Best Practices in Nucleic Acid Removal from Vaccine Processes

Frank Appel[1] and Priyabrata Pattnaik[2] *1 Merck Millipore, Frankfurter Strasse 250, Darmstadt, Germany; 2 Merck Pte. Ltd., Biomanufacturing Sciences and Training Centre, Singapore*

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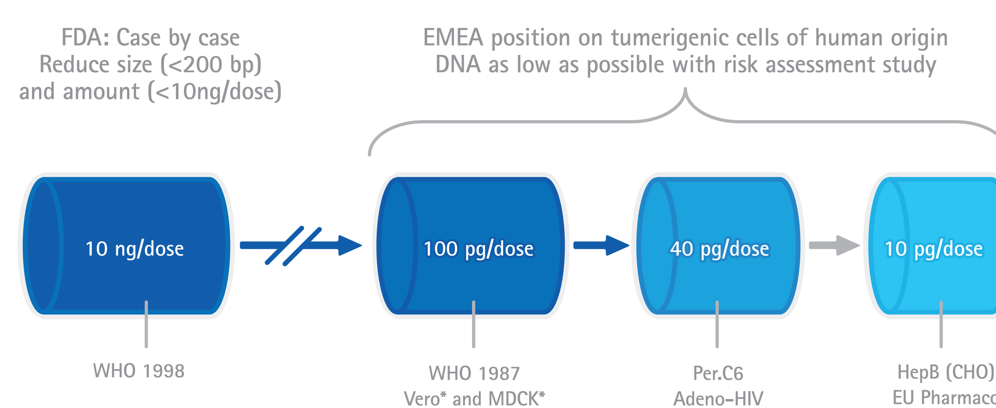
Introduction

The production of viral vaccines at commercial scale requires large quantities of viruses as an antigenic source. There are different production platforms suitable for viral replication – mammalian, avian and insect cells. Host cell residuals in the final product – nucleic acid in particular – create a significant concern due to a potential transfer and integration into the living cell's genetic material, potentially leading to various harmful effects including cancer. Health authorities and regulatory bodies continue to increase safety standards for biopharmaceutical products, providing stringent guidelines on removal of residual nucleic acid from the vaccine manufacturing processes.

Several physicochemical methods exist to reduce nucleic acid during the manufacturing process, each with its unique strengths and weaknesses. One of the methods to reduce nucleic acid contaminants is via enzymatic degradation with endonucleases. It acts on nucleic acid by specifically catalyzing the hydrolysis of internal phosphodiester bonds in DNA and RNA chains breaking them into smaller nucleotides. Smaller nucleotides/nucleic acid fragments, so also the endonucleases, can be easily removed from the process during subsequent downstream processing using ultrafiltration/diafiltration and chromatography. This poster highlights recent advances in nucleic acid removal from vaccine processes.

Regulatory requirement on purity and safety

Residual DNA content



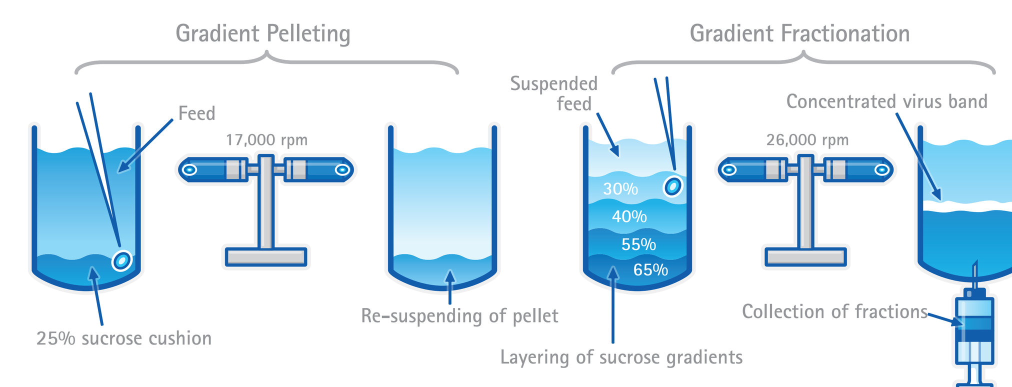
* Non tumerigenic at the passage of production.
** DNA <10 ng/dose commonly accepted

