Analytical & Clinical Performance of the cobas® HIV-1/2 Qualitative Nucleic Acid Test

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1 — Abstract

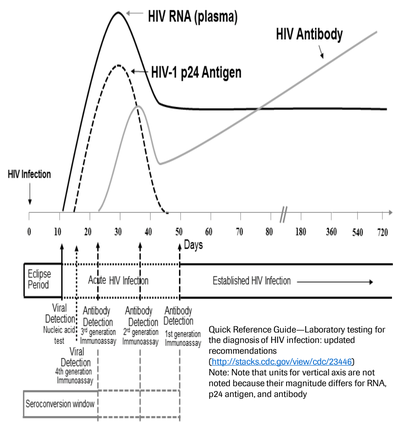
Background: The identification of acute HIV infection is important for early treatment initiation and to control the risk of the spread of the disease, in particular in high risk populations. Since the window period between the infection and the identification of antigen/antibodies to HIV is still a limitation of serology-based screening tests, a high quality molecular approach is crucial.

Methods: Commercially available seroconversion panels were tested with the **cobas**® HIV-1/2 Qual test and with the Bio-Rad Geenius™ HIV 1/2 Confirmatory Assay and the individual and mean delta sojourn time for the first identification of a positive result between the two tests were calculated. Limit of detection was determined with WHO International Standards for HIV-1 and HIV-2. Assay inclusivity was determined on an additional 16 HIV-1 and HIV-2 groups and subtypes.

Results: cobas® HIV1/2 Qual detected all HIV-1/HIV-2 subtypes and groups tested. For the seroconversion panels, the assay detected HIV an average of 18.9 days earlier than the serology-based test. The limit of detection for **cobas**® HIV-1/2 Qual test was verified as 12.6 cp/mL for plasma and 12.1 cp/mL for serum, for HIV-1 and 27.9 cp/mL for plasma, and 23.4 cp/mL for serum, for HIV-2, respectively.

Conclusion: The results indicate that the **cobas**® HIV-1/2 Qual test is highly sensitive and specific for diagnosing HIV-1 and HIV-2. The assay may detect and discriminate HIV infections over two weeks earlier than serology-based test, enabling earlier HIV treatment and prevention of ongoing HIV transmission.

2 — Sequential appearance of different laboratory markers



Quick Reference Guide—Laboratory testing for the diagnosis of HIV infection: updated recommendations (http://stacks.cdc.gov/view/cdc/23446)
Note: Note that units for vertical axis are not noted because their magnitude differs for RNA, p24 antigen, and antibody

a) ~ 10 days after infection, HIV-1 RNA detectable by NAT, quantities increase to very high levels.

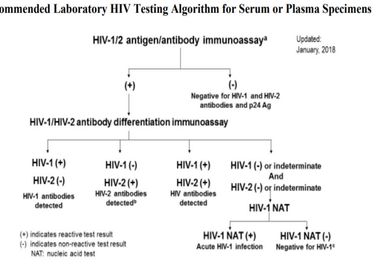
b) HIV-1 **p24 antigen** expressed and can be detected by 4th generation immunoassays 4 to **10 days after** the initial detection of **HIV-1 RNA.**

c) HIV antibodies are expressed:

i. **IgM 3 to 5 days** after p24 antigen, **10 to 13 days after HIV RNA.**

ii. **IgG 18 to 38 days** or more **after HIV RNA**

3 — CDC HIV Diagnostic Algorithm



4 — Analytical performance including Limit of Detection (LOD)

Parameter	Performance		
Sample types	EDTA plasma, serum and dried blood spot (DBS)*		
Minimum amount of sample required	650 µL for EDTA plasma and serum samples or one DBS* sample (70 µL dried blood per spot) in 1150 µL **cobas**® Specimen Pre-Extraction Reagent (SPER)		
Sample process volume	500 µL for EDTA plasma and serum samples or 850 µL for DBS* samples		
Analytical sensitivity (LOD)		HIV-1M	HIV-2
	EDTA plasma	12.6 cp/mL	27.9 cp/mL
	Serum	12.1 cp/mL	23.4 cp/mL
	DBS*	255 cp/mL	984 cp/mL
Specificity	100% (one-sided 95% confidence interval: 99.5%) (EDTA plasma/serum)		
	100% (one-sided 95% confidence interval: 99.5%) (DBS*)		
Groups/subtypes – inclusivity	HIV-1M (A–D, F–H, J, K, CRF01_AE, CRF02_AG, CRF12_BF, CRF14_BG), HIV-1O, HIV-1N, HIV-2 (A and B)		

LOD (by PROBIT at ≥ 95% Hit Rate) studies conducted with WHO standard (HIV-1 group M and HIV-2) and Roche Primary Standard for HIV-1 group O, and verified with clinical or cultured HIV samples for HIV-1 group M (A, C, D, F, G, H) and circulating recombinant forms (CRF01_AE, CRF02_AG), HIV-1 group N and HIV-2 group B.

