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On-Line influenza virus quantification for viral production processes thanks to affinity-based surface plasmon resonance biosensor

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ON-LINE INFLUENZA VIRUS QUANTIFICATION FOR APPLICATION TO VIRAL PRODUCTION PROCESSES THANKS TO AFFINITY-BASED SURFACE PLASMON RESONANCE BIOSENSOR

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Influenza virus seasonal epidemics, associated with the constant threat of new pandemic outbreak, challenge vaccine manufacturers to develop responsive processes that can outreach the limitations of traditional egg-based technology. Recent progress made regarding cell culture bioprocesses allowed for numerous alternative strategies to developed future vaccine candidates, as for example the recombinant HA or Virus—like Particles (VLP) vaccines. However, while cell culture allows for more versatility than ovoculture, regarding process development and monitoring, these alternatives still require optimization to seriously concurrence the traditional process. To drive these developments, WHO and regulatory agencies underlined the need for developing better influenza vaccine potency assays[1,2]. Actual influenza vaccine formulation and lot release rely on single-radial immunodiffusion (SRID) assay, which requires strain-specific reference sera and antigen reagents. However, the annual preparation of these reagents takes between 2 to 6 months and constitutes a critical bottleneck for the release of vaccine lots[3]. Additionally, SRID is not implementable for process development as such technique cannot handle in-process low concentrated and non-purified material. We developed an assay for rapid and label-free quantification of influenza hemagglutinin (HA) antigen and influenza virus based on surface plasmon resonance (SPR). The method is based on affinity capture of hemagglutinin antigen by sialic-acid terminated glycans present at the surface of the fetuin-functionalized sensor. Conditions were optimized for the regeneration of the surface, in order to run multiple sequential analyses on a unique sensor. Two types of purified standard were used during the development of the assay. Commercial trivalent inactivated vaccine ("TIV") has been used for the determination of optimal analytical conditions, while a stock of split inactivated H1N1 virus has been produced and calibrated in our laboratory to study the specific response obtained toward this HA subtype. This assay offers a quantification of influenza hemagglutinin within minutes with a wide dynamic range (30 ng/mL-20 µg/mL). Also, the technique provides a limit of detection (LOD) 100 times lower than SRID, and a better reproducibility than SRID and its potential alternatives recently proposed (<5% RSD vs 6-15%)[1,4,5]. Additionally, the applicability of this assay for an on-line vaccine production monitoring has been validated by off-line measurement of influenza H1N1 virus particles derived from cell culture supernatant. Such a test allowed to achieve a LOD of 10^6 Infectious Viral Particles/mL Thus, our assay provides an innovative tool to evaluate influenza new vaccine bioprocesses, from viral production kinetics in mammalian cell culture to vaccine potency evaluation.

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