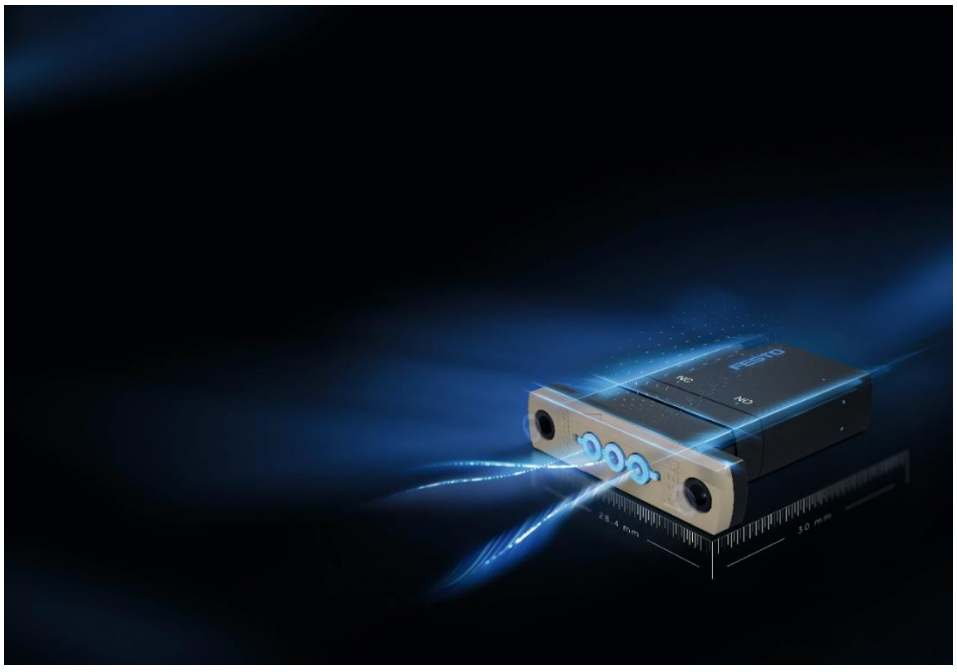
Application Note

FESTO

Cleanability of media-separated solenoid valves in application with bacteria, proteins and other biological compounds

Cleaning of bacteria, endotoxins and TOC shown using the example of contamination with *E.coli* bacteria suspension and a commercially available PCR-Kit

VYKA
VYKB
VYKC
VAVN
VABS



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1 Material compatibility and low dead volumes

Media-separated valves are ideal for use with aggressive media. The mechanics of the valve are protected from the medium by a diaphragm so that the medium only is in contact with the diaphragm, which is made of high-performance polymers. The high material resistance and low dead volumes make these valves ideal for handling biological substances such as cells or proteins. Examples of possible application areas range from downstream processes such as protein purification, analytical methods such as PCR analysis, up to the dosing of liquids such as media, acids and bases into the bioreactor. Here, low dead volumes and high material resistance to aggressive chemicals are of great importance for cleaning.

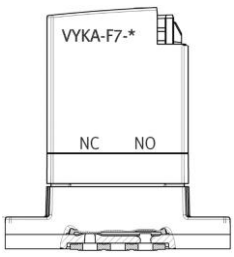
Media-separated diaphragm valves from Festo, such as the VYKA or VYKC, can be used flexibly in a wide range of applications thanks to their FDA-listed materials. Depending on the application, you can choose between three different diaphragms, FKM, FFKM and EPDM. Together with the sub-base made of PEEK, all materials in contact with the product are highly resistant to strong cleaning chemicals such as ethanol or sodium hydroxide. Further material compatibilities can be found in our Festo media resistance table at [Media Resistance | Festo DE].

Product contacting components

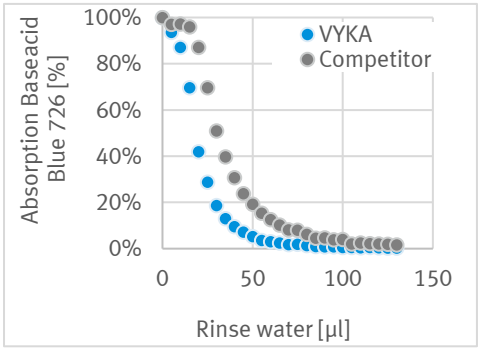
VYKA VYKB VYKC VABS VAVN

The compact design with low dead volumes ensures good flushability. This minimizes the risk of biofilms.

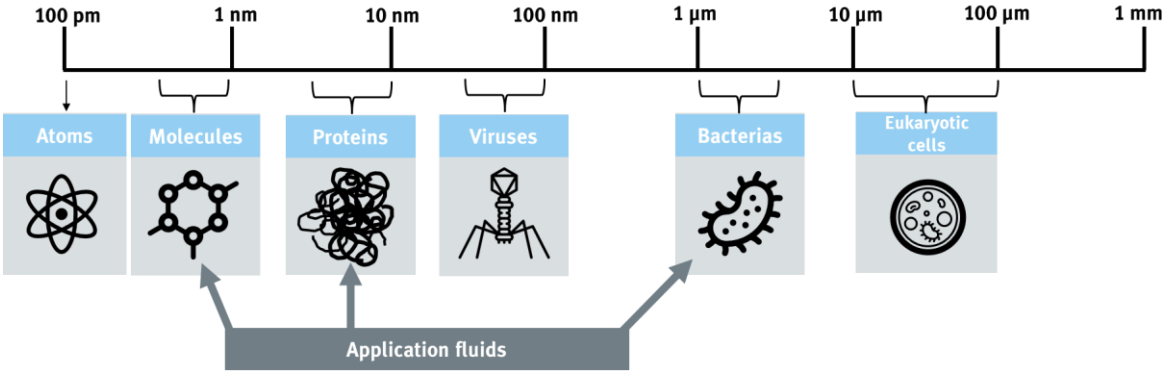
Excellent rinsability!



Flow behavior that wets the entire fluid chamber at high speed



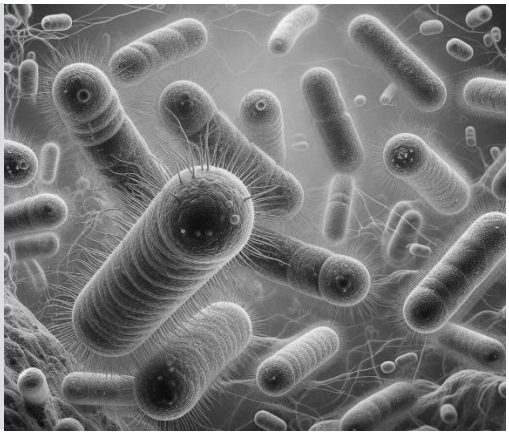
In addition to the good design and flushability, tests have shown that the valves are cleanable against bacteria, proteins and other biomolecules. To this end, the valves were contaminated with various biomolecules at different size levels and tested for cleanability. The success of the cleaning was evaluated using detection methods based on the European Pharmacopoeia (Ph. Eur.).



2 Cleanability after contamination with bacteria und endotoxins

For the contamination at cell level *Escherichia coli (E. coli*) was used. It is one of the most biochemically researched organism in science.

Escherichia coli - a bacterium at home in the human intestinal tract - has contributed to many ground-breaking discoveries and today serves as an important tool in industry and research.



The bacterial suspension was rinsed into the valves and then cleaned using a suitable cleaning process (Fig. 1). Ethanol (EtOH) was used to clean the bacterias and endotoxins. Doint that, all test valves were exposed to a specific incubation time.

