Sanitization of ÄKTA flux 6 cross flow filtration system

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Sanitization of ÄKTA™ flux 6 cross flow filtration system

This application note describes the sanitization of ÄKTA flux 6 cross flow filtration (CFF) system (Fig 1). The system was tested for the efficiency of microbe removal using 0.5 M sodium hydroxide (NaOH) as sanitization agent.

For the challenge test, ÄKTA flux 6 was equipped with a Kvick Lab™ filter holder and a cleaning-in-place (CIP) dummy. The system was challenged with high concentration of *E. coli* for 16 to 20 h at room temperature. Before sampled, the system was sanitized with 0.5 M NaOH. The results fulfilled the acceptance criteria of the study, indicating a highly effective sanitization procedure.

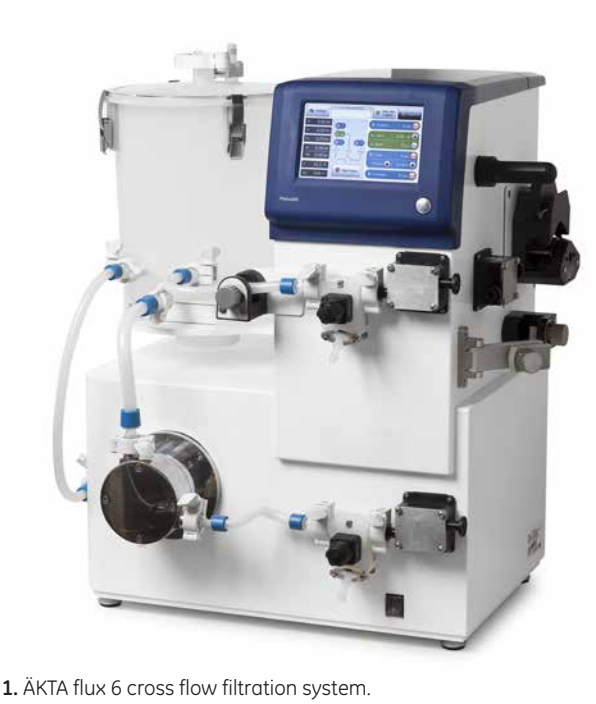

Fig 1. ÄKTA flux 6 cross flow filtration system.

Introduction

As products processed with the ÄKTA flux 6 might be used for clinical applications, stringent levels of purity and present microorganisms are required. With sanitization, high hygienic standards can be achieved. This application note describes sanitization using NaOH, a widely used and inexpensive cleaning agent commonly used for cleaning of filters and filtration systems.

Sanitization

Sanitization is the use of a chemical agent to reduce a microbial population to an acceptable, predetermined level. Efficient sanitization of filter systems is required to minimize the risk of contamination of the end product. According to good manufacturing practice (GMP), the efficiency of sanitizing agents should be evaluated, and this is generally performed using microbial challenge tests.

Challenging testing

The principal of challenge testing is to introduce high concentrations of microorganisms into the equipment to be investigated. The equipment is incubated with the challenging organisms for a specific time, after which it is treated with the sanitizing agent. Following treatment, samples are taken at predetermined sites, and the numbers of viable cells remaining are calculated. The objective of this study was to achieve results that fulfill acceptable, predetermined criteria. Here, a high concentration of challenging organism was used (1×10^9 viable organisms/mL) to mimic a typical bacterial concentration process performed on CFF systems.

Materials and methods

Microbial challenge test

The materials used for the microbial challenge test are listed in Table 1.

Table 1. Materials used for microbial challenging test

Equipment	ÄKTA flux 6 equipped with the Line tubing kit (0.375 i.d./0.625 o.d.) and a Kvick Lab filter holder with a CIP dummy
Challenging organism	*E. coli* (Gram-negative bacteria) ATCC 8739 (1×10^9 viable organisms/mL)
Sanitizing agent	0.5 M NaOH
Solutions	Ethanol (70%), sterile water Sterile NaCl (9 mg/mL)
Sampling materials	Peptone water (pH 7.2) Tryptic soy broth (TSB) Tryptic soy agar (TSA) Melted tryptic soy agar Alginate swabs in isotonic solution Sterile 0.45 µm cellulose nitrate membrane filters Sterile NaCl (9 mg/mL)

Test method 1. Viable count

Samples of challenging organism suspension were diluted and incubated on TSA plates at 30°C to 35°C for 1 to 2 days, after which colony forming units (CFU) were counted and the number of viable microorganisms in the samples calculated.

