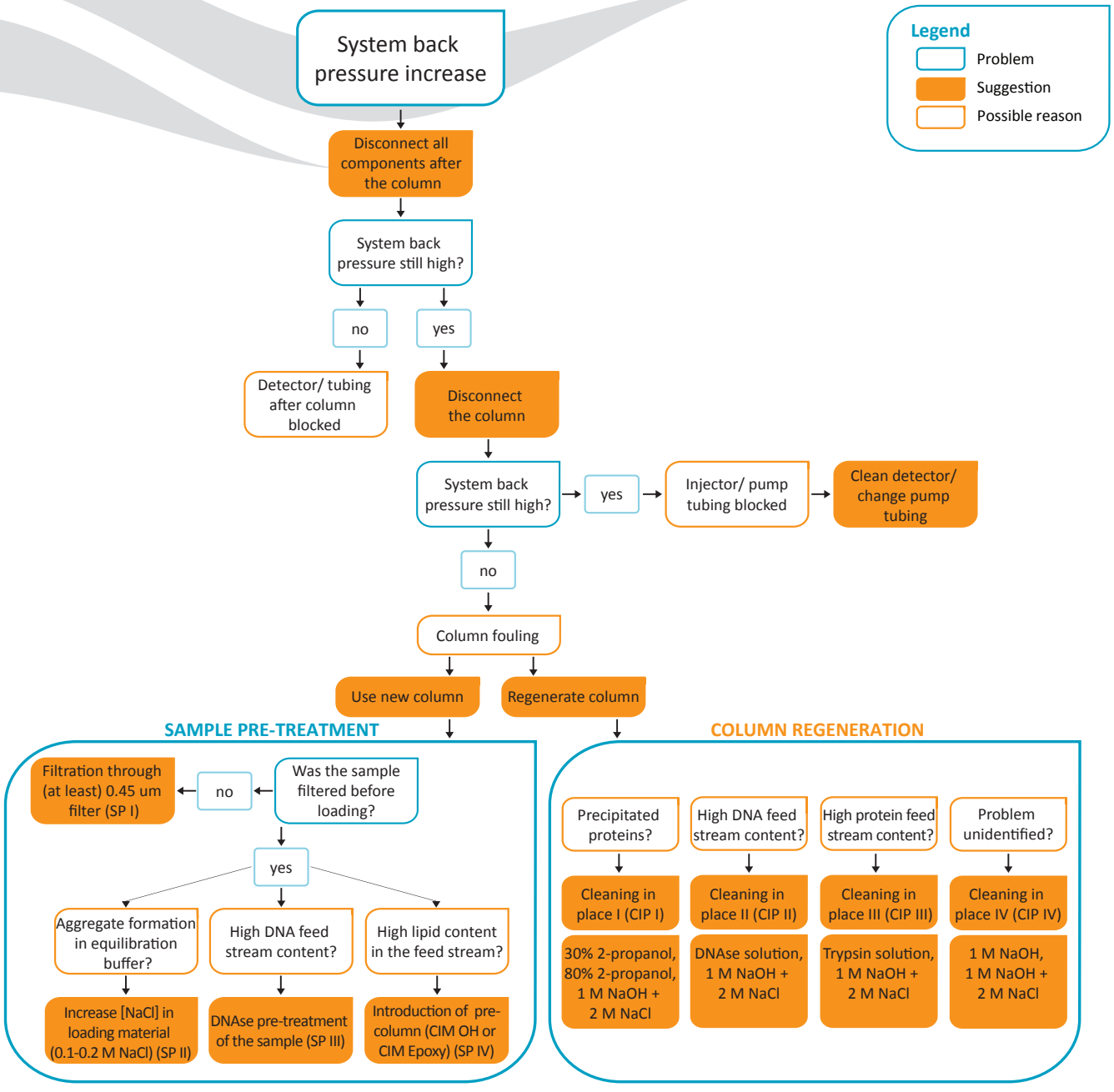
CIM Convective Interaction Media®
GUIDELINE

Pre-treatment of complex biological samples before column purification and regeneration procedures for columns with increased backpressure

It is a general recommendation to carefully consider pre-treatment steps that your sample undergoes before column purification. For viruses, VLPs, phages and other large biomolecules, pre-treatment is essential (suggestions below) to prevent an increase in backpressure and to avoid unnecessary cleaning-in-place procedures.

