Residual DNA analysis in influenza vaccine processing

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Abstract

In cell-based influenza vaccine production, high concentrations of virus are used to prepare a vaccine containing a sufficient amount of virus for the required efficacy. The virus used for vaccine production is usually a recombinant virus, which has a nucleotide DNA sequence of less than 10 ng per dose. To reliably measure residual DNA in both process samples and final vaccine by quantitative PCR (qPCR), DNA preparation prior to analysis is necessary. Samples from the vaccine purification process contain different buffers, salts, and host cell compounds, and vary 3–4 logs in DNA concentration from harvest to the final product, which all put strain on the DNA preparation method.

For accurate determination of DNA concentration, recovery is of high importance. There are many commercially available DNA preparation kits that use different techniques to bind DNA from spin columns with a DNA-binding membrane or resin to magnetic beads. However, these kits are developed mainly for purification of DNA fragments from gel electrophoresis or genomic DNA from tissues such as blood or cultured cells, and do not have recovery as a priority. Few kits are intended for residual DNA determination in samples with high concentration of a protein or virus product.

In this study, prototype chromatography resins for DNA preparation, in capture and batch modes, were evaluated for recovery, hands-on time, and throughput. In batch mode, recoveries of >80% were achieved, but the technique exhibited matrix effects on real process samples. In capture mode, recoveries of 40%–60% were achieved after elution. However, recovery could be improved by concentration determination of DNA bound to the resin.

Results

Resins were screened in capture and batch modes using purified genomic DNA from Chinese hamster ovary (CHO) cells (Fig 2). In capture mode, Captos™ Q and Prototype 3 resins bound DNA well at the two DNA concentrations and in the different buffers tested (Fig 2A). Prototype 3 was further used for DNA preparation from an influenza virus purification process (Table 1). The results showed DNA concentrations similar to when using a commercially available sample preparation kit. Proteinase K treatment did not have any observed effect on the qPCR analysis. In batch mode, Capto Core 700 and Prototype 3 showed a recovery of >80% (Fig 2B). Prototype 3 was selected for further testing using genomic DNA spiked with a mAb at different concentrations in various buffers (Fig 3). PBS and conditions for proteinase K treatment were tested using real samples from a mAb purification process. The results showed low DNA recoveries, probably due to matrix effects from the buffer (data not shown).

Conclusions

- Capture and batch mode sample preparations worked well for samples spiked with purified qDNA.
- Optimization of batch procedure needed for real DNA samples from influenza and mAb purification processes to minimize buffer matrix effects.
- For mAb-containing samples, including a protein A-mAb capture step could be beneficial.
- Using Prototype 3 in DNA preparation, a simple method with high DNA recovery (> 80%) could be obtained.

Introduction

DNA sample preparation can be performed with two different strategies: capture and batch modes, having different pros and cons (Fig 1). The goal with this study was to optimise DNA preparation to be a simple procedure in one tube over maximum 30 min without the need for a centrifugation step. Target DNA preparation to be a simple procedure in one tube over maximum 30 min without the need for a centrifugation step. Target DNA recovery was set to > 80%. The method should be robust and sensitive enough to enable detection of low concentrations of DNA in high concentration of protein.

High risk of matrix effects as buffer is not exchanged
- Quick: 25 min preparation time
- DNA binding increases yield
- No centrifugation
- Assay in one tube

No centrifugation
- Quick: 20 min preparation time
- Assay in one tube
- No elution increases yield
- High risk of matrix effects as buffer is not exchanged

Schematic view of sample preparations tested. (A) Capture protocol: DNA in high concentration of protein. Sensitive enough to enable detection of low concentrations of DNA in high concentration of protein. DNA recovery was set to > 80%. The method should be robust and sensitive enough to enable detection of low concentrations of DNA in high concentration of protein.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Buffer</th>
<th>DNA concentration (ng/mL)</th>
<th>Recovery in FT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>After clarification</td>
<td>PBS</td>
<td>0.3 M NaCl</td>
<td>20</td>
</tr>
<tr>
<td>After chromatography</td>
<td>Protein A-mAb</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Capture protocol for DNA preparation from influenza virus samples (Gibberella-attenuate mAb) propagated in CHO cells. Genomic DNA fromvero cells was used as reference in the analyses.

Conclusions

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