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Using quantitative HIV RNA viral load to diagnose HIV infection

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BACKGROUND AND OBJECTIVES

The third generation enzyme immunoassays (EIA) currently used in Canada as the initial screen for HIV antibodies have an estimated diagnostic window period of 4-6 weeks. However, the Canadian case definition for HIV is based on a positive confirmatory Western Blot (WB) which is less sensitive than EIA. Early diagnosis of HIV infection optimizes individual patient management and clinical outcomes. Moreover, acute HIV seroconverters usually have a very high viral load, which is an important determinant of infectivity. Another challenge using serology as the diagnostic criterion is the recognition of vertical transmission because of transplacental maternal antibodies in infants. While quantitative HIV RNA viral load (QVL) is only licensed for the clinical monitoring of HIV infected patients, it has also been shown to be sensitive and specific in previous studies as a diagnostic test. We examined the performance of QVL as a HIV diagnostic test in two populations:

1) infants born to HIV infected mothers, and

2) patients with reactive EIA and negative/indeterminate (neg/ind) WB.

METHODS

Provincial Public Health Laboratory (ProvLab) performs all diagnostic HIV serology tests for the province of Alberta using an algorithm that includes two EIA assays and WB for patients with a positive EIA screen (Figure 1). Each clinician is phoned by the virologist to discuss the WB result of a patient and to obtain HIV risk history, reason for testing and presenting symptoms. QVL is recommended when there is a high suspicion for acute seroconversion or if the QVL is deemed to be useful in the clinical management and/or counselling. QVL was performed by both regular and ultrasensitive Roche Cobas® Amplicor® HIV-1 Monitor (v1.0 and v1.5). The ProvLab database contains data of diagnostic HIV serology for Northern Alberta from Feb 1998 to Feb 2002 and for the whole province from Mar 2002 to present. Information on two specific patient population groups was extracted from the database:

1) All infants born to HIV infected mothers from 1998 to 2006 who had QVL testing prior to 18 months of age, and

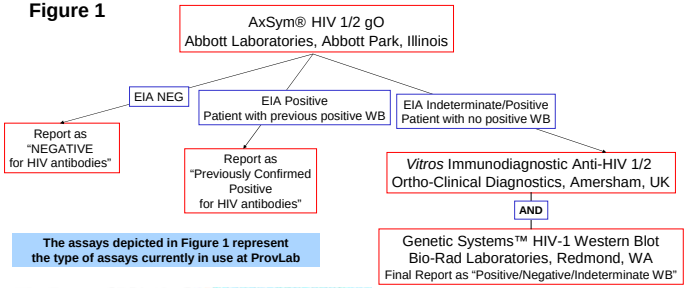
2) All persons with reactive HIV antibodies by EIA and neg/ind by WB from 1998 to 2005 who also had QVL performed within 150 days of their neg/ind WB. This population was further categorized as:

a) patients with risk history and/or clinical symptoms suggestive of acute HIV seroconversion

b) patients with no risk factors for HIV infection and suspected to have false reactive result by the screening EIA

Sensitivity and specificity of QVL was determined using serological diagnosis as a gold standard, and 95% confidence intervals were calculated using binomial distribution.

Figure 1

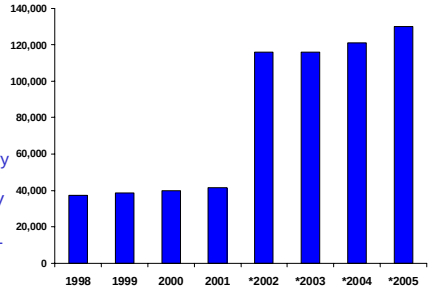


RESULTS AND DISCUSSION

The number of HIV screening EIA performed per year from Feb 1998 to 2005 and the percentage of indeterminate and positive EIA results are shown in Figure 2 and the percentage of EIA positive specimens that were confirmed as WB positive is shown in Figure 3.

Figure 2

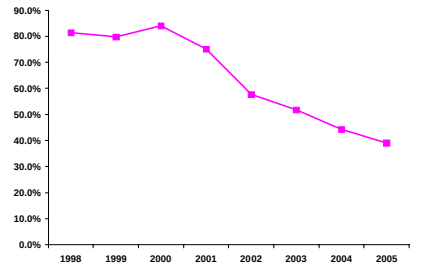
* Three changes occurred in 2002:
- Change of the screening EIA from AxSym® HIV-1/HIV-2 MEIA to AxSym® HIV 1/2 gO in March
- Consolidation of the provincial HIV laboratory database in March
- ProvLab providing HIV screening for the Provincial Prenatal Opt-out HIV Program in August



Year	1998	1999	2000	2001	*2002	*2003	*2004	*2005
Indeterminate EIA (s/co 0.9 -<1.0)	0.03%	0.03%	0.06%	0.04%	0.05%	0.05%	0.04%	0.06%
Positive EIA (s/co ≥ 1.0)	0.83%	0.83%	0.86%	0.98%	0.87%	0.70%	0.64%	0.71%

Figure 3

After the screening EIA was changed from AxSym® HIV-1/HIV-2 MEIA to AxSym® HIV 1/2 gO in March 2002, a decrease in the percentage of EIA positive specimens that would confirm as WB positive was noted.



