Quantitative PERT Assay: Method Validation of Real-Time PCR Analysis

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Abstract

Detection of retrovirus in cell substrate is an important issue in quality control of biological products. The biological assay and the RT (reverse transcriptase) assay were most commonly used methods for detecting adventitious retrovirus. Here, we describe the development and application of a sensitive TaqMan fluorescent probe-based product enhanced RT assay for the quantitative detection of RT activity (Q-PERT) including the method validation of Q-PERT assay to ensure confidence in the analytical data according ICH and FDA guidelines. Specificity, accuracy, linearity, precision, range, detection limit and robustness of the method were studied using the commercial RT. After establishing optimal assay conditions, linearity with good correlation coefficients (R^2〉 0.99) were found between the C_T values and the concentrations of RTase in the range of (1.25～ 1.25×10^{-6} Unit/reaction). The limit of detection (LOD) for the assay was ～10^{-6} Units of retrovirus RT activity, corresponding approximately to 1 retrovirus particle in a 1～10 μL sample. LOD result also imply that this assay has enough sensitivity. The precision and the accuracy were satisfactory with good recovery ranges from 91～107%. The level of RT activity in culture supernatant of several cells was determined by validated Q-PERT assay. RT activity was not detected in supernatant from primary cultured immature dendritic cells, whereas it was detected in primary cultured chicken embryonic fibroblast supernatant. These results imply that Q-PERT assay can be used as substitution for conventional RT assays.

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