Quality control assessment of human immunodeficiency virus type 2 (HIV-2) viral load quantification assays: results from an international collaboration on HIV-2 infection in 2006

Damond, F; Benard, A; Ruelle, J; Alabi, A; Kupfer, B; Gomes, P; Rodes, B; Albert, J; Böni, J; Garson, J; Ferns, B; Matheron, S; Chene, G; Brun-Vezinet, F

Damond, F; Benard, A; Ruelle, J; Alabi, A; Kupfer, B; Gomes, P; Rodes, B; Albert, J; Böni, J; Garson, J; Ferns, B; Matheron, S; Chene, G; Brun-Vezinet, F (2008). Quality control assessment of human immunodeficiency virus type 2 (HIV-2) viral load quantification assays: results from an international collaboration on HIV-2 infection in 2006. Journal of Clinical Microbiology, 46(6):2088-2091.

Postprint available at:
http://www.zora.uzh.ch

Posted at the Zurich Open Repository and Archive, University of Zurich.
http://www.zora.uzh.ch

Originally published at:
Quality control assessment of human immunodeficiency virus type 2 (HIV-2) viral load quantification assays: results from an international collaboration on HIV-2 infection in 2006

Abstract

Human immunodeficiency virus type 2 (HIV-2) RNA quantification assays used in nine laboratories of the ACHI(E)V(2E) (A Collaboration on HIV-2 Infection) study group were evaluated. In a blinded experimental design, laboratories quantified three series of aliquots of an HIV-2 subtype A strain, each at a different theoretical viral load. Quantification varied between laboratories, and international standardization of quantification assays is strongly needed.
Quality Control Assessment of Human Immunodeficiency Virus Type 2 (HIV-2) Viral Load Quantification Assays: Results from an International Collaboration on HIV-2 Infection in 2006

Florence Damond, Antoine Benard, Jean Ruelle, Abraham Alabi, Bernd Kupfer, Perpetua Gomes, Berta Rodes, Jan Albert, Jürg Böni, Jeremy Garson, Bridget Ferns, Sophie Matheron, Geneviève Chene, and Françoise Brun-Vezinet for the ACHI EV2E (A Collaboration on HIV-2 Infection) Study Group

APHP, Hôpital Bichat—Claude Bernard, Laboratoire de Virologie, Paris, 75018 France; INSERM, U897.ISPED, Université Victor Segalen Bordeaux 2, Bordeaux, F-33076, France; AIDS Reference Laboratory, Université Catholique de Louvain, Louvain-la-Neuve, Belgium; Medical Research Council Laboratories, Banjul, Gambia; Institute of Virology, Bonn, Germany; Laboratório de Biologia Molecular, CHLO—Hospital Egas Moniz, Lisboa, Portugal; Department of Infectious Diseases, Hospital Carlos III, Madrid, Spain; Department of Virology, Swedish Institute for Infectious Disease Control, Solna, and Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden; Swiss HIV Cohort Study, Zurich, Switzerland; Centre for Virology, Royal Free & University College London Medical School, London, United Kingdom; and APHP, Hôpital Bichat—Claude Bernard, Service de Maladies Infectieuses et Tropicales, Paris, France

Received 22 January 2008/Accepted 14 April 2008

The human immunodeficiency virus type 2 (HIV-2) epidemic remains essentially confined to West Africa (4). In Europe, most of the patients live in France, Portugal, and Spain, although patients are also found in The Netherlands, Belgium, Luxembourg, Germany, Switzerland, Italy, the United Kingdom, and Sweden (5, 10–13). Many questions remain unanswered concerning the evolution of virological, immunological, and clinical parameters under treatment. Answering these questions requires the largest possible number of observations and standardization of outcome measurements such as that of the HIV-2 load in plasma. There is currently no available commercial assay for the quantification of plasma HIV-2 RNA. In this study, we evaluated the validity of assays used for the quantification of HIV-2 RNA among nine centers in the ACHI EV2E (A Collaboration on HIV-2 Infection) study group were evaluated. In a blinded experimental design, laboratories quantified three series of aliquots of an HIV-2 subtype A strain, each at a different theoretical viral load. Quantification varied between laboratories, and international standardization of quantification assays is strongly needed.

