NADAL® HIV Ag/Ab (4th Generation) Test (test cassette)

REF 452015N-30

IVD

Version 2.0, 2017-11-27

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NADAL® HIV Ag/Ab (4th Generation) Test (Ref. 452015N-30)

1. Intended Use

The NADAL® HIV Ag/Ab (4th Generation) Test is a lateral flow chromatographic immunoassay for the qualitative detection of IgG, IgM and IgA to anti-HIV-1 (including O) and anti-HIV-2 antibodies as well as HIV-1 p24 antigens in human serum, plasma or whole blood. The test is intended to be used as a screening test and as an aid in the diagnosis of HIV infections.

Any reactive specimen with the NADAL® HIV Ag/Ab (4th Generation) Test should be confirmed with alternative testing method(s) and clinical findings.

2. Introduction and Clinical Significance

Human immunodeficiency virus type I and type II (HIV-1 and HIV-2) are enveloped, single-stranded, positive-sense RNA viruses. The causative relationship between HIV-1 and HIV-2 viruses and acquired immunodeficiency syndrome (AIDS) has been established over decades. HIV-1 has been isolated from patients with AIDS and AIDS-related complex and from healthy individuals with a high risk for developing AIDS.[1] HIV-2 has been isolated from West African AIDS patients and from sero-positive asymptomatic individuals.[2] HIV-1 is much more prevalent worldwide than HIV-2. Recent studies have shown that over 30 million people have been infected with HIV-1.

Both HIV-1 and HIV-2 viruses can elicit strong immune responses[3] including the production of antiviral antibodies. The presence of specific anti-HIV-1 or HIV-2 virus antibodies in whole blood, serum or plasma indicates the exposure of the individual to HIV-1 or HIV-2 which is of great value for clinical diagnosis.[4] Tests that detect HIV p24 antigen may be useful for the early diagnosis of HIV as p24 antigen is one of the earliest markers of HIV infection. It has been reported that HIV infection is detectable with a p24 antigen test 6 days earlier than with an antibody test.[5]

The NADAL® HIV Ag/Ab (4th Generation) Test utilises recombinant gp-120-41, gp36 and anti-p24 antibodies and can be used for the qualitative detection of IgG, IgM and IgA to anti-HIV-1 (including O) and anti-HIV-2 antibodies as well as HIV-1 p24 antigens in patient serum, plasma or whole blood within 15 minutes.

The NADAL® HIV Ag/Ab (4th Generation) Test can be performed without cumbersome laboratory equipment.

3. Test Principle

The NADAL® HIV Ag/Ab (4th Generation) Test is a lateral flow chromatographic immunoassay. The test strip in the test cassette consists of: 1) a burgundy conjugate pad containing recombinant HIV-gp-120-41 and gp-36 antigens conjugated with colloidal gold (HIV-conjugates), monoclonal anti-HIV-p24 antibodies conjugated with colloidal gold (p24-conjugates) and rabbit IgG-gold conjugates (for control line), 2) a nitrocellulose membrane strip containing two test lines ('Ag' and 'Ab') and a control line ('C'). HIV-gp-120-41 and HIV-2 gp-36 antigens for the detection of antibodies to anti-HIV-1 (including O) or anti-HIV-2 antibodies are pre-coated on the test line 'Ab'. Monoclonal anti-HIV-p24 antibodies for the detection of p24 antigens are precoated on the test line 'Ag' and goat anti-rabbit IgG are pre-coated on the control line 'C'.

When an adequate volume of specimen is dispensed into the sample well (S), the specimen migrates by capillary action along the test cassette. If IgG, IgM and IgA to anti-HIV-1 or anti-HIV-2 antibodies are present in the specimen, they bind to the HIV conjugates. The immunocomplexes are then captured on the membrane by the pre-coated HIV-1+2 antigens, forming a burgundy 'Ab' line, indicating a positive test result. The absence of the 'Ab' line suggests an HIV-1 and HIV-2 antibody negative result.

If HIV-p24 antigens are present in the specimen, they also bind to the p24-conjugates. The immunocomplexes are then captured on the membrane by the pre-coated anti-HIV-p24 antibodies, forming a burgundy 'Ag' line, indicating a positive test result. The absence of both test lines ('Ag' and 'Ab') suggests a negative result. The absence of the 'Ag' line suggests an HIV-p24 antigen negative result.

