RAPID VIRUS TITRATION USING FLOW CYTOMETRY

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Rapid high throughput virus titration methods are essential for facilitating continuous process monitoring and rapid decision making in viral bioprocess development. In spite of repeated efforts to address this need, the industry continues to reply on well understood and trusted plaque assays and end point dilution assays, or variations thereof. Together with the University of Waterloo, we have developed a flow cytometry based assay that is able to give infectious virus titers in a fraction of the time as compared to conventional virus titration assays.

The developed method utilizes the phenomenon of increased granularity in cells after virus infection, with the increase in granularity proportional to the multiplicity of infection of the virus. The assay has been adapted to a 96 well plate format which, in combination with the use of a flow cytometer with an automated sampler, results in a high throughput assay with much reduced operator effort as compared to traditional assays.
Two different cell and virus systems have been examined using this assay. Assay variations in both systems were measured to be ~20%, and assay accuracy was highly comparable to traditional "gold standard" assays such as the plaque assay. Assay analysis was found to be simple and amenable to automation through the use of R scripts. Operator effort was reduced by approximately half per sample, and the assay time was reduced by 75%, when compared to traditional assays. In addition, the simplicity of the assay greatly reduces operator training time.

Studies by other groups provide confidence that the phenomenon of increased cell granularity with virus infection is present in several virus-cell systems. Therefore, the developed method has great potential to be used as a routine high throughput screening technique for a wide range of viruses.