Different methods for residual DNA removal

- Ultracentrifugation
- Precipitation (Acid/base treatment, organic solvent)
  - Example Conjugated polysaccharide vaccine
- Treatment by β-propiolactone (BPL)
  - Example Killed viral vaccine
- Adsorptive Depth Filters (1-2 log reduction of DNA by using Millistak+® HC)
  - Inactivated Polio
- Chromatographic methods
  - Bind and elute (chromatography media)
- Nuclease treatment
  - HepA, LAIV, Rabies, HPV
- Tangential Flow Filtration (TFF)
  - Several vaccines

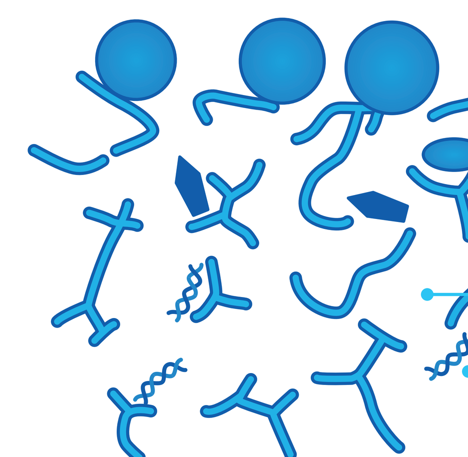
Traditional method for nucleic acid removal

Density gradient centrifugation



Steps	% Overall Flu Recovery	% HCP removal	% DNA removal
Step -1: Gradient pelleting	60	99.9%	67-75%
Step-2: Gradient fractionation	30	Below detection limit	Below detection limit

DNA removal mostly by adsorption-based retention mechanism



- DNA is adsorbed by a combination of electrostatic and hydrophobic interaction
- Not size-dependent
- Adsorptive capacity is limited and "breakthrough" eventually occurs
- DNA adsorption depends on solution composition. pH and conductivity plays a major role

Use of Benzonase® endonuclease for nucleic acid digestion

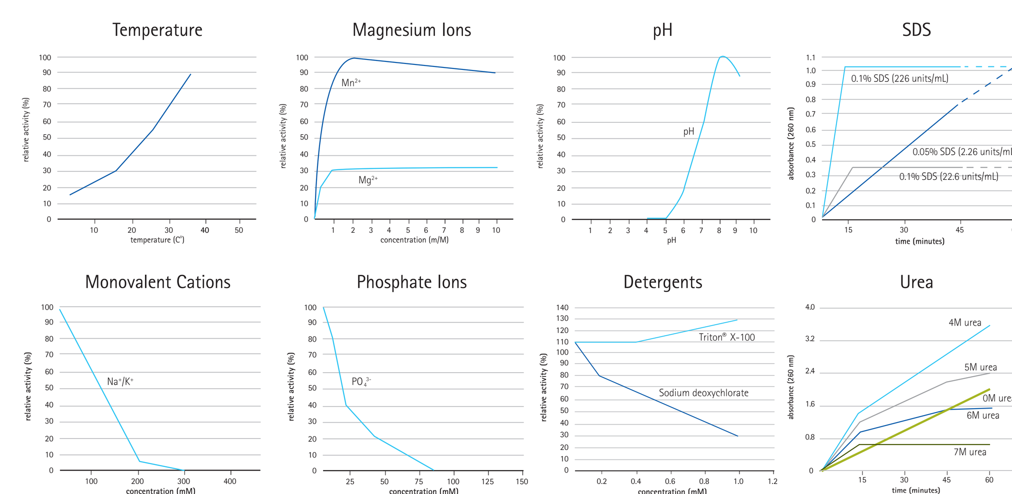
Genetically engineered endonuclease that cleaves all forms of DNA and RNA

Origin	*Serratia marcescens*
Expression	*E.coli* K -12 mutant
Molecular mass	ca. 30 kD (subunit, exist as dimer)
Isoelectric point (pI)	6.85
Functional in pH range	6–10
Temperature	0 - 42 °C

Presence of Mg^{2+} (1-2 mM) is required for enzyme activity.

One unit of Benzonase® endonuclease degrades approximately 37 µg DNA in 30 min to as low as 3–8 base pairs (<6 kDa).