The test contains an internal control (control line 'C') which should always exhibit a burgundy line of goat anti-rabbit IgG/rabbit IgG-gold immunocomplex conjugates regardless of the colour development of the test lines ('Ag' and 'Ab'). Otherwise, the test result is invalid and the specimen should be retested with another test cassette.

4. Reagents and Materials Supplied

- 30 NADAL® HIV Ag/Ab (4th Generation) test cassettes (incl. desiccant)
- 30 disposable capillary tubes (20 µl)
- 1 Buffer (5 mL/bottle)
- 1 package insert

5. Additional Materials Required

- Clock or timer
- Lancing device for whole blood testing

6. Storage & Stability

All reagents are ready to use as supplied. Store unused test cassettes unopened at 2-30°C. If tests are stored at 2-8°C, ensure that they are brought to room temperature before opening. The test cassette is stable until the expiration date printed on the sealed foil pouch. Do not freeze test kits or expose them to temperatures over 30°C.

7. Warnings and Precautions

- For professional, *in-vitro* diagnostic use only.
- Carefully read through the test procedure prior to testing.
- Do not use the test beyond the expiry date indicated on the package.
- Do not use the test if the foil pouch is damaged.
- Do not reuse tests.
- Do not add samples to the reaction area (result area).
- In order to avoid contamination, do not touch the reaction area (result area).
- Do not substitute or mix components from different test kits.
- Do not use haemolysed specimens for testing.
- Do not eat, drink or smoke in the area where specimens and test kits are handled.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being assayed. Wash hands thoroughly after performing the test.

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- Handle all specimens as if they contain infectious agents. Observe established precautions for microbiological risks throughout all procedures and standard guidelines for the appropriate disposal of specimens.
- The test results should be read 15 minutes after the specimen is applied to the sample well of the test cassette. Reading the results after more than 20 minutes may give erroneous results.
- Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air-conditioning.

8. Specimen Collection and Preparation

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

Plasma

- Collect a blood specimen into a collection tube (containing EDTA, citrate or heparin) by venipuncture.
- Separate plasma by centrifugation.
- Carefully withdraw the plasma into a new, pre-labeled tube.

Serum

- Collect a blood specimen into a collection tube (containing no anticoagulants) by venipuncture.
- Allow the blood to clot.
- Separate serum by centrifugation.
- Carefully withdraw the serum into a new, pre-labeled tube.

Test specimens as soon as possible after collection. If specimens are not to be tested immediately, store them at 2-8°C for up to 5 days. Specimens should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Do not use samples demonstrating gross lipemia, gross haemolysis or turbidity in order to avoid interference with result interpretation.

Blood

Drops of whole blood can be obtained by either fingertip puncture or venipuncture. Collect a blood specimen into a collection tube (containing EDTA, citrate or heparin). Do not use haemolysed blood for testing.

Specimens must be tested within 24 hours of collection. Whole blood specimens should be refrigerated (2-8°C) if they are not tested immediately.

9. Test Procedure

Bring specimens and test components to room temperature if refrigerated or frozen. Once specimens are thawed, mix them well prior to performing the assay.

1. When you are ready to test, open the pouch and remove the test cassette. Place it on a clean, level surface.
2. Label the test cassette with the specimen ID number.
3. Fill a capillary tube with a specimen, without exceeding the filling line. The volume of the specimen is approximately 20 µL.

Note: For optimum precision, transfer the specimen, using a pipette capable of delivering a 20 µL volume.

4. Holding the capillary tube vertically, dispense the entire specimen into the center of the sample well (S), making sure that there are no air bubbles.

5. Holding the bottle vertically, immediately add 2 drops (about 60-80 µL) of buffer to the the sample well (S). Make sure that there are no air bubbles.
6. Start the timer.
7. Read test results after 15 minutes. Positive results may be visible in as little as 1 minute. Do not read results after more than 20 minutes. To avoid confusion, discard the test cassette after interpreting the result.

