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Review

Optimization and validation of the TZM-bl assay for standardized assessments of neutralizing antibodies against HIV-1

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ABSTRACT

The TZM-bl assay measures antibody-mediated neutralization of HIV-1 as a function of reductions in HIV-1 Tat-regulated firefly luciferase (Luc) reporter gene expression after a single round of infection with Env-pseudotyped viruses. This assay has become the main endpoint neutralization assay used for the assessment of pre-clinical and clinical trial samples by a growing number of laboratories worldwide. Here we present the results of the formal optimization and validation of the TZM-bl assay, performed in compliance with Good Clinical Laboratory Practice (GCLP) guidelines. The assay was evaluated for specificity, accuracy, precision, limits of detection and quantitation, linearity, range and robustness. The validated manual TZM-bl assay was also adapted, optimized and qualified to an automated 384-well format.

Contents

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1. Introduction

The process of assay optimization and validation provides assurance that assay results are as reliable as possible and facilitate compliance with Good Clinical Laboratory Practice (GCLP) (Stiles et al., 2003; Ezzelle et al., 2008; Sarzotti-Kelsoe et al., 2009) and the acceptance of data by regulatory agencies. Here we describe the formal optimization and validation of the TZM-bl assay as it is currently performed in the Laboratory for AIDS Vaccine Research and Development at Duke University Medical Center (Duke Laboratory). This assay is widely used for standardized assessments of vaccine-elicited neutralizing antibodies, for studies of monoclonal antibodies and the neutralizing antibody response in HIV-1 infected people, as well as for studies of SIV and SHIV infected non-human primates. The assay measures neutralization as a function of reductions in HIV-1 Tat-regulated firefly luciferase (Luc) reporter gene expression after a single round of infection with Env-pseudotyped viruses (Montefiori, 2009).

Assay optimization determines how a range of test conditions affect assay parameters and performance. Optimization data, along with scientific judgment, are used to set the acceptance criteria for assay validation. Assay validation provides documented evidence that the method is operating accurately and consistently, is sensitive enough for its intended application and is suitable for its intended purpose, i.e. the method is "fit for purpose". Parameters addressed by validation include specificity, precision, linearity, range, accuracy, limit of detection, limit of quantitation, and robustness (Guideline, 2010).

The validated TZM-bl assay was formally transferred to multiple laboratories around the world (Ozaki et al., 2012), preceded by one laboratory, the Vaccine Research Center at the NIH (USA), National Institute of Allergy and Infectious Disease (NIAID) Vaccine Immune T-Cell and Antibody Laboratory (NVITAL), which conducted independent assay validation using the prospectively established acceptance criteria based upon the validation data generated by the Duke Laboratory. The ultimate goal of such independent validation was to demonstrate assay strength and reproducibility, and to perform a further qualification of the assay using an automated 384-well format, designed to provide high throughput results for clinical trial testing. Both laboratories operated in compliance with GCLP guidelines. Combined results of the validation of the manual TZM-bl assay from both Duke Laboratory and NVITAL are presented here, together with the qualification of the assay using an automated 384-well format.

2. Materials and methods

Many of the methods described in this report have been published previously (Montefiori, 2009). Detailed protocols and other supporting materials may be found at http://www.hiv.lanl.gov/content/nab-reference-strains/html/home.htm. The purpose of this report is to describe the key initial elements that went into the formal optimization and validation of the TZM-bl assay and is not meant to be an exhaustive summary of all optimization and validation experiments that have been performed.

2.1. Cell lines

TZM-bl cells (also called JC53BL-13) were obtained from the NIH AIDS Research and Reference Reagent Program (Cat. no. 8129). The TZM-bl cell line is derived from a HeLa cell clone that was engineered to express CD4, CCR5 and CXCR4 (Platt et al., 1998) and to contain integrated reporter genes for firefly Luc and *Escherichia coli* β-galactosidase under the control of an HIV-1 long terminal repeat (Wei et al., 2002), permitting sensitive and accurate measurements of infection. The cells are highly permissive to infection by most strains of HIV, SIV and SHIV, including primary or molecularly cloned viral isolates and molecularly cloned Env-pseudotyped viruses, which provide the advantage of greater reagent stability and traceability. The 293T/17 cell line was obtained from the American Type Culture Collection (catalog no. 11268).

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