The sample panel was prepared in a single virology laboratory (Bichat-Claude Bernard Hospital, Paris, France) by performing serial dilution of the NIHx HIV-2 subtype A supernatant, quantified by electron microscopy (ABI Technologies), in HIV-negative human plasma to obtain 10 1-ml aliquots each with final HIV-2 RNA concentrations of 1.7, 2.3, and 3.0 log10 copies/ml.

The accuracy and reproducibility of assays were estimated for the three theoretical concentrations. A quantification assay was defined as accurate if at least 9 of the 10 measurements fell within a clinically acceptable interval based on the NIHx theoretical concentrations. The interval was defined as one-third of the theoretical viral load to three times the theoretical viral load for theoretical viral loads of 3.0 and 2.3 log10 copies/ml and as 0 to three times the theoretical viral load for a theoretical viral load of 1.7 log10 copies/ml.

Reproducibility of assays was evaluated by using the intralaboratory coherence coefficient (ILCC) and the coefficient of variation (CV) at each of the three theoretical concentrations (1). A quantification heterogeneity was defined as an ILCC greater than the theoretical value L, extracted from a reference table for p laboratories and n repetitions (1).

Each of the participating laboratories quantified 35 coded and randomized aliquots. All laboratories reported an undetectable viral load for the five HIV-negative aliquots.

At a theoretical level of 3.0 log10 copies/ml, median quantifications varied from 2.9 log10 copies/ml in laboratory 6 to 4.2 log10 copies/ml in laboratory 8 (Fig. 1A). Five laboratories reported accurate measurements (i.e., 9 of the 10 quantifica-
The four other assays overquantified viral loads (laboratories 1, 3, 7, and 8) (Fig. 1A). Seven laboratories reported reproducible measurements (CV/H100, 3 to 6%; ILCC/H100, 0.5 to 0.9; L/H100, 1.35) (laboratories 1, 2, 3, 6, 7, 8, and 9). Laboratories 2, 6, and 9 reported both accurate and reproducible measurements. At a theoretical viral load of 2.3 log10 copies/ml, the accuracy and reproducibility of the tests at laboratories 4 and 5 could not be evaluated, since 10 and 9 negative results were reported, respectively. Median quantifications varied from 2.4 log10 copies/ml in laboratory 9 to 3.4 log10 copies/ml in laboratory 8 (Fig. 1B). Three laboratories reported accurate measurements (i.e., 9 of the 10 quantifications fell in the interval from 1.82 to 2.78 log10 copies/ml). The other assays overquantified viral loads (laboratories 1, 3, 7, and 8) (Fig. 1A).