For the study period, 130 patients had QVL performed < 150 days of their negative/indeterminate (neg/ind) WB (Table 1).

Fifty-five patients had at least one detectable QVL: 46 were subsequently confirmed to be HIV infected by positive WB, 8 had multiple detectable QVL supporting an HIV diagnosis and one patient had only one specimen submitted for QVL and no follow-up serology. One of the patients who had a follow-up positive WB had a very low viral load set point with the first viral load tested as <50 copies/ml and a second viral load tested as 85 copies/ml. Overall, the median first detectable viral was 87,000 copies/ml (range: 85 to 2.3E7 copies/ml).

Seventy-five patients had undetectable QVL: 41 had at least one HIV serological tests after the QVL to confirm their uninfected state, 22 showed no evidence of acute seroconversion based on the serial EIA results around the time when the QVL was performed, and 12 patients only had one HIV antibody test and one QVL. For those 12 patients, the EIA signal/cut-off ratio (s/co) of both EIA tests were low (<2.5) and 11 of the second EIA tests were negative.

Table 1. WB, EIA results and demographics of adults with QVL

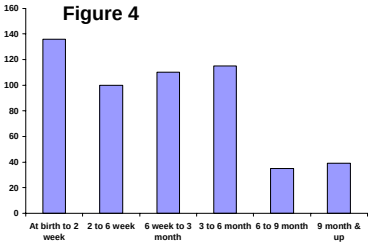
QVL Results	WB Results	No. (%) tested positive by both EIA	No. (%) of Male	Age (All cases) median (range)	Age (Male) median (range)	Age (Female) median (range)
Patients with Detectable QVL	All cases (n=55)	† 50 (90.0%)	40 (72.7%)	35.3 (16.2-61.3)	*37.7 (22.6-56.4)	*31.0 (16.2-45.9)
	NEG WB (n=11)	† 8 (72.7%)	7 (63.6%)	41.7 (21.2-56.4)	42.4 (26.4-56.4)	35.9 (21.2-45.9)
	IND WB (n=44)	† 42 (95.4%)	33 (75.0%)	34.8 (16.2-61.3)	*37.0 (22.6-61.4)	*31.0 (16.2-40.4)
Patients with Undetectable QVL	All cases (n=75)	§ 7 (21.3%)	32 (42.7%)	31.0 (15.3-80.3)	31.6 (17.5-55.7)	31.0 (15.3-80.3)
	NEG WB (n=36)	§ 9 (25.7%)	‡15 (41.7%)	29.9 (15.3-54.2)	32.1 (22.3-54.2)	27.5 (15.3-49.3)
	IND WB (n=39)	§ 7 (17.9%)	‡17 (43.6%)	33.5 (16.3-80.3)	31.0 (17.5-55.7)	36.4 (16.3-80.3)

† One patient had NSQ sample for the second EIA. The four patients whose second EIA were negative occurred in 1998-1999 before a change in the second EIA assay.
§ One patient had NSQ sample for the second EIA and one patient tested negative by the first EIA but the second EIA was performed because of being from HIV-2 endemic country and the second EIA was positive.
* P<0.05 Mann Whitney U test
‡ One patient had unknown gender

For the diagnosis of vertical transmission, QVL was performed on 176 HIV-exposed infants. Six infants had a detectable QVL with the median age of first detectable WB at 2 months and the median first detectable viral load at 4.3E5 copies/ml (280 to 1.7E6). For the 170 infants (male=96, 56.4%) with undetectable QVL, 131 (77.1%) had negative follow-up serology while the remainder either had no follow-up serology (n=28) or have not reached 18 months of age (n=11).

A total of 535 QVL test were performed for the 170 infants with undetectable QVL; 143 infants had 2 or more tests (median=3, range 1-6) with most of the specimens submitted at less than 6 months of age.

Figure 4



Based on the first result of QVL after the neg/ind WB in the acute seroconvertors and the first detectable QVL in the infants, the sensitivity and specificity of QVL is shown in Table 2:

Table 2	HIV-Exposed Infants (n=137)	Acute Seroconverters (n=117)
No. Positive by QVL / No. Positive by Serology and/or Repeat Detectable QVL	6/6 *	53/54 †
Sensitivity (95% CI)	100% (54-100)	98.1% (90-100)
No. Negative by QVL / No. Negative by Serology	131/131	63/63
Specificity (95% CI)	100% (97-100)	100% (94-100)

* None of the infants had confirmatory WB, all had repeat detectable QVL
† Eight of these patients had no confirmatory WB, but had multiple detectable QVL supporting HIV diagnosis

CONCLUSION

QVL is sensitive and specific for the diagnosis of HIV in HIV-exposed infants and suspect acute seroconvertors. Our data support the use of QVL as a diagnostic test for HIV.

ACKNOWLEDGEMENT

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