**Dried Blood Spot (DBS) will not be an approved sample type in US.*

cobas® HIV-1/HIV-2 Qualitative nucleic acid test for use on **cobas**® 6800/8800 Systems Instructions for Use: 08020655001-01EN , page 30 and 39

5 — Inclusivity Using 2nd WHO HIV Inclusivity *International Reference Panel* for NAT

Subtype	Number of Eligible Results (E)	Number of Valid Results (V)	Number of HIV Reactive Replicates (R)	Percent of HIV Reactive Replicates % [R/V]
A	5	5	5	100
B	5	5	5	100
C	5	5	5	100
D	5	5	5	100
A/E	5	5	5	100
F	5	5	5	100
G	5	5	5	100
AG-GH	5	5	5	100
Group				
N	5	5	5	100
O	5	5	5	100
Total	50	50	50	100

- 2nd WHO HIV inclusivity reference panel (NIBSC code: 12/224)
- Each panel member was tested using 5 neat replicates

6 — Evaluation of each assay's relative effectiveness in relation to pre- seroconversion window period using seroconversion panels (ZeptoMetrix, SeraCare)

6a) cobas® HIV 1/2 Qual Test Clinical Agreement (as Compared to CoA) by Seroconversion Panels and Overall. *(Sojourn Time Calculated as Median Days by Subject)*

HIV-1/HIV-2 Seroconversion Panel (tested neat for HIV-1)	Total Number of Eligible Results (E)	Number of Valid Results (V)	Number of Correctly Identified Valid Results (Y)	Percent Correct Detection Rate			First day of NAT detection in CoA	Last Visit Day of Negative Detection from cobas	First Visit Day of Positive Detection from cobas	Sojourn Time (days)*
				Percent Agreement	95% CI					
				% [Y/V]	LL	UL				
600	7	7	7	100 (7/7)	59	100	10	7	10	8.5
9012	8	8	6	75.0 (6/8)	34.9	96.8	0	7	9	8
9031	19	19	17	89.5 (17/19)	66.9	98.7	97	123	127	125
9089	6	6	5	83.3 (5/6)	35.9	99.6	9	9	16	12.5
PRB961	9	9	8	88.9 (8/9)	51.8	99.7	21	14	19	16.5
PRB963	7	7	6	85.7 (6/7)	42.1	99.6	14	7	9	8
PRB967	6	6	6	100 (6/6)	54.1	100	3	0	3	1.5
PRB968	10	10	10	100 (10/10)	69.2	100	15	10	15	12.5
PRB969	10	10	9	90.0 (9/10)	55.5	99.7	55	48	53	50.5
PRB977	4	4	3	75.0 (3/4)	19.4	99.4	0	0	0	0
	Total			Percent				Mean		
	86	86	77	89.5 (77/86)	81.1	95.1	0	22.5	26.1	24.3
Excluding 9031	67	67	60	(60/67)	79.7	95.7	0	11.3	14.9	13.1

CoA=Certificate of Analysis; CI=Confidence Interval; LL=Lower Limit; UL=Upper Limit.
*Sojourn time for each subject sample is calculated as the mid-point of the last visit day negative and the first visit day positive.

6b) Geenius HIV-1/2 Confirmatory Assay Clinical Agreement (as Compared to CoA) by Seroconversion Panels and Overall. *(Sojourn Time Calculated as Median Days by Subject)*

