

Standardization of quantitative virological assays for antiretroviral clinical trials

Abbreviations: ACTG, AIDS Clinical Trial Group; NIAID, National Institute of Allergy and Infectious Diseases; PBMCs, Peripheral Blood Mononuclear Cells; DAIDS, Division of AIDS; VRL, Virology Reference Laboratory; VQA, Virology Quality Assurance; ICD, Immune Complex Dissociation

Editorial

The AIDS Clinical Trial Group (ACTG) was formed and funded by the National Institute of Allergy and Infectious Diseases (NIAID) in 1987 to enable multi-center clinical trials for the evaluation of treatments for HIV. As there were no licensed anti-retroviral drugs at that time large numbers of subjects and many testing sites were needed to adequately demonstrate clinical efficacy and make the drugs available for general treatment. Initially trial endpoints were based on evaluation of symptoms some of which were based on subjective judgment by clinicians. Measurements of CD4 positive cells were used to demonstrate progression from asymptomatic to symptomatic disease to AIDS. In order to shorten the length of the trials and obtain objective results it was necessary to identify virus specific markers of efficacy. Some of the first viral markers to be evaluated included HIV-1 p24 antigen as measured in serum, and the infectious outgrowth of HIV from peripheral blood mononuclear cells (PBMCs). These tests paved the way for more sensitive tests using HIV nucleic acid amplification to determine the copies of HIV genome in plasma. HIV RNA continues to be the best surrogate marker used today to evaluate the efficacy of new combinations of antiretroviral drugs.

Clinical efficacy is demonstrated by “substantial evidence” from adequate and well-controlled investigations with a consistently manufactured product. HIV infection results in a multifaceted disease which complicates the evaluation of treatment efficacy. In the early stages of antiretroviral drug development, research focused on the urgency for identifying a treatment that slowed the progression of disease and reduced spread of the virus, thus multiple clinical sites were needed to enroll large numbers of subjects. This necessitated the use of multiple laboratories and the development and standardization of multiple tests for measuring virus load. The first test to be used for the detection of infectious HIV involved the co-culture of PBMC from HIV-infected subjects with the stimulated PBMC from uninfected donors. Initially, laboratories used their own procedures with cultures being held for up to 60 days, and the HIV-1 p24 antigen assay used as the read-out for a positive culture. Because uniform results across labs were needed, NIH’s Division of AIDS (DAIDS) set up a Virology Reference Laboratory (VRL) which later became the Virology Quality Assurance (VQA) laboratory to standardize procedures across laboratories and access the uniformity of results using proficiency testing. Each laboratory’s performance was evaluated for accuracy to the expected value and only those laboratories that passed proficiency testing for a specific assay were certified to perform that assay for evaluation of clinical trials. HIV culture procedures were standardized by the VRL and proficiency testing was initiated across all ACTG laboratories.¹ Efforts to refine the early testing procedures for efficiency and sensitivity were evaluated through the use of Virology Working Groups made up of members from the ACTG laboratories also sponsored by DAIDS.² As a result of this standardization, the

Volume 1 Issue 3 - 2014

Janet L Lathey
Consultant, USA

Correspondence: Janet L Lathey, Consultant, Montgomery Village, Maryland, USA, Tel 240-477-6273, Email jlathey@hotmail.com

Received: July 12, 2014 | **Published:** July 14, 2014

HIV qualitative culture assay for infectious virus was reduced to a 21 day culture period.³ In addition, qualitative and quantitative HIV micro cultures were developed and added to the proficiency testing program.⁴⁻⁶ Finally, PBMC culture of plasma from HIV infected subjects for the quantification of infectious virus was developed and standardized.⁷

Besides using the HIV-1 p24 antigen ELISA assay to determine culture results, this assay was also used to quantitate the amount of HIV-1 antigen present in the serum of HIV infected subjects. As there were multiple commercial ELISA kits available for use, not all laboratories used the same kits. Thus, passing proficiency testing was again essential for laboratories to participate in testing of clinical trial samples. However, there was another complication, because each manufacturer utilized a unique standard curve in order to quantify the amount of HIV-1 p24 antigen in the samples, the quantitative results from different kits were not necessarily comparable, making it difficult to combine results from multiple laboratories. Again the VRL/VQA was called on to standardize the assay. A single set of external standards was developed and required for use with all ELISA kits to determine HIV-1 p24 levels for clinical trial samples. Thus, culture results and HIV antigen levels would be comparable across laboratories and across clinical trials and could be performed in real-time. Real-time vs. batch testing is important because it not only speeds up the completion of testing, but eliminates sample stability problems. Detection of HIV-1 p24 antigen in serum and plasma can deteriorate over time with storage at 4°C and -20°C.^{8,9} However, pre-treatment of the sample with acid resulted in immune complex

dissociation (ICD), and deterioration was not observed and sensitivity was increased⁹ making batch or real-time testing possible. Culture and HIV-1 p24 antigen testing were effective tools for use in the early trials, but as subjects received newer, more effective anti-retroviral treatments there was a need for assays with increased sensitivity for the quantification of HIV. Plasma HIV RNA-PCR was the logical next step, but with increased sensitivity and increased variation associated with PCR amplification, the need for increased standardization became even more important than for culture and HIV-1 p24 antigen ELISA. Manufacturers of early HIV RNA assays utilized different enzymes and methodologies to quantify HIV RNA in a sample. Thus, external controls were again developed for use in all laboratories regardless of the type of kit used. In conjunction with the standards, a proficiency testing program was developed, enabling the pooling of results from multiple laboratories for clinical trial evaluation.¹⁰ The parameters used for scoring proficiency testing panels first focused on batch testing as it was being done in early clinical trials and was 'modernized' to meet the needs of real-time testing and patient management.

