Residual DNA analysis in influenza vaccine processing

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Residual DNA analysis in influenza vaccine processing

Camilla Estmer Nilsson, Hanna Kraft, Christine Sund Lundström, Anna Mattsson, and Elisabet Wallby

GE Healthcare Bio-Sciences AB, SE-75184 Uppsala, Sweden

Abstract

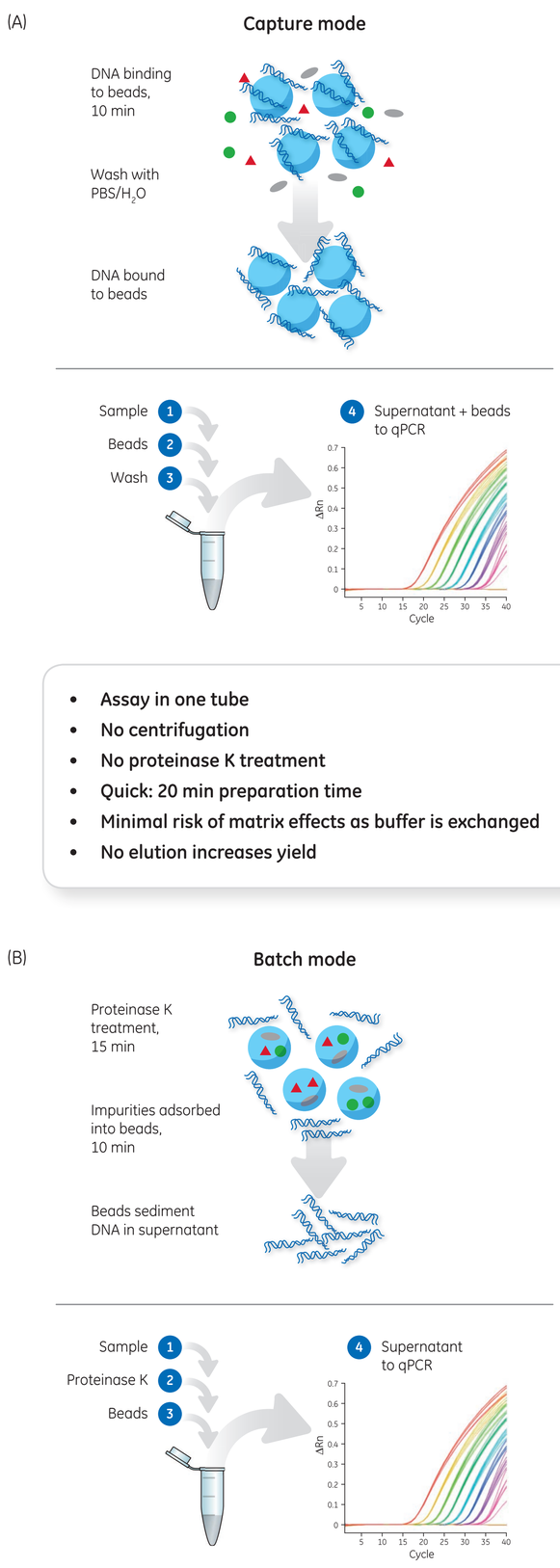
In cell-based influenza vaccine production, the European Pharmacopoeia demands a host cell residual DNA concentration of less than 10 ng per dose. To reliably measure residual DNA in both process samples and final vaccine by quantitative PCR (qPCR), DNA preparation prior to analysis is a necessity. Samples from the vaccine purification process contain different buffers, salts, and host cell compounds, and varies 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.

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 optimize 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.



- **Assay in one tube**
- **No centrifugation**
- **No proteinase K treatment**
- **Quick: 20 min preparation time**
- **Minimal risk of matrix effects as buffer is exchanged**
- **No elution increases yield**

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

Fig 1. Schematic view of sample preparations tested. (A) Capture protocol: DNA is bound to beads, washed, and subjected to qPCR, preferably without elution. (B) Batch protocol: impurities are adsorbed into the beads, while DNA is maintained in the supernatant.

Results

Resins were screened in capture and batch modes using purified genomic DNA from Chinese hamster ovary (CHO) cells (Fig 2). In capture mode, Capto™ 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 1 showed a recovery of > 80% (Fig 2B). Prototype 1 was selected for further tests 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).

