PURIFICATION OF MEASLES VIRUS BY COMBINATION OF SALT-ACTIVE NUCLEASE TREATMENT AND HEPARIN-AFFINITY CHROMATOGRAPHY

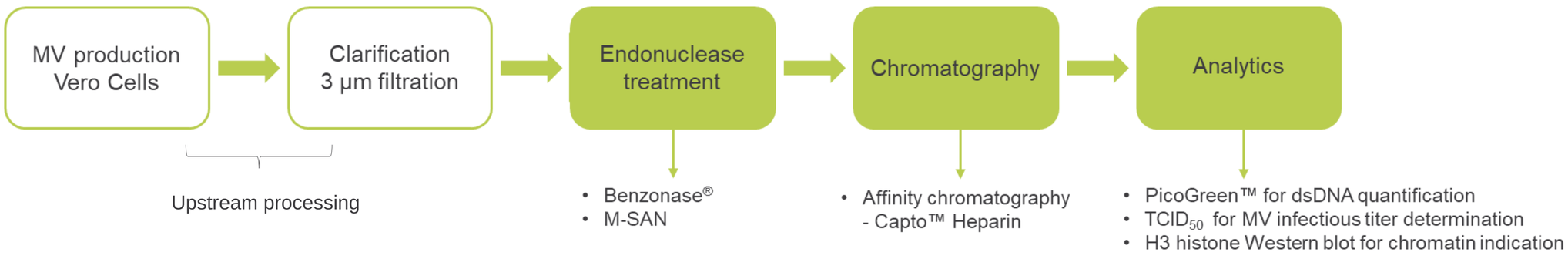
Viktoria Mayer[1,2], Florian Steiner[1], Alois Jungbauer[1,2],Patricia Pereira Aguilar[1,2]

[1] Austrian Centre of Industrial Biotechnology (ACIB), Muthgasse 11, 1190 Vienna, Austria, [2] IBSE, BOKU University, Muthgasse 18, 1190 Vienna, Austria

INTRODUCTION

- Host cell DNA is a critical impurity in downstream processing of enveloped viruses.
- Enveloped viruses have an overall negative net charge and binding properties similar to DNA, making their separation cumbersome.
- In addition to DNA in its naked form, host cell DNA in virus preparations is present in the form of chromatin.
- Chromatin is often similar in size to viruses, complicating their separation.
- We evaluated the performance of heparin-affinity chromatography combined with endonuclease treatment for measles virus (MV) purification.

MATERIALS & METHODS



RESULTS & DISCUSSION

Purification of MV using Heparin affinity chromatography

- **Heparin affinity chromatography allows capture of MV directly from clarified cell culture supernatant.**
- **Partial separation of MV and chromatin can be achieved using a salt linear gradient (Figure 1A).**
- **Endonuclease digestion was performed to improve chromatin removal and MV recovery and purity.**
- **A traditional endonuclease (Benzonase®, Figure 1B) and a salt-active nuclease (M-SAN, Figure 1C) were investigated.**

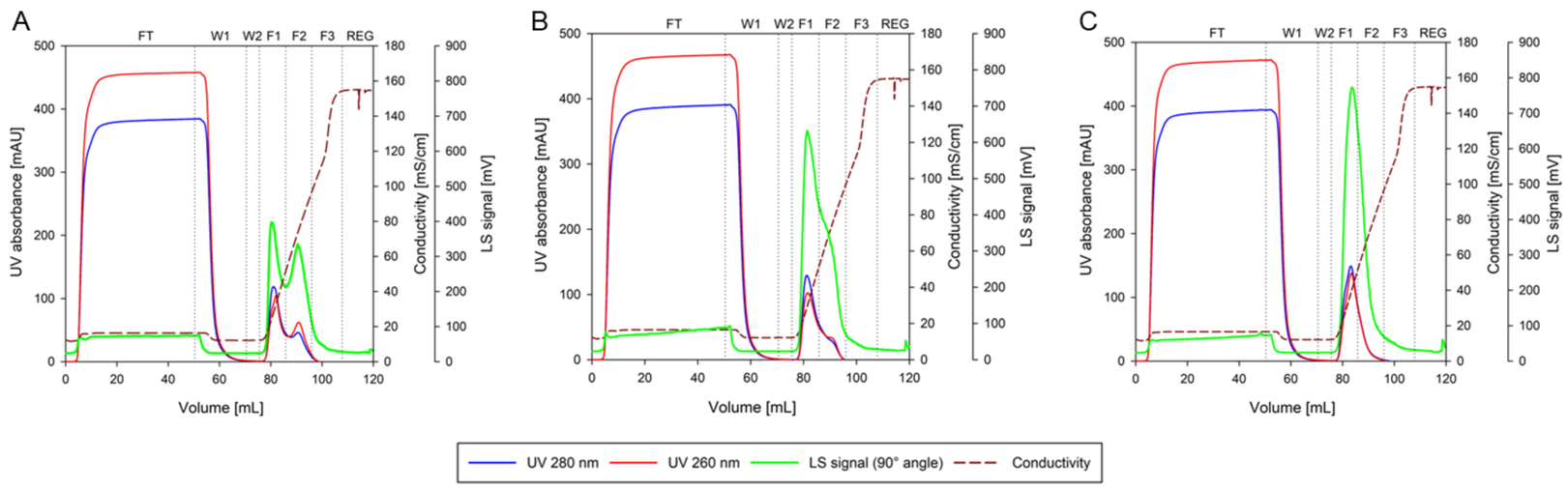


Figure 1: Chromatograms of MV purification using Capto™ Heparin: A: no endonuclease treatment; B: Benzonase® treatment; C: M-SAN treatment