10. Result Interpretation

Positive/reactive for anti-HIV-1 and/or anti-HIV-2 antibodies:

A coloured line develops in the control line region (C) and a coloured line develops in the 'Ab' test line region. The test result indicates the presence of anti-HIV-1 and/or anti-HIV-2 antibodies. The test result is positive or reactive for anti-HIV-1 and/or anti-HIV-2 antibodies.

Positive/reactive for HIV p24:

A coloured line develops in the control line region (C) and a coloured line (including a faint one) develops in the 'Ag' test line region. The test result indicates the presence of HIV p24 antigens. The test result is positive or reactive for HIV p24.

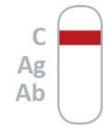
Positive/reactive for anti-HIV-1 and/or anti-HIV-2 antibodies and HIV p24:

In addition to the control line (C), two coloured 'Ab' and 'Ag' test lines develop. The test result is positive or reactive for anti-HIV-1 and/or anti-HIV-2 antibodies as well as for HIV p24 antigens.

Samples with reactive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.

Negative/non-reactive:

Only the control line (C) appears. The absence of the 'Ab' and 'Ag' test lines indicates that no detectable anti-HIV-1 and/or anti-HIV-2 antibodies nor HIV p24 antigens are present in the specimen.



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The test result is negative or non-reactive.

Invalid:

If no control line (C) appears, the test is invalid regardless of any colour development in the 'Ab' and 'Ag' test lines. Repeat the assay with a new test cassette.

Results from any test which has not produced a control line at the specified reading time must be discarded. Please review the procedure and repeat the test with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your distributor.

Insufficient specimen volume, incorrect operating procedure or expired tests are the most likely reasons for the control line failure.

11. Quality Control

Internal Control:

A coloured line appearing in the control line region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

External Control:

The use of external positive and negative controls is recommended to ensure proper assay performance.

12. Limitations

- The sections 'Test Procedure' and 'Result Interpretation' must be followed closely when testing for the presence of anti-HIV antibodies and/or HIV p24 antigens in serum, plasma or whole blood from individual subjects. Failure to follow correct procedure may give inaccurate results.
- The NADAL® HIV Ag/Ab (4th Generation) Test is limited to the qualitative detection of anti-HIV-1 and anti-HIV-2 antibodies and/or HIV p24 antigens in human serum, plasma or whole blood. The intensity of the test line has no linear correlation with the antibody titer in the specimen.
- A non-reactive result for an individual subject indicates the absence of detectable anti-HIV-1 and anti-HIV-2 antibodies and/or HIV p24 antigens. However, a non-reactive test result does not preclude the possibility of exposure to or infection with HIV-1 or HIV-2.
- A non-reactive result can occur if the quantity of anti-HIV-1 and anti-HIV-2 antibodies and/or HIV p24 antigens present in the specimen is below the detection limit of the test, or if the antibodies/antigens detected were not yet present during the stage of disease in which the sample was collected.
- If symptoms persist, while the result obtained with the NADAL® HIV Ag/Ab (4th Generation) Test is non-reactive, it is recommended to retest the patient a few days later or with an alternative testing method.
- Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
- The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

13. Performance Characteristics

A total of 350 clinical samples were collected and tested using the NADAL® HIV Ag/Ab (4th Generation) Test and an HIV 1+2 Ab reference kit licensed by the SFDA.

The results are presented in the following table:

		HIV 1+2 Ab reference kit		
NADAL® HIV Ag/Ab (4th Generation) Test		+	–	Total
	+	105	0	105
	–	0	245	245
	Total	105	245	350

Relative sensitivity: >99.9%

Relative specificity: >99.9%

Overall agreement: >99.9%

Specificity

The specificity of the NADAL® HIV Ag/Ab (4th Generation) Test was evaluated with 1,000 specimens from a normal population and 200 specimens from pregnant women. No false positive results were detected.

Boston Biomedica Inc (BBI) Seroconversion Panel

The performance of the NADAL® HIV Ag/Ab (4th Generation) Test was evaluated using BBI seroconversion panel PRB967.

