IBC Policy: Testing Lentiviral Vectors for Replication Competent Virus

The lentiviral vector (HIV) preps used in animals or for experiments involving sharps must be evaluated for the appearance of replication-competent retrovirus (RCR) using the three independent assays described below:

1. Tat-transfer assay – This assay is based on a reporter HeLa-CD4-LTR-βgal cells containing one integrated copy of the HIV-1 LTR (nts -138 through +80) linked to the β-galactosidase gene. The reporter cell line is highly susceptible to infection. In the case of viral genome recombination that results in the reconstitution of replication-competent HIV-1, the recombined vector will be capable of generating functional tat protein. The tat-expression will lead to activation of the viral LTR-promoter driven the expression of β-galactosidase gene of the reporter cell line. The assay sensitivity has been determined to be as low as 20 tat-transducing units per ml of test medium. The assay is performed as follows: The cells transduced with lentiviral vector are serially passaged three-four times (about 2 weeks), after which the supernatant of the cells is transferred to a reporter HeLa-CD4-LTR-βgal cells. The HIV-1-tat activity is determined by X-Gal (5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside) staining. By this method, vector preparation is considered helper negative when no expression of βgal is detected.

2. Gag-transfer assay – This assay is based on the detection of p^{24}gag-protein of the virus in conditioned media obtained from vector-transduced cells. The cells are serially passaged three-four times (about 2 weeks), after which the supernatant of the cells is collected for assessing the level of p24gag by ELISA (p24 ELISA kit, NIH). The detection limit of this method is ≥100 pg/mL of p^{24}, which is about 10^3 copies of viral genome per mL. By this method, vector preparation is considered helper negative when p24 concentrations are below detection levels.

3. Marker-rescue assay – This assay is based on the direct detection of GFP or other reporter following the transfer. Viral vector stocks are assessed as follows. The cells transduced with lentiviral vector harboring a reporter (GFP) are serially passaged three-four times (about 2 weeks), after which the supernatant of the cells is collected and transferred to HEK293T cells cultured in a 10-cm plate. Seventy-two hours post-transduction, the cells are scored for a reporter expression. Vector stock was considered helper free when no reporter is detected.

References:

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