>
</tr>
<tr>
<td><strong>AND</strong></td>
<td>• If higher intensity reaction in proteins coming from “gag” and/or “pol” genes than in proteins coming from “env” genes, perform a specific HIV-2 serology test and consider an infection due to HIV-1 O group or other possible variant.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Probable positivity (usually profile of early seroconversion)</th>
<th>Actions to be taken and interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 anti-p24 Ab AND 1 anti-gp160 Ab</td>
<td>• New collection required one or two weeks later:</td>
</tr>
<tr>
<td>• New collection required one or two weeks later:</td>
<td>- If an evolution is observed: HIV-1 seroconversion.</td>
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<tr>
<td></td>
<td>- If no evolution and negative HIV-2 WB: probable false positive.</td>
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<tr>
<td></td>
<td>(exceptional) or HIV-1 O group or other possible variant (rare profile).</td>
</tr>
<tr>
<td></td>
<td>- If no evolution and positive HIV-2 WB: HIV-2 seropositivity (rare profile).</td>
</tr>
<tr>
<td>2 anti-“env” Ab (anti-gp120 + anti-gp160)</td>
<td>• New collection required one or two weeks later:</td>
</tr>
<tr>
<td></td>
<td>- If negative sample: contamination of the 1st sample or identification error.</td>
</tr>
<tr>
<td></td>
<td>- If an evolution is observed: HIV-1 seroconversion (rare profile).</td>
</tr>
<tr>
<td></td>
<td>- If no evolution and positive HIV-2 WB: HIV-2 seropositivity (rare profile).</td>
</tr>
<tr>
<td>Profiles to be controlled</td>
<td>• Perform a specific HIV-2 serology especially if both HIV screening tests are clearly positive. Collect a new sample one or two weeks later.</td>
</tr>
<tr>
<td>Isolated anti-gp160 Ab</td>
<td>• If no evolution and negative HIV-2: false positive reaction or HIV-1 variant (exceptional).</td>
</tr>
<tr>
<td>Isolated anti-p24 Ab (+/- anti-p55)</td>
<td></td>
</tr>
<tr>
<td>Isolated anti-p34 Ab (+/- anti-p24)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Negativity</th>
<th>Actions to be taken and interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-p17 Ab</td>
<td>• No reactivity on the WB associated with clearly positive screening tests should be considered as an early HIV.</td>
</tr>
<tr>
<td>Other profiles not considered No Ab seroconversion</td>
<td>• A new bleeding is required 1 to 2 weeks later.</td>
</tr>
</tbody>
</table>

HIV-1 proteins coming from the following genes: “env”: gp 160, gp120, gp41; “gag”: p55, p24, p18; “pol”: p68, p34.

Based on the ANAES (French Agence Nationale d'Accréditation et d'Evaluation en santé) recommendations: Stratégies du diagnostic biologique de l’infection due au VIH chez les sujets âgés de plus de 18 mois (à l'exclusion du dépistage sur les dons de sang et chez les organes ou de tissus) - January 2000.