Optimization of Benzonase® endonuclease reaction conditions



Removal and detection of residual Benzonase® endonuclease

Benzonase® endonuclease is not an API or Excipient. Like any other process additive, Benzonase® endonuclease needs to be removed from the vaccine process.

- Benzonase® endonuclease pass in flow through of TMAE, DMAE, or DEAE Fractogel® pH 7-9, 50 – 250 Mm NaCl in 50 mM Tris buffer.
- Benzonase® endonuclease binds to Fractogel SO_3- between pH 4.0 – 6.0, containing 100 – 300 mM NaCl in 20 mM acetate buffer and is not bound to weak cation exchange resin at pH 6.0.
- Benzonase® endonuclease tend to elute from cation exchange resins (Fractogel® SO_3-) at different conditions (salt concentration, i.e., 200 mM NaCl at pH 6.0) resulting in pooled fractions of product of interest (elutions) free from Benzonase® endonuclease.
- Ultrafiltration 300 kDa Biomax® membrane
  - Retains Viral Particle
  - Diafilter out Benzonase® endonuclease and small nucleic acid base pairs

Corresponding Author: frank.appel@merckgroup.com

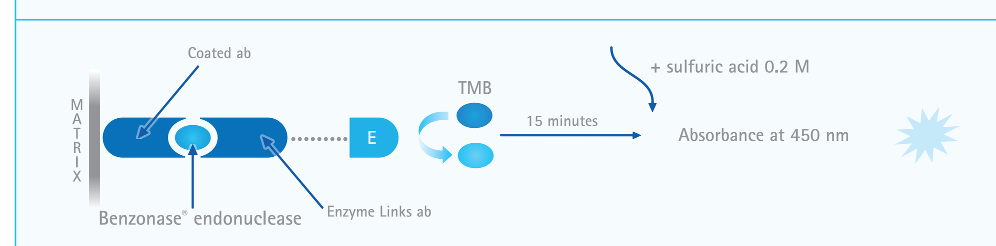
Detection of residual Benzonase® endonuclease

Benzonase® ELISA Kit II

Description: Immunological detection of Benzonase® endonuclease

Sensitivity: ca. 0.2 ng/ml Benzonase® endonuclease (0.2 ng/ml (correspond to < 1 ppm in the presence of other proteins at conc. > 0.5mg/ml.)

Validation: Test method is validated. Nevertheless, vaccine manufacturers also develop orthogonal detection methods that can offer higher sensitivity.

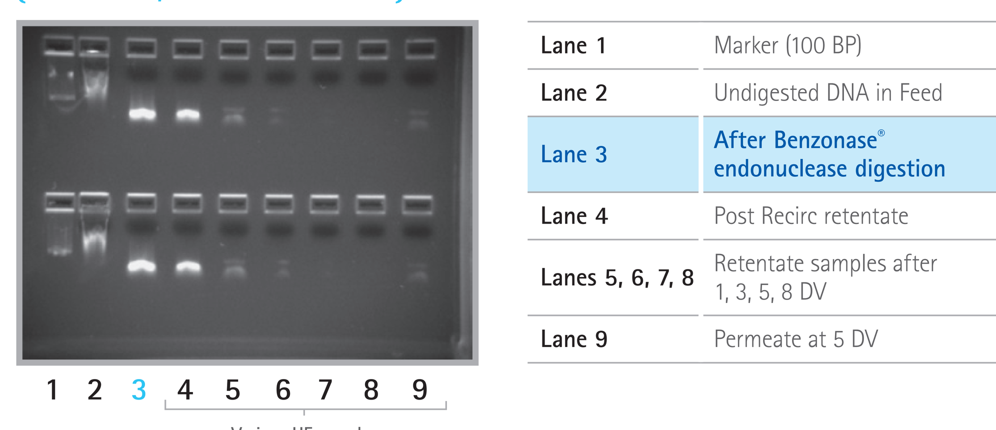


Flow through separation of Benzonase® endonuclease using ion-exchange chromatography

