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A method for concentration and purification of human coronavirus HCoV-OC43 using CIM QA monolithic columns

Human coronavirus OC43 (HCoV-OC43) is a frequent cause of respiratory tract illness, ranging from common cold to severe disease. The research on coronaviruses and medical application of coronaviral vectors/vaccines requires a quality material of high purity. Unfortunately, virus preparations are highly contaminated with cell debris and purification requires laborious, cost-ineffective procedures.

Here, we report a simple and efficient method for coronavirus concentration and purification by the example of HCoV-OC43. To achieve this, virus chromatography was performed on CIM QA monolithic columns (BIA Separations), with immobilized positively charged quaternary amines. The quality of the obtained virus stock was assessed with SDS Page electrophoresis, followed by Western blot analysis. Finally, infectivity of recovered virus was evaluated by titration (Reed e Muench, 1938).

SAMPLE PREPARATION

HCoV-OC43 was propagated in human ileocecal colorectal adenocarcinoma cells (HCT-8) in DMEM, supplemented with 2% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were maintained at 37°C, while the infection was carried out at 32°C in an atmosphere containing in 5% CO_2. Six days post infection 100% confluent cells in a 75 cm^2 flask were lysed by 2 freeze and thaw cycles. Mock-infected HCT-8 cells were used as a negative control.

RESULTS

Column:	CIM® QA-1 Tube Monolithic Column (Quarternary amine) (Pores 1.3 um)
Column chemistry:	Strong anion exchanger; quaternary amine (QA)
Instrumentaion:	Syringe
Mobile phases:	Buffer A: 100 mM NaCl in 20 mM phosphate buffer, pH = 6.5 Buffer B: 2 M NaCl in 20 mM phosphate buffer, pH= 6.5
Loading material preparation:	Collect virus-containing cell lysate and medium was centrifugated (4,500 × g at 4°C, 5 min), supernatant was then filtrated through Ø 45 µm PES syringe filter. The virus stock was concentrated (from 10 ml to 2 ml) on a centrifugal filter unit (MWCO = 10 000 Da; e.g., Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-10 membrane UFC901008 from Merck Millipore) at 4,500 × g at 4°C for 5 min
Column loading volume:	5 mL
Flow:	Column washes and virus elution was generated using a syringe
Method:	Step gradient elution: 35% -100% – 2M NaCl in MPB

At each step a sample of material is collected. Noteworthy, in the presented experiment there were 5 Buffer A wash samples (1 ml each; fractions 13-17), 4 35% Buffer B elution samples (2.5 ml each; fractions 18-21), and 7 100% Buffer B elution samples (1.4 ml each; fractions 22-28).

All of the samples were thermally inactivated (5 min at 96˚C) and analyzed by SDS Page electrophoresis and Western blot for the presence of viral proteins.

Western Blot Analysis

Samples were separated on the SDS-Page gel and subsequently proteins were transferred onto the PVDF membrane at 100 V for 2 h. The membrane was blocked with 5% bovine serum albumin in TBST buffer for 2 h at room temperature. Next, virus nucleocapsid protein was immunostained with primary mouse anti-coronavirus antibodies and secondary anti-mouse rabbit polyclonal antibodies conjugated with horseradish peroxidase. Chemiluminescence was visualized using photographic film exposed for 5 min to the membrane developed with Immobilon Western Chemiluminescent HRP Substrate. The resulting pattern was analyzed with ImageJ.