HIV-1/HIV-2 Seroconversion Panel (tested neat for HIV-1)	Total Number of Eligible Results (E)	Number of Valid Results (V)	Number of Correctly Identified Valid Results (Y)	Number of Indeterminate Results (Y)	Percent Correct Detection Rate			First day of NAT detection in CoA	Last Visit Day of Negative Detection from Geenius	First Visit Day of Positive Detection from Geenius	Sojourn Time (days)*
					Percent Agreement	95% CI					
					% [Y/V]	LL	UL				
600	7	7	3	0	42.9 (3/7)	9.9	81.6	10	21	C	NA
9012	8	8	2	0	25.0 (2/8)	3.2	65.1	0	21	23	22
9031	19	19	14	0	73.7 (14/19)	48.8	90.9	97	146	153	149.5
9089	6	6	3	0	50.0 (3/6)	11.8	88.2	9	20	24	22
PRB961	9	9	6	0	66.7 (6/9)	29.9	92.5	21	29	C	NA
PRB963	7	7	4	0	57.1 (4/7)	18.4	90.1	14	21	C	NA
PRB967	6	6	3	0	50.0 (3/6)	11.8	88.2	3	17	19	18
PRB968	10	10	6	0	60.0 (6/10)	26.2	87.8	15	28	33	30.5
PRB969	10	10	7	0	70.0 (7/10)	34.8	93.3	55	63	70	66.5
PRB977	4	4	1	0	25.0 (1/4)	0.6	80.6	0	15	C	NA
	Total				Percent				Mean		
	86	86	49	0	57.0 (49/86)	45.8	67.6	0	38.1	53.7	51.4
Excluding 9031	67	67	35	0	52.2 (35/67)	39.7	64.6	0	26.1	33.8	31.8

C = Censored observation. At the last visit, subject remained negative. Estimate is a lower bound. NA=Not Available. CoA=Certificate of Analysis; CI=Confidence Interval; LL=Lower Limit; UL=Upper Limit.
*Sojourn time for each subject sample is calculated as the mid-point of the last visit day negative and the first visit day positive.

- Median Sojourn Time calculated for the **cobas**® HIV-1/HIV-2 Qual test ranged **from 0 to 125 days**. The Mean Sojourn Time (MST) was 24.3 days. Excluding subject panel 9031, the MST drops to 13.1 days.
- Median Sojourn Time for the Geenius HIV-1/2 Confirmatory Assay for each subject ranged from **18 to 149.5 days**. The Mean Sojourn Time (MST) was 51.4 days. Excluding subject panel 9031, the MST drops to 31.8 days.

6c) Delta Sojourn Time – Geenius™ HIV-1/2 Confirmatory Assay minus cobas® HIV-1/HIV-2 Qual test

HIV-1/HIV-2 Seroconversion Panel (tested neat for HIV-1)	Delta (Geenius – cobas) Sojourn Time (days)	
	Median	LOCF*
600	NA	NA
9012	14	14
9031	24.5	26
9089	9.5	8
PRB961	NA	NA
PRB963	NA	NA
PRB967	16.5	16
PRB968	18	18
PRB969	16	17
PRB977	NA	NA
Overall Deltas		
Delta Mean 1	27.1	27.6
Delta Mean 2**	18.7	18.9

or first day positive.
**Excluding Seroconversion Panel #903
*LOCF = Last Observation Carried Forward 1.

7 — Assay Correlation using Clinical Specimens

- **HIV-1 Clinical Specimens:** EDTA plasma or serum specimens provided by the testing site from patients undergoing HIV management. These specimens may be HIV detectable (n=160) or non-detectable (n=48) depending on response to treatment.
- **HIV-2 Clinical Specimens:** Approximately 30 EDTA plasma or serum specimens previously determined to be HIV 2 positive. Provided to the testing site.
- **HIV Negative Specimens:** 51 EDTA plasma or serum specimens negative for HIV screening, provided by the testing site.

cobas HIV-1/2 Qual Test Result	Geenius HIV Overall Result						
HIV-1 / HIV-2	**HIV1–/HIV2–**	**HIV-1 +**	**HIV-2 +**	**HIV-2 Indet**	**HIV-2+ (with HIV-1 cross-reactivity)**	**HIV+ Un-typable**	**Total**
+ / –	0	158	0	0	0	2	**160**
– / +	0	0	0	0	**14**	0	**14**
– / –	51	47	2*	2*	13*	0	**115**
Failed/Invalid	0	1	0	0	0	0	**1**
Total	**51**	**206**	**2**	**2**	**27**	**2**	**290**

HIV-2 serology + samples: 10 NAT negative, 7 VL<LLOQ (40 cp/mL) in the CoA

8 — Conclusions

- **cobas**® HIV-1/2 Qual test enables accurate and sensitive diagnosis of HIV-1 and HIV-2 across sample types.
- **cobas**® HIV-1/2 Qual test detected and discriminated HIV infections over two weeks compared to the serology-based test, that could help avert transmission during acute HIV infection, expand access to NAT HIV testing for adults and lead to simplification of HIV diagnostic algorithms.
- **cobas**® HIV-1/2 Qual test could make a substantial contribution to reaching the 'first 90' UNAIDS goal, the testing of 90% of the infected population.

The **cobas**® HIV-1/2 Qualitative nucleic acid test for use on the **cobas**® 6800/8800 Systems is commercially available in countries accepting the CE and currently undergoing the FDA registration process (the test is not approved in the United States).