**TABLE 1. Characteristics of the different quantification assays assessed by the ACHIEV2E Collaboration in 2006**

<table>
<thead>
<tr>
<th>Laboratory System</th>
<th>Primer(s) and probe localization</th>
<th>RNA extraction Standard used</th>
<th>Threshold (log10 copies/ml)</th>
<th>Subtypes tested</th>
<th>Primer supplier(s)</th>
<th>Probe supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 LightCycler</td>
<td>LTR region (SYBR green detection)</td>
<td>Nuclisens-MiniMag</td>
<td>External, synthetic RNA (ROD seq)</td>
<td>1.7</td>
<td>A, B</td>
<td>Eurogentec</td>
</tr>
<tr>
<td>2 LightCycler</td>
<td>Gag gene</td>
<td>Magnapure</td>
<td>External, NIHZ quantified by electron microscopy</td>
<td>2.0</td>
<td>A, B</td>
<td>MWG, Proligo, and Applied Proligo</td>
</tr>
<tr>
<td>3 In-house PCR</td>
<td>ELONA</td>
<td>Boom CBL23</td>
<td>Internal control</td>
<td>2.0</td>
<td>A, B</td>
<td>MWG</td>
</tr>
<tr>
<td>4 LightCycler</td>
<td>Gag gene</td>
<td>Qiagen, viral RNA</td>
<td>External, NIHZ quantified by electron microscopy</td>
<td>2.7</td>
<td>A, B</td>
<td>TIB MolBiol</td>
</tr>
<tr>
<td>5 In-house QCRT-PCR</td>
<td>Env gene and biotin/digoxigenin</td>
<td>easyMag</td>
<td>Internal control</td>
<td>2.3</td>
<td>A, B</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>6 Nuclisens EasyQ v1.1</td>
<td>Gag gene</td>
<td>Nuclisens EasyMAG</td>
<td>Internal</td>
<td>2.3</td>
<td>A, B</td>
<td>bioMerieux</td>
</tr>
<tr>
<td>7 ExaVir Load Analyzer v1.61</td>
<td>ExaVir RT test from Cavidi</td>
<td>ExaVir RT test from Cavidi</td>
<td>ExaVir RT test from Cavidi</td>
<td>2.6</td>
<td>A, SIVsm</td>
<td></td>
</tr>
<tr>
<td>8 TaqMan Gag leader HIV Monitor</td>
<td>External, ST isolate, only relative quantification</td>
<td>NT</td>
<td>A, B</td>
<td>Microsynth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 ABI Prism 7000</td>
<td>TaqMan probe LTR</td>
<td>Qiagen viral RNA</td>
<td>CBL22 external BMV internal</td>
<td>2.0</td>
<td>A, B</td>
<td>MWG</td>
</tr>
</tbody>
</table>

a LTR, long terminal repeat.
b QCRT-PCR, quality control reverse transcription-PCR.
c NT, not tested.

**FIG. 1.** Accuracy of quantification assays evaluated by the ACHIEV2E Collaboration in 2006. Quantification results are reported for each participating laboratory. The accuracy interval is represented by the white area for each of the three theoretical viral loads used. Panels: A, theoretical viral load 3.0 log10 copies/ml; B, theoretical viral load 2.3 log10 copies/ml; C, theoretical viral load 1.7 log10 copies/ml.
log_{10} copies/ml) and four laboratories overquantified viral loads (laboratories 1, 3, 7, and 8) (Fig. 1B). Six laboratories reported reproducible measurements (CV = 5 to 8%; ILCC = 0.5 to 1.1; \( L = 1.35 \)). Only two laboratories, 2 and 9, reported both accurate and reproducible measurements.

At a theoretical level of 1.7 log_{10} copies/ml, the accuracy and reproducibility of the assays used by laboratories 4, 5, 6, and 7 could not be evaluated since 9, 10, 7, and 9 negative results were reported, respectively. The median quantifications varied from 1.4 log_{10} copies/ml in laboratory 2 to 2.9 log_{10} copies/ml in laboratory 8 (Fig. 1C). Two laboratories, 2 and 9, reported accurate measurements (i.e., 9 of the 10 quantifications were below 2.18 log_{10} copies/ml), and three laboratories overquantified viral loads (laboratories 1, 3, and 8) (Fig. 1C). These three laboratories reported reproducible measurements (CV = 5 to 11%; ILCC = 0.4 to 0.8; \( L = 1.35 \)). At this level, no laboratories reported both accurate and reproducible measurements.

Our study is the first international evaluation of the validity of HIV-2 RNA quantification assays. We found a fair homogeneity of results of assays evaluated at a theoretical level of 3.0 log_{10} copies/ml and a decreasing accuracy and reproducibility of assays with decreasing viral loads, as previously shown in a quality control study of HIV-1 RNA quantification assays (6). Overall, four laboratories systematically overquantified viral loads, no. 1, 3, 7, and 8. Two laboratories, 4 and 5, reported 18 negative results out of 20 quantifications at theoretical viral loads of both 2.3 and 1.7 log_{10} copies/ml. The thresholds of these two assays had been evaluated at 2.7 and 2.3 log_{10} copies/ml, respectively. The cutoff values of the methods used by laboratories 5 and 7 may also explain their results for the 1.7-log_{10}-copy/ml aliquots. One laboratory (no. 6) reported both accurate and reproducible results at a theoretical viral load of 3.0 log_{10} copies/ml but heterogeneous quantifications at 2.3 log_{10} copies/ml and negative results at 1.7 log_{10} copies/ml. Two laboratories, 2 and 9, reported both accurate and reproducible results at theoretical viral loads of 3.0 and 2.3 log_{10} copies/ml and accurate but relatively poorly reproducible quantifications at 1.7 log_{10} copies/ml. The aliquots were made from the NIH2 HIV-2 supernatant, which is the standard used for the calibration of the laboratory 2 assay. That may have improved the results reported by this laboratory and decreased those observed in other laboratories such as no. 8, which has not yet been evaluated with calibrated specimens. However, laboratory 9, which did not use NIH2 as a standard, reported results very similar to those obtained by laboratory 2.