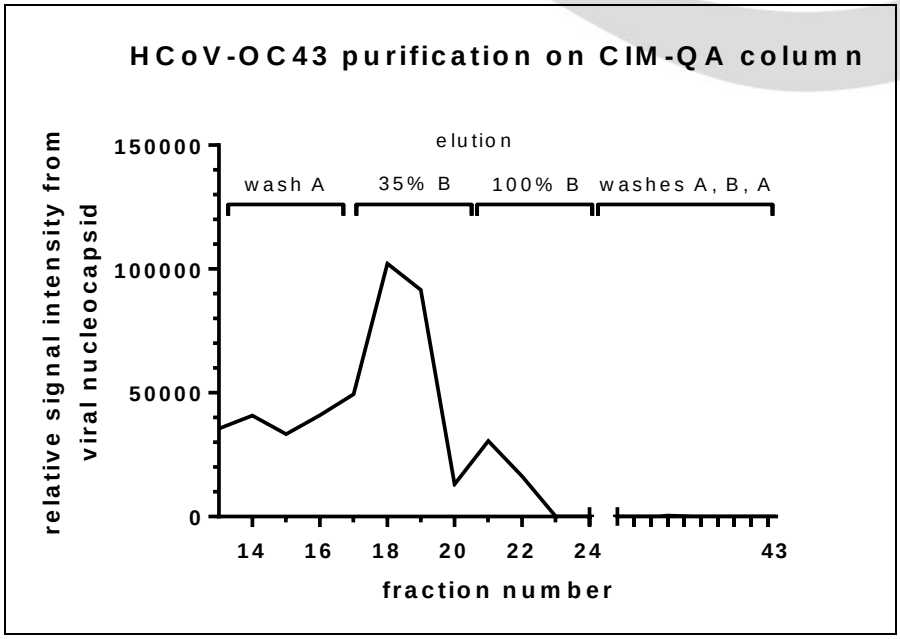

Figure 1: 1 HCoV-OC43 purification of CIM QA column. The relative quantity of the HCoV-OC43 protein was assessed by densitometry and is presented on y axis. The wash and elution fractions are presented on the x axis.

One may hypothesized that the signal obtained from the first wash with buffer A corresponds to the nucleocapsid protein released from lysed cells, while the following two peaks (C_{max} at fraction 18 and 21, respectively) corresponds to intact virions purified by CIM-QA chromatography.

SDS Page

Second, the purity of virus-containing fractions was assessed by SDS Page electrophoresis. As shown in Figure2, only fractions 18, 21 and 22 contained significantly less contaminants than the control sample of the non-purified initial stock.

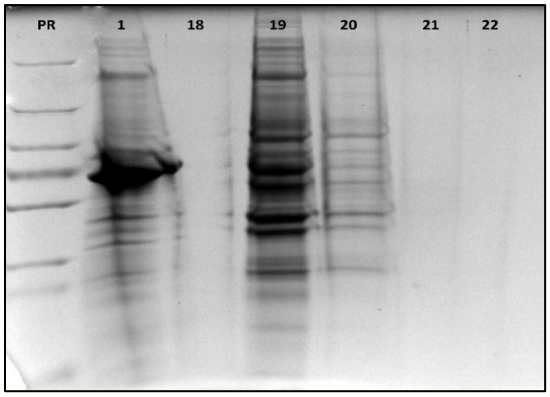

Figure 2: SDS Page electrophoresis of the virus-containing fractions eluted from CIM QA column. PR – PageRuler prestained protein ladder (Thermofisher Scientific); 1 - non-purified HCoV-OC43 stock; 18-21 – 4 samples of the elution with 35% Buffer B; 22 – sample of the first fraction from elution with 100% Buffer B

Purified coronavirus titration

Third, the HCoV-OC43 titration assay was performed. Briefly, confluent HCT-8 cells were cultured in 96-well plates. Serial fivefold dilutions of the chosen purified virus samples were prepared in DMEM supplemented with 2% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin, and 100 µl of the diluted virus was added into each well. The cells were incubated at 32°C under 5% CO_2 for 5 days. Afterwards, the number of wells, where cytopathic effect was noted was scored and $TCID_{50}$ values were calculated according to the Reed–Muench formula. Obtained results are presented in Table 1.

Fraction number	$TCID_{50}$
1 (control)	346 000
18	346 000
19	190 000
20	70 000
21	14 000
22	70 000

Table 1: TCID50 assessment of the chosen fractions eluted from CIM QA column

The highest concentration of infectious HCoV-OC43 particles was found in the first fraction of 35% elution (fraction 18) and was gradually decreasing within the remaining fractions. However, along with the change to 100% elution there went a slight increase in the infectious virus yield in fraction 22, which was also considered the purest, according to Figure 2.

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