Fractogel® resin	pH	Sample and equilibration buffer
TMAE	7.0	50 mM Tris + 200 mM NaCl, and 50 mM Tris + 50 mM NaCl
TMAE	8.0	50 mM Tris + 250 mM NaCl, and 50 mM Tris + 100 mM NaCl
TMAE	9.0	50 mM Tris + 200 mM NaCl, and 50 mM Tris + 100 mM NaCl
DEAE	7.0	50 mM Tris + 200 mM NaCl, and 50 mM Tris + 50 mM NaCl
DEAE	8.0	50 mM Tris + 250 mM NaCl, and 50 mM Tris + 100 mM NaCl
DEAE	9.0	50 mM Tris + 250 mM NaCl, and 50 mM Tris + 50 mM NaCl
DMAE	8.0	50 mM Tris + 250 mM NaCl, and 50 mM Tris + 50 mM NaCl
SO_3-	6.0	20 mM phosphate + 200 mM NaCl
SO_3-	5.0	20 mM acetate + 700 mM NaCl
SO_3-	4.0	20 mM acetate + 800 mM NaCl
COO-	6.0	20 mM phosphate
COO-	5.0	20 mM acetate + 100 mM NaCl
COO-	4.0	20 mM acetate + 400 mM NaCl

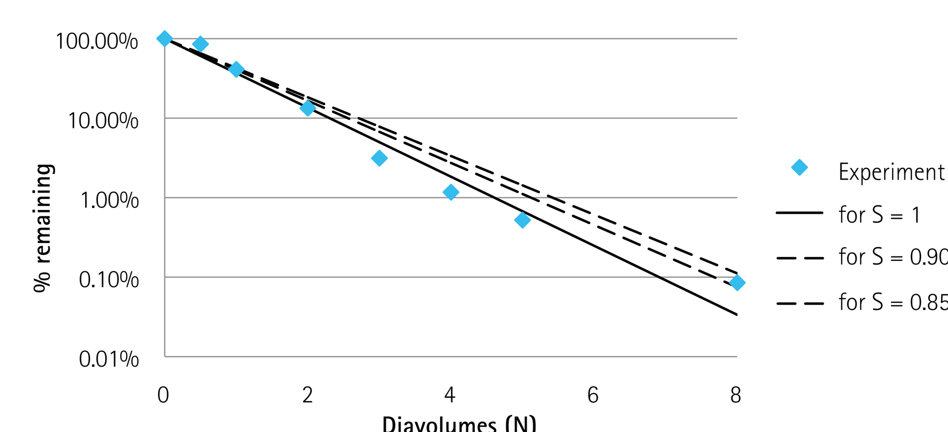
Residual Benzonase® endonuclease and hcDNA removal by TFF

Clearance of Benzonase® endonuclease digested DNA across TFF (Pellicon® 2, Biomax® 300 kDA)



Lane	Sample
Lane 1	Marker (100 BP)
Lane 2	Undigested DNA in Feed
Lane 3	After Benzonase® endonuclease digestion
Lane 4	Post Recirc retentate
Lanes 5, 6, 7, 8	Retentate samples after 1, 3, 5, 8 DV
Lane 9	Permeate at 5 DV

Diafiltration of residual Benzonase® endonuclease



- 99.5% clearance at 5 diavolumes and > 99.9% (3 log) clearance after 8 diavolumes across the UF/DF step

Summary

There are multiple methods for efficient DNA removal from vaccine processes:

- Adsorptive depth filter (Millistak+® depth filters) can be considered as an effective method to remove nucleic acid from vaccine processes
- Benzonase® endonuclease is the proven endonuclease for digestion of nucleic acid in vaccine processes
- Optimization of reaction conditions using Benzonase® endonuclease is critical for the success of DNA digestion
- The combination of Chromatography (Fractogel® resin) and TFF (Pellicon® 2 cassettes) work well for the removal of residual DNA and residual Benzonase® endonuclease
- Multiple analytical methods (Benzonase® ELISA Kit II) are available for quantization of residual Benzonase® endonuclease in final product
- Benzonase® endonuclease is proven as a safe process aid, as it:
  - attacks and degrades all forms of DNA and RNA
  - is free of detectable proteolytic activity
  - is effective over a wide range of operating conditions
  - is manufactured under cGMP conditions in order to meet industrial requirements for a reliable supply and consistent high quality
  - is supported by our EMPROVE® bio dossier and a DMF type II file (FDA Reg. No. BBMF 5403; current version 2013, available in eCTD format)

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