> Figure 1

Troubleshooting scheme for system/column back pressure increase during/after virus material purification on CIM® monolithic column



Pressure increase on the column can be efficiently prevented by performing one of the following pre-treatment steps. It is recommended to optimise the selected pre-treatment step for your particular sample type, as introducing sub-optimal pre-treatments into downstream purification scheme can lead to a decrease in overall recovery. In order to maximise the recovery of your target molecule, we recommend that one of the following methods be tested:

SAMPLE PRETREATMENT:

SP I: Filtration of virus material

- 0.45 µm filtration of virus material is recommended; for larger viruses (e.g. MVA), a filter with larger pore size should be used (e.g. 0.8 µm or 1.0 µm filter).
- Cellulose Acetate (CA) filters typically offer the lowest unspecific binding, but different filter materials can be screened to find the optimal one.
- Virus loss due to filtration is minimal, but depends on the filter material, filter pore size and size of the virus.

SP II: Addition of NaCl into virus material

- NaCl in the loading material and equilibration buffer minimizes virus aggregation and/or formation of complexes between virus particles and impurities.
- Binding conditions for a specific virus need to be determined first; typically 0.1-0.2 M NaCl can be used without compromising virus binding to the matrix.
- Addition of NaCl into equilibration buffer and loading material decreases non-specific binding (mostly proteins) and increases the dynamic binding capacity for the target virus.

SP III: DNase (e.g. benzonase) treatment of virus material*

- Applicable for virus feeds that contain high concentrations of host cell (HC) DNA.
- A likely consequence of DNase treatment before column purification is a change of DNA elution profile, leading to co-elution of the DNA fragments with the target virus, therefore the chromatographic method should be optimized accordingly.
- DNase treatment should be followed with filtration or ultrafiltration/diafiltration (UF/DF) step. The UF step removes small DNA fragments and the DF step leads to buffer exchange to the buffer which will be used for column purification of virus material.
- Procedure: DNase is added to the virus material (e.g. 50 U/500 µg total proteins, 10 mM $MgSO_4$) and incubated at 37 °C, followed by filtration (0.45 µm) and adjustment to the equilibration IEX conditions (e.g. UF/DF). Note: optimal DNAse concentration and treatment conditions should be determined according to the DNase used (DNA activity specified by the manufacturer) and HC DNA content in the virus material.

SP IV: Use of pre-columns*

- CIM® Epoxy or CIM® OH pre-columns protect the main column and the column lifetime is prolonged.
- Procedure: CIM® Epoxy or OH column are used in negative mode, therefore the flow through fraction from the pre-column is collected, adjusted to the loading condition for CIM® IEX column (if necessary) and used for virus purification.

***NOTE:** See the example of MDCK cell-derived influenza A virus purification (Figure 2).

COLUMN REGENERATION:

When increased column back pressure is observed after purification of the virus material, one of several cleaning in place (CIP) procedures can be applied:

CIP I: Organic solvent followed by 1 M NaOH + 2 M NaCl

NOTE: Organic solvent CIP is appropriate only for IEX columns, and should be avoided with C4 chemistry.

STEP 1: 30% 2-propanol

i. Record column pressure (using the equilibration buffer) at the following flow rates: 0.5, 1 and 2 CV/min.
ii. Wash the column with 20 CVs of deionized water.
iii. Wash the column with 20 CVs of 30% 2-propanol (prepared with deionized water) with the flow rate of 0.5-1 CV/min.
iv. Wash the column with deionized water (20-50 CV).
v. Wash the column with equilibration buffer (until the pH and the conductivity of the buffer at the outlet are the same as for the equilibration buffer).

vi. Record the pressure drop on the column at the same flow rates as used in i).
vii. If the pressure does not decrease to the initial level (pressure on the column before the first use), continue with Step 2.

STEP 2: 80% 2-propanol

i. Wash the column with deionized water (20 CVs).
ii. Wash the column with 20 CVs of 80% 2-propanol (prepared with deionized water) with the flow rate of 0.5-1 CV/min.
iii. Wash the column with deionized water (20-50 CVs).
iv. Wash the column with equilibration buffer (until the pH and the conductivity of the buffer at the outlet are the same as for the equilibration buffer).
v. Record the pressure drop on the column at the same flow rates as used in i).
vi. If the pressure does not decrease to the initial level (pressure on the column before the first use), continue with Step 3.