Test method 2. Swab

Surface samples were taken with alginate swabs. The swab was inserted into the tube containing the isotonic swab rinse solution. After dissolution, the whole solution including the swab tip was poured into molten TSA, which was subsequently let to solidify in petri dishes. The plates were incubated at 30°C to 35°C for 5 days before the number of CFU was counted and the number of viable cells in the sample was calculated.

Test method 3. Peptone water filtration

Detachable system parts were aseptically removed, placed in 50 mL sterile peptone water, and vigorously shaken for at least 20 min. The solutions were filtered through a 0.45 µm cellulose nitrate membrane filter, which was washed with 100 mL sterile NaCl solution (9 mg/mL) and incubated on TSA plates at 30°C to 35°C for 5 days, after which CFU were counted and the number of viable microorganisms in the sample calculated.

Test method 4. Direct filtration

Sample solutions collected in sterile tubes were filtrated through a 0.45 µm cellulose nitrate membrane filter. The filter was washed with 100 mL sterile NaCl solution (9 mg/mL) and incubated on TSA plates at 30°C to 35°C for 5 days, after which CFU were counted and the number of viable microorganisms in the sample calculated.

Preparation of bacterial suspension

E. coli ATCC 8739 (stored in freezer) was grown on a nutrient agar plate at 30°C to 35°C for 18 to 24 h. Organisms were inoculated in 20 mL TSB and incubated in 30°C to 35°C for 5 to 6 h. A 1 mL sample of the culture was further inoculated in approximately 500 mL TSB and incubated in 30°C to 35°C for 18 to 24 h before centrifuged 10 min at 4500 rpm. The pellets were suspended in sterile peptone water. The suspension was diluted in sterile peptone water to obtain 10^9 CFU/mL. Microorganism concentration was determined by viable count (see Test method 1. Viable count).

Preparation of the system

As a precleaning process, detachable parts (i.e., the outer parts of the pump, membrane of the pressure sensor housing, pressure sensor housing, as well as the tank and drain valves) were removed, disassembled and soaked in 1 M NaOH for 20 to 24 h, rinsed with sterile water, and left to dry in a laminar flow (LAF) hood before assembly. Cassette filter holders were treated in the same manner as detachable parts before assembly. The tank was rinsed with sterile water, sprayed with 70% ethanol, and left to dry in a LAF hood before assembly. The pump parts not removed from the system (i.e., the pistons and diaphragm) were sprayed with sporicide (Klercide™-CR Biocide, Shield Medicare) and left for 1 h before carefully wiped with sterile water and thereafter with 70% ethanol before assembly.

Assembly

A filter dummy was placed in the Kvick Lab filter holder connected to the system and the vent filter and the pressure relief valve were detached from the tank lid. A 2 m tubing was attached to the tank lid, replacing the pressure relief valve, and to the permeate pressure sensor (tubing can be attached to upper permeate port on the Kvick Lab filter holder if permeate pressure sensor is not used), enabling recirculation of the sanitization solution from the permeate port to the tank.

A second tubing was attached to the top of the tank lid (replacing the vent filter), using a reducer (i.e., a plastic 51 mm TC to 25 mm TC connector). This tubing was used as an air inlet during filling of the tank and enabled overfilling of the tank during sanitization.

Drain tubing was connected to the upper and lower drain valve on ÄKTA flux 6 and a drain tubing with a valve closing the tubing (a pinch clamp can also be used) was connected to the lower permeate port on the Kvick Lab filter holder.

To pressurize the system during sanitization, the tube between permeate and tank was placed in the permeate pressure control valve and restricted during sanitization.