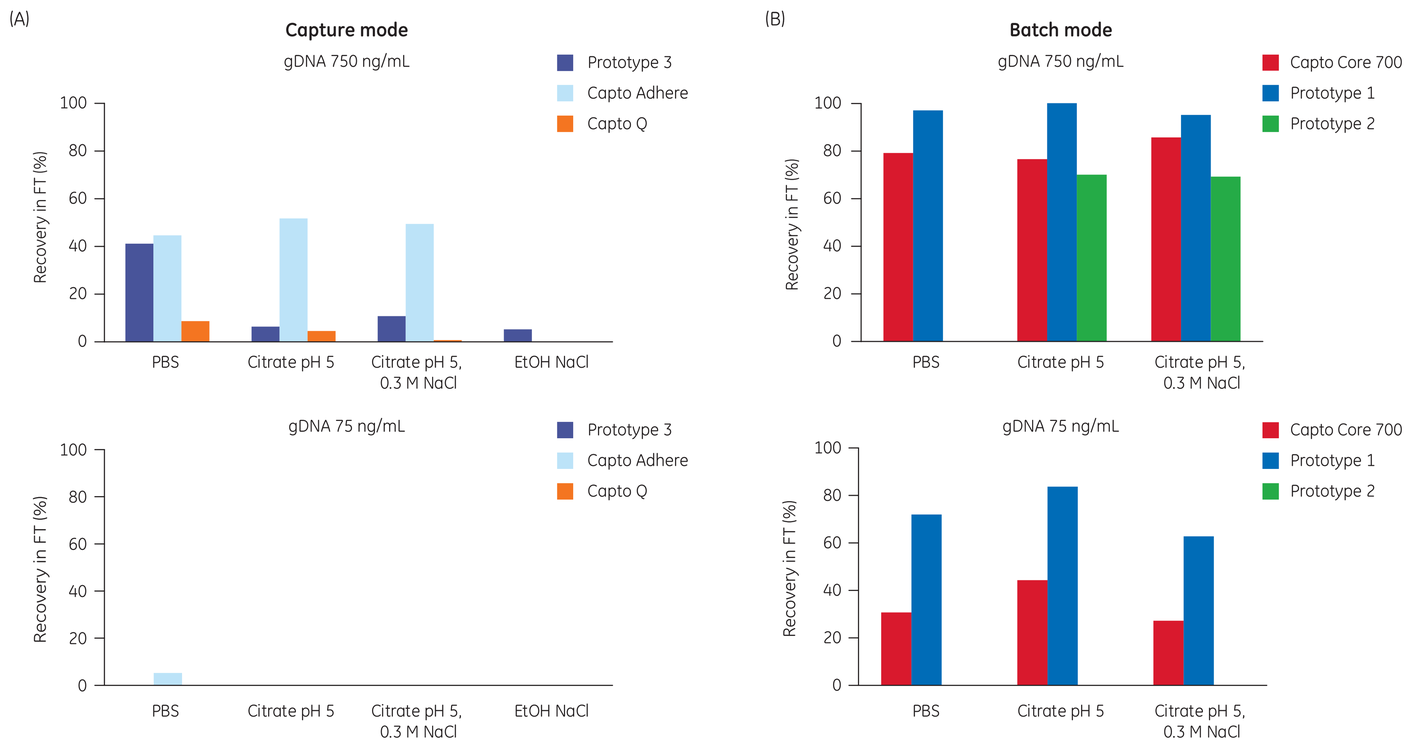


Fig 2. Screening of resins for DNA sample preparation. (A) Capture mode: CHO gDNA is bound to the resin. Low DNA concentration in supernatant indicates high binding. (B) Batch mode: impurities are adsorbed into the beads, while gDNA is maintained in the supernatant. Two concentrations of gDNA were tested: 750 and 75 ng/mL. Analysis of gDNA was performed by qPCR.

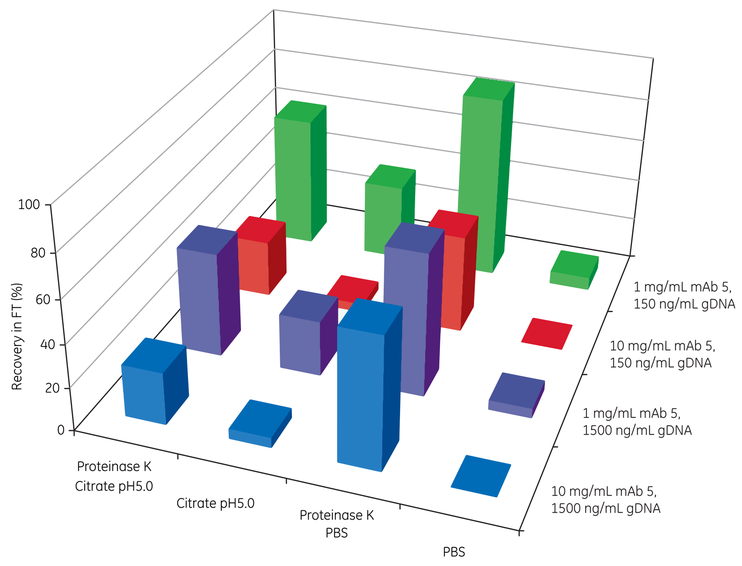


Fig 3. Batch protocol using Prototype 1. Samples used were different combinations of various buffers with varying concentrations of mAb and purified genomic DNA (CHO).

Table 1. Capture protocol for DNA preparation from influenza virus samples (Solomon Islands H1N1 propagated in Vero cells). Genomic DNA from Vero cells was used as reference in the analyses.

Sample	HA concentration (µg/mL)	DNA content Commercial prep kit (ng/mL)	DNA content Prototype 3 (ng/mL)	Proteinase K treatment
After clarification	5.8	530	500	No
After clarification	5.8	530	430	Yes
After chromatography	52	< 2.4	1.4	No
After chromatography	52	< 2.4	1.4	Yes

Conclusions

- Capture and batch modes sample preparations worked well for samples spiked with purified gDNA.
- 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.

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