STEP 3: 1 M NaOH + 2 M NaCl

i. Wash the column with deionized water (20 CVs).
ii. Wash the column with 20 CVs of 1 M NaOH + 2 M NaCl with the flow rate of 0.5-1 CV/min.
iii. Leave the column in in the cleaning solution for 2 hours (up to 12 hours if needed).
iv. Wash with 20 CVs of a concentrated buffer (e.g. 0.1 - 0.5 M buffer) in order to restore the appropriate pH.
v. Wash the column with equilibration buffer (until the pH and the conductivity of the buffer at the outlet are the same as for the equilibration buffer).
vi. Record the pressure drop on the column at the same flow rates as used in i).

CIP II: Benzonase treatment followed by 1 M NaOH + 2 M NaCl

i. Record column pressure (using the equilibration buffer) at the following flow rates: 0.5, 1 and 2 CV/min.
ii. Wash the column with 10 CVs of benzonase solution (50-200 U of benzonase/mL of 50 mM Tris buffer + 0.1 M NaCl + 2 mM MgCl2, pH 7.4) with the flow rate of 0.5-1 CV/min.
iii. Leave the column in the benzonase solution for 2 hours at 37 °C (disconnect the column from the HPLC system, seal it with »end fittings« and incubate at 37 °C).
iv. Re-connect the column to the HPLC system.
v. Wash the column with equilibration buffer (20-50 CVs).
vi. Record pressure drop on the column at the same flow rates as used in the first point.
vii. If the pressure does not decrease to the initial level (pressure on the column before the first use), continue with the NaOH/NaCl step (described in STEP 3 of CIP I protocol).

CIP III: Trypsin treatment followed by 1 M NaOH + 2 M NaCl

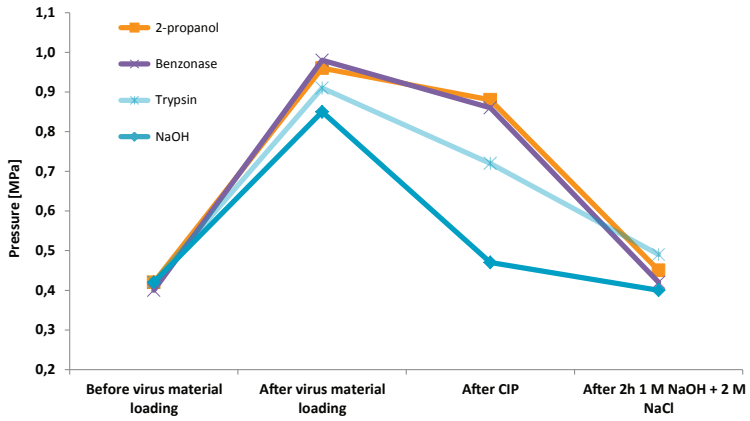
i. Record column pressure (using the equilibration buffer) at the following flow rates: 0.5, 1 and 2 CV/min.
ii. Wash the column with trypsin solution (5%, w/v) at room temperature at flow rate of 0.1 CV/min. Contact time should be 2-5 minutes.
iii. Wash the column with equilibration buffer (20-50 CVs).
iv. Record pressure drop on the column at the flow rates as used in i).
v. Compare pressure drop on the column before and after the trypsin treatment.
vi. If the pressure on the column does not decrease to the initial level (pressure drop on the column before the first use), continue with the NaOH/NaCl step (described in STEP 3 of CIP I protocol).

CIP IV: 1 M NaOH followed by 1 M NaOH + 2 M NaCl

i. Wash the column with deionized water (20 CVs).
ii. Wash the column with 20 CVs of 1 M NaOH with the flow rate of 0.5-1 CV/min.
iii. Leave the column in in the cleaning solution for 2 hours (up to 12 hours if needed).
iv. Wash with 20 column volumes of a concentrated buffer (e.g. 0.1 - 0.5 M buffer) in order to restore the appropriate pH.
v. Wash the column with equilibration buffer (until the pH and the conductivity of the buffer at the outlet are the same as for the equilibration buffer).
vi. Record pressure drop on the column at the same flow rates as used in i).
vii. If the pressure on the column does not decrease to the initial level (pressure drop on the column before the first use), continue with the NaOH/NaCl step (described in STEP 3 of CIP I protocol).

> Figure 2

An example of different CIP protocols applied after purification of MDCK cell-derived influenza A virus on CIM QA 1 mL column. Loading material: 15 mL of non-filtered influenza virus material, diluted 1:2 (v/v) with 50 mM MES + 0.1 M NaCl, pH 6.7.



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