The results are presented in the following table:

PRB-967 panel		BioMerieux HIV Ag pg/ml	Abbott HIV1/2 Ab s/co	NADAL® HIV Ag/Ab (4th Generation) Test	
Patient ID	Days bleed			Ag reactivity	Ab reactivity
PRB967-04	17	>400.0	2.5	Pos.	Pos.
PRB967-05	19	>400.0	8.3	Neg.	Pos.
PRB967-06	24	10.5	8.4	Neg.	Pos.

Note: s/co < 1: Negative, s/co >=1: Positive

Cross-reactivity

Specimens from other infectious diseases were tested for cross-reactivity using the NADAL® HIV Ag/Ab (4th Generation) Test according to the standard procedure. No cross-reactivity was observed with the the following specimens:

Samples	No. of samples	NADAL® HIV Ag/Ab (4th Generation) Test	
		Ag reactivity	Ab reactivity
HBsAg positive	20	Negative	Negative
HCV positive	10	Negative	Negative
Syphilis positive	10	Negative	Negative
HAV positive	10	Negative	Negative
HEV positive	10	Negative	Negative
H. pylori positive	10	Negative	Negative
TB positive	10	Negative	Negative
ANA positive	6	Negative	Negative
HAMA positive	4	Negative	Negative
RF positive (<2,500 IU/ml)	10	Negative	Negative

Interference

The interference of common substances (such as pain and fever medication, blood components) listed below with the performance of the NADAL® HIV Ag/Ab (4th Generation) Test

was studied by spiking 3 levels of HIV Ag and HIV Ab standard controls with these substances.

The results are presented in the following table:

Note: - = Negative; + = Weak positive; +++ = Strong positive

Potentially interfering substances	HIV Ag reactivity			HIV Ab reactivity		
	Negative	Weak positive	Strong positive	Negative	Weak positive	Strong positive
Control	-	+	+++	-	+	+++
Bilirubin 20 mg/dL	-	+	+++	-	+	+++
Glucose 55 mmol/L	-	+	+++	-	+	+++
Haemoglobin 2 g/L	-	+	+++	-	+	+++
Salicylic acid 4.34 mmol/L	-	+	+++	-	+	+++
Heparin 3,000 U/L	-	+	+++	-	+	+++
EDTA 3.4 µmol/L	-	+	+++	-	+	+++
Human IgG 150 mg/dL	-	+	+++	-	+	+++
Sodium citrate 3.8%	-	+	+++	-	+	+++

The results demonstrate that, at the concentrations tested, the substances studied do not affect the performance of the NADAL® HIV Ag/Ab (4th Generation) Test.

14. References

1. Chang, SY, Bowman, BH, Weiss, JB, Garcia, RE and White, TJ. The origin of HIV-1 isolate HTLV-IIIB. Nature (1993) 3/363:466-9.
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Rev. 0, 2017-11-27 OM/KBe

Symbol	Deutsch	English	Français	Español	Italiano	Polski
	Gebrauchsanweisung beachten	Consult instructions for use	Consulter la notice d'utilisation	Consúltense las instrucciones de uso	Consultare le istruzioni per l'uso	Przestrzegać instrukcji obsługi
	In-vitro-Diagnostika	In-vitro diagnostic medical device	Dispositif médical de diagnosticc in vitro	Producto sanitario para diagnóstico in vitro	Dispositivo medico-diagnostico in vitro	Tylko do diagnostyki in vitro
	Temperaturbegrenzung	Temperature limitation	Limites de température	Límites de temperatura	Limiti di temperatura	Temperatura przechowywania
	Chargenbezeichnung	Batch code	Code du lot	Código de lote	Codice lotto	Numer serii
	Nicht zur Wiederverwendung	Do not reuse	Ne pas réutiliser	No reutilizar	Non riutilizzare	Tylko do jednorazowego użytku
	Verwendbar bis	Use by	Utiliser jusqu'au	Fecha de caducidad	Utilizzare entro	Data ważności
	Bestellnummer	Catalogue Number	Référence du catalogue	Número de catálogo	Riferimento di Catalogo	Numer katalogowy
	Hersteller	Manufacturer	Fabricant	Fabricante	Fabbricante	Producent
	Ausreichend für <n> Ansätze	Sufficient for <n> tests	Suffisant pour "n" tests	Suficiente para <n> utilizaciones	Sufficiente per "n" saggi	Wystarczający na <n> Powtórzeń

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