Pretreatment

The system was reassembled and the tank was filled with approx. 10 L of 1 M NaOH solution and left for 18 to 24 h. Before challenged, the system was drained and rinsed with sterile water at a feed flow of 8.8 L/min (CIP flow) until pH neutral as measured in the sterile water flushed through the drain valves and the permeate tubing.

Challenge

The ÄKTA flux 6 system was challenged with *E.coli* suspension at a feed flow of 3 L/min. The suspension was recirculated in the system at a pressure of 2 bar for 3 min and the drain valves and permeate outlet were flushed through with the suspension. After recirculation, the pump was stopped and the challenged ÄKTA flux 6 system was incubated for 16 to 20 h at room temperature before sanitization.

Sanitization procedure

The sanitization run started by repeatedly rinsing the system (e.g., overfilling the tank and then draining the system through the permeate tubing and drain valves) with sterile water until the outflow was visually clean. Thereafter, the system was rinsed with 0.5 M NaOH before recirculated with one tank volume of 0.5 M NaOH for 2 h at 2 bar. The valves were rinsed with approx. 400 mL 0.5 M NaOH. A feed pump flow of 1600 rpm using the system CIP feature was used throughout the whole process. When using the CIP feature of ÄKTA flux 6, the feed pump will run at maximum speed as part of the CIP process. The system was rinsed with sterile water before sampling.

Table 2 summarizes the general sanitization procedure.

Table 2. Summary of sanitization process

Sanitization step	Solution	Volumes (L)	Feed pumpflow rate (L/min)	Tank filling
1	Sterile water	~ 24	8.8	The tank was overfilled by pumping the solution trough the transfer inlet.
2	0.5 M NaOH	20 (2 × 10)	8.8	
3	0.5 M NaOH (recirculation 2 h at 2 bar)	~ 10 (tank overfilled)	8.8	
4	Sterile water	~ 24	8.8	

Microbial testing

Samples for microbial testing were taken at several predetermined sites on ÄKTA flux 6 and Kvick Lab filter holder (Fig 2, 3). Concentration of challenging organism in the permeate tubing flowthrough was determined by viable count (see Test method 1. Viable count) after challenge as well as before and after the sanitization procedure.

Criteria for acceptance

For the sanitization study, the number of microbial challenging organisms was to be reduced from 1×10^9 CFU/mL to ≤ 9 CFU/mL or sampling location. In addition, remaining organisms at ≤ 5% of sampling locations were allowed, and no more than 10% of the test points were allowed to be contaminated (i.e., show growth of other organisms than the challenging organism).

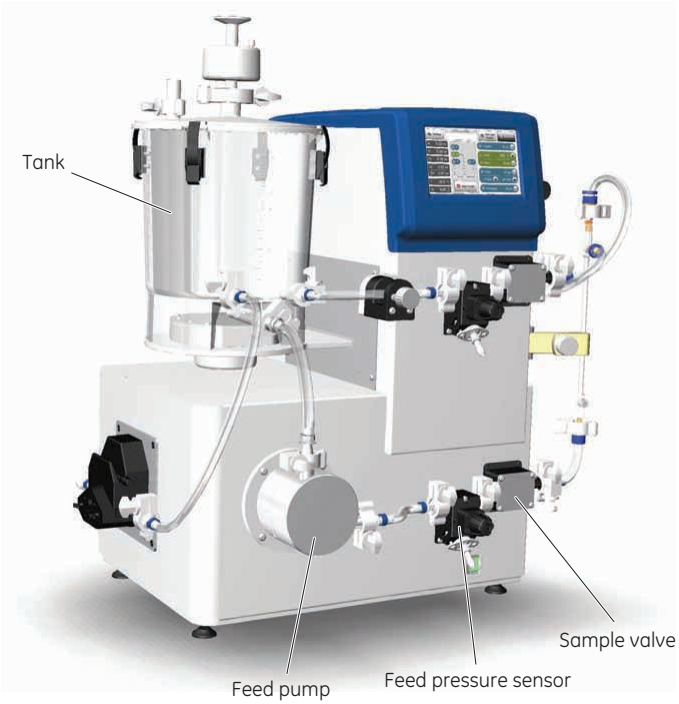

Fig 2. General location of the predetermined sampling sites on ÄKTA flux 6.