With a battery of quantitative virology tests in addition to the original evaluation of CD4 cell number it was important to determine the virological parameter that was most predictive of efficacy in order to streamline testing and be confident that the trial results accurately determine the effectiveness of the antiretroviral treatment. To accomplish this, all the tests were performed in conjunction with a clinical trial that met the predicted efficacy endpoints for reduction in disease progression. A subset of subjects participating in ACTG 175 "A trial comparing nucleoside mono therapy with combination therapy in HIV-infected adults with CD4 cell counts from 200 to 500 per cubic millimeter" had blood samples collected to be tested in multiple quantitative virological assays.¹¹⁻¹⁴ Among the assays performed were the assays described above: Infectious HIV PBMC micro culture, serum HIV-1 p24 antigen ELISA (ICD), and plasma HIV RNA PCR. In multivariate proportional hazards models HIV RNA and infectious HIV were significantly associated with progression, however, HIV-1 p24 antigen was never significantly associated with disease progression when HIV RNA was included in the model. Thus, HIV RNA and infectious HIV were shown to be independent markers for HIV disease progression and could be used as surrogate markers for anti-retroviral efficacy. Because of the complexity of PBMC processing and the inherent variability in bioassays, this study recommended going forward with plasma HIV RNA as the principal virologic surrogate marker for efficacy of anti-retroviral therapies.¹³

As antiretroviral therapies improved, combination therapies were able to reduce plasma RNA viral loads to levels undetectable by PCR and other nucleic acid amplification procedures. There is hope that current therapies could result in a cure. Thus, testing has come full circle. To demonstrate a cure it will be necessary to standardize a new, more sensitive PBMC culture assay able to detect any residual HIV hiding in mononuclear cells and capable, under the appropriate conditions, of replicating and spreading to new cells. Development of this ultra-sensitive culture assay will be followed by the establishment of external quality assurance for the assay.

Acknowledgment

I'd like to acknowledge the Virology Quality Assurance Laboratory Program sponsored by DAIDS and Patricia S Reichelderfer previously of DAIDS, for their support in assay development and standardization allowing for the success of the ACTG antiretroviral clinical trials.

Conflict of interest

None.

References

- Hollinger FB, Bremer JW, Myers LE, et al. Standardization of sensitive human immunodeficiency virus co-culture procedures and establishment of a multicenter quality assurance program for the AIDS Clinical Trials Group. The NIH/NIAID/DAIDS/ACTG Virology Laboratories. *J Clin Microbiol.* 1992;30(7):1787-1794.
- Hammer S, Crumpacker C, D'Aquila R, et al. Use of virologic assays for the detection of HIV in clinical trials: recommendations of the AIDS Clinical Trials Group Virology Committee. *J Clin Microbiol.* 1993;31(10):2557-2564.
- Fiscus SA, Welles SL, Spector SA, et al. Length of incubation time for human immunodeficiency virus cultures. *J Clin Microbiol.* 1995;33(1):246-247.
- Erice A, Sannerud KJ, Leske VL, et al. Sensitive microculture method for isolation of human immunodeficiency virus type 1 from blood leukocytes. *J Clin Microbiol.* 1992;30(2):444-448.
- Dimitrov DH, Melnick JL, Hollinger FB. Microculture assay for the isolation of human immunodeficiency virus type 1 and for titration of infected peripheral blood mononuclear cells. *J Clin Microbiol.* 1990;28(4):734-737.
- Scott WA, Brambilla D, Siwak E, et al. Evaluation of an infectivity standard for real-time quality control of human immunodeficiency virus type 1 quantitative microculture assays. Participating Laboratories of The AIDS Clinical Trials Group. *J Clin Microbiol.* 1996;34(9):2312-2315.
- Lathey JL, Fiscus SA, Rasheed S, et al. Optimization of quantitative culture assay for human immunodeficiency virus from plasma. *J Clin Microbiol.* 1994;32(12):3064-3067.
- Lathey JL, Marschner IC, Kabat B, et al. Deterioration of detectable human immunodeficiency virus serum p24 antigen in samples stored for batch testing. *J Clin Microbiol.* 1997;35(3):631-635.
- Arens M, Meyer W, Brambilla D, et al. Stabilities of free and complexed human immunodeficiency virus p24 antigens during short and long term storage. *J Clin Microbiol.* 1997;35(9):2413-2416.
- Yen-Lieberman B, Brambilla D, Jackson B, et al. Evaluation of a quality assurance program for quantitation of human immunodeficiency virus type 1 RNA in plasma by the AIDS Clinical Trials Group virology laboratories. *J Clin Microbiol.* 1996;34(11):2695-2701.
- Hammer SM, Katzenstein DA, Hughes MD, et al. A trial comparing nucleoside monotherapy with combination therapy in HIV-infected adults with CD4 cell counts from 200 to 500 per cubic millimeter. *N Engl J Med.* 1996;335(15):1081-1090.
- Katzenstein DA, Hammer SM, Hughes MD, et al. The relationship of virologic and immunologic markers to clinical outcomes after nucleoside therapy in HIV-infected adults with 200 to 500 per cubic millimeter. *N Engl J Med.* 1996;335(15):1091-1098.
- Lathey JL, Hughes MD, Fiscus SA, et al. Variability and prognostic values of virologic and CD4 cell measures in Human Immunodeficiency Virus Type 1-infected patients with 200-500 CD4 Cells/mm³ (ACTG 175). AIDS Clinical Trials Group Protocol 175 Team. *J Infect Dis.* 1998;177(3):617-624.
- Fiscus SA, Hughes MD, Lathey JL, et al. Changes in virologic markers as predictors of CD4 cell decline and progression of disease in Human Immunodeficiency Virus Type 1-infected adults treated with nucleosides. *J Infect Dis.* 1998;177(3):625-633.