This study provides an overview of the performance of different HIV-2 RNA quantification assays. If the HIV-2 supernatant counted by electron microscopy is accepted as a standard, only two laboratories reported accurate and reproducible measurements at both 3 and 2.3 log_{10} copies/ml. This heterogeneity may yield difficulties in the comparison of results between the different cohorts of the ACHIEVE2 Collaboration. The ACHIEV2 network is working toward the standardization of quantification assays to improve the interpretation of results for case management and collaborative clinical trials.

This work was supported by the Agence Nationale de Recherche sur le SIDA (ANRS). The members of the ACHIEV2 study group in Belgium are Patrick Goubau and Jean Ruelle (AIDS Reference Laboratory, Université Catholique de Louvain). Those in France are François Brun-Vezinet, Pauline Campa, Florence Damond, Diane Descamps, Sophie Matheron (Bichat—Claude Bernard Hospital, Paris), François Simon (Saint-Louis Hospital, Paris), Antoine Benard, Geneviève Chene, Audrey Taieb (INSERM U897, Bordeaux2 University, Bordeaux), and Brigitte Autran (Pitié-Salpêtrière Hospital, Paris). Those in Gambia are Abraham Alabi, Matt Cotten, Assan Jallow, Kevin Peterson, and Sarah Rowland-Jones (Medical Research Council Laboratories). Those in Germany are Bernd Kupfer (Institute of Virology, Bonn), Jürgen Rockstroh, and Carolyne Schwarze-Zander (Department of Internal Medicine I, University of Bonn, Bonn). Those in The Netherlands are Frank De Wolf, Ard Van Sighem (National AIDS Therapy Evaluation Center, AMC, Amsterdam), Peter Reiss, Maarten Schim Van der Loeff (AMC-CPE, Amsterdam; MRC, Gambia), and Martin Schütten (Department of Virology, Erasmus MC, Rotterdam).

Those in Portugal are Ricardo Camacho, Perpetua Gomes, Kamal Mansinho (Egas Moniz Hospital, Lisbon), Francisco Antunes, Luís Franca, and Emília Valadas (Clínica Universitaria de Doenças Infecciosas, Lisboa). Those in Spain are Berta Rodes, Carlos Toro, and Vicente Soriano (Department of Infectious Diseases, Hospital Carlos III, Madrid). Those in Sweden are Jan Albert (Department of Virology, Swedish Institute for Infectious Disease Control, Solna), Katarina Gyllensten, Anders Sonnerborg (Divisions of Clinical Virology and Infectious Diseases, Huddinge University Hospital, Karolinska Institute, Stockholm), Aylin Yilmaz, and Magnus Gisslén (Department of Infectious Diseases, Sahlgrenska University Hospital, Göteborg).

Those in Switzerland are Greg Böni, Alexandra Calmy, and Martin Rickenbach (Swiss HIV Cohort Study). Those in the United Kingdom are Deenan Pillay, Bridget Ferns, Jeremy Garson (Centre for Virology, Royal Free & University College London Medical School, London), Jennifer Tosswell (Centre for Infections, Health Protection Agency, London), Jane Anderson (Centre for the Study of Sexual Health and HIV, Homerton University Hospital, London), and David Chadwick (James Cook University Hospital, Middlesbrough).

REFERENCES