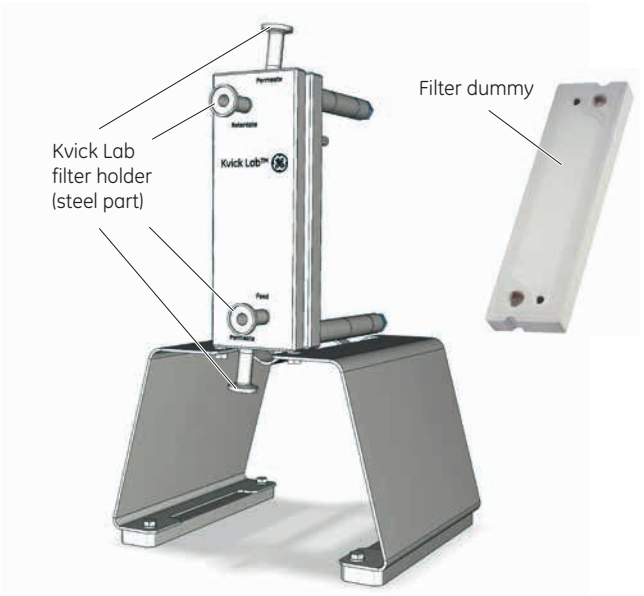

Fig 3. General location of the predetermined sampling sites on Kvick Lab filter holder.

Results

Table 3 shows the number of CFU after challenge as well as before and after sanitization. Table 4 shows the number of CFU at the sampling sites after sanitization. Despite the high initial amount of the challenging organism, the results fulfilled the acceptance criteria of the study.

Table 3. Number of *E.coli* CFU in permeate tubing flowthrough after challenge and before and after sanitization

Sanitization phase	Test method	Run 1 (CFU/mL)	Run 2 (CFU/mL)
Inoculum sample (start concentration)	1	1.4×10^9	0.8×10^9
Post challenge (flowthrough sample after challenge)	1	1.9×10^9	0.9×10^9
Presanitization (flowthrough sample before sanitization)	1	1.4×10^9	1.4×10^9
Post sanitization (flowthrough sample after sanitization)	4	0	1*

* Sample contained 7 CFU/50 mL (< 1 CFU/mL) of challenging organism and fulfills acceptance criteria ≤ 9 CFU/mL or sampling location.

Table 4. Number of *E.coli* CFU after sanitization

Sampling site	No. of sampling points	Test method	Run 1 (CFU/mL)	Run 2 (CFU/mL)
Sample valve	9	2	0*	0
Feed pressure sensor	7	2	0*	0
Tank	3	2	0	0
Feed pump	17	2, 3	0†	0
Kvick Lab filter holder (steel part)	12	2	0	0†
Filter dummy	1	2	0	0

* One sample was contaminated with other organism than *E.coli.*

† Two samples were contaminated with other organism than *E.coli.*

Conclusion

The performed study shows that ÄKTA flux 6 can be efficiently sanitized by recirculation of 0.5 M NaOH for 2 h at a pressure of 2 bar. Despite the high initial level of challenging organism, the results obtained with this sanitization procedure fulfilled the acceptance criteria of the study. However, system design alone cannot guarantee good hygienic status in a filtration process. Methods like those described here should always be applied in conjunction with other well-designed and carefully controlled hygienic routines, together with rigorous control of buffers, water, and other input material.

Ordering information

Product	Quantity	Code number
ÄKTA flux 6 system	1	29-0384-38
Transfer pump	1	29-0940-19
Permeate control pump	1	29-0946-76
Tank assembly	1	29-0946-80
Line tubing kit	1	29-0946-81
Kvick Lab holder II	1	29-0946-74
Pressure sensor	1	29-0951-52
Drain valve kit	10	29-0979-55

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