Sample Legend: FT – Flowthrough; W1-2 – Wash; F1-3 – Peak fraction; REG – Regeneration

Yield

Quantification of virus infectivity by $TCID_{50}$

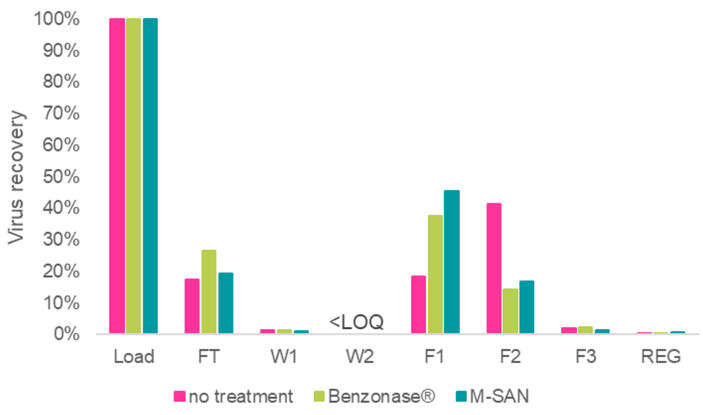


Figure 2: Virus infectivity determination by $TCID_{50}$ for all three purification runs.

Purity

Determination of residual dsDNA (ng dsDNA per 10^5 infectious particles)

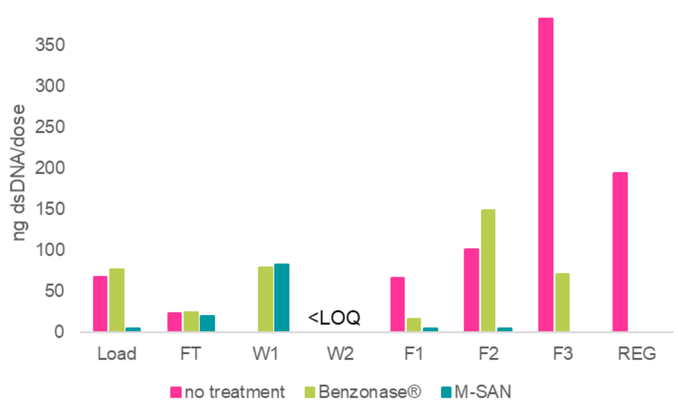


Figure 3: Calculation of ng dsDNA per dose (assuming a dose of 10^5 infectious virus particles).

Identification of MV and chromatin presence

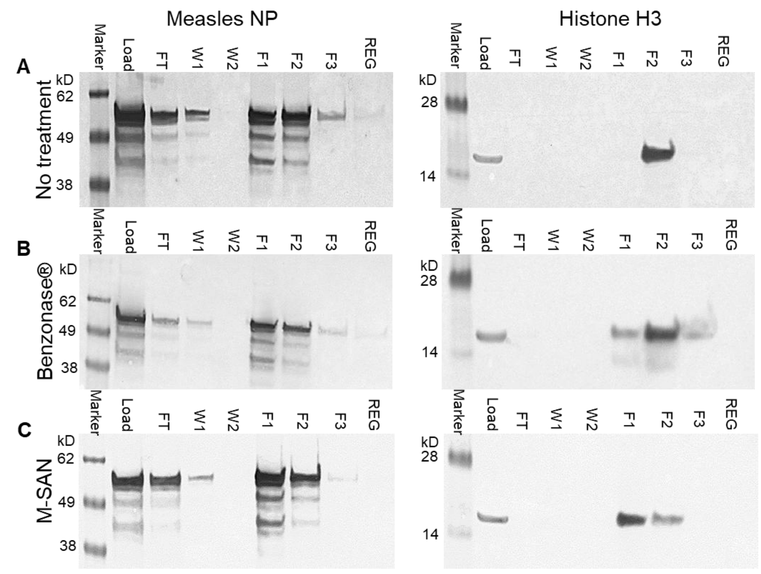


Figure 4: Western blots against MV nucleoprotein (NP) and histone H3 for the respective affinity chromatography after no (A), Benzonase® (B) or M-SAN (C) treatment.

- **Salt-active nuclease (M-SAN) showed higher reduction of dsDNA content in purified samples (Figure 3) and chromatin, indicated by the absence of second LS elution peak (Figure 1C).**
- **The use of salt-active endonuclease treatment resulted in higher yield of measles virus.**
- **Remaining H3 histone proteins after M-SAN digestion (Figure 4C) could be removed in a polishing step.**

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CONCLUSION

Additional questions? Send me an e-mail: viktoriamayer@acib.at

- M-SAN treatment of MV supernatant increased both, purity and yield, in a single purification step.
- As the developed method is simple and scalable it could be integrated in a downstream process train for measles virus manufacturing.

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