HIV Diagnostic Testing Algorithm

**Step 1. HIV-1/2 Ag/Ab combo immunoassay (4th generation)**

- **(+) = Reactive (or repeatedly reactive) test result, in accordance with manufacturer’s instructions**
- **(–) = Nonreactive test result, in accordance with manufacturer’s instructions**

**(-) = Negative for HIV-1 and HIV-2 antibodies and HIV-1 p24 Ag**

**Step 2. HIV-1/HIV-2 antibody differentiation immunoassay**

- HIV-1 (+) HIV-2 (–) **Positive for HIV-1 antibodies**
- HIV-1 (–) HIV-2 (+) **Positive for HIV-2 antibodies**
- HIV-1 (+) HIV-2 (+) **(Undifferentiated) Positive for HIV antibodies**
- HIV-1 (–) or indeterminate HIV-2 (–)

**Step 3. HIV-1 RNA assay**

- **RNA (+) Positive for HIV-1**
- **RNA (–) Negative for HIV-1**

**Italics = Final interpretation; No further testing indicated for the specimen**

*For 3rd generation HIV-1/2 immunoassay, interpretation is ‘Negative for HIV-1 and HIV-2 antibodies’.

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**Step 1. 4th generation HIV-1/2 Ag/Ab combo immunoassay (preferred) or 3rd generation HIV-1/2 immunoassay (acceptable).** If the result from this test is ‘Nonreactive’, no further testing of the specimen is indicated. If the result is ‘Reactive’, this is considered to be a preliminary positive result and supplemental testing must be performed, beginning with an HIV-1/HIV-2 antibody differentiation immunoassay (step 2).

**Step 2. HIV-1/HIV-2 antibody differentiation immunoassay.** If the initial HIV-1/2 immunoassay (step 1) was reactive and the result of HIV-1/HIV-2 antibody differentiation immunoassay is ‘Reactive’ for HIV-1 or HIV-2 antibodies, the interpretation is ‘Positive for HIV-1 antibodies’ or ‘Positive for HIV-2 antibodies’, respectively. No further testing of the specimen is required and medical care is recommended. If the result is ‘Reactive’ for both HIV-1 and HIV-2 antibodies (i.e. HIV Positive, Undifferentiated), the interpretation is ‘Positive for HIV antibodies’ and medical care is recommended. Additional testing for HIV-1 RNA and HIV-2 RNA or DNA is recommended at the initial clinical evaluation to verify or rule-out HIV-1/HIV-2 dual infection. If the result of the HIV-1/HIV-2 antibody differentiation test is ‘Nonreactive’ or ‘Indeterminate’, testing of the specimen should reflex to an HIV-1 RNA assay (step 3).

**Step 3. HIV-1 RNA assay.** If the initial HIV-1/2 immunoassay (step 1) was reactive and HIV-1 RNA is detected, the final interpretation is ‘Positive for HIV-1’ and medical care should be initiated. If HIV-1 RNA is not detected, the final interpretation is ‘Negative for HIV-1’. The initial HIV-1/2 immunoassay result was most likely a false positive. If there is reason to suspect recent HIV-2 infection, follow-up testing for HIV-2 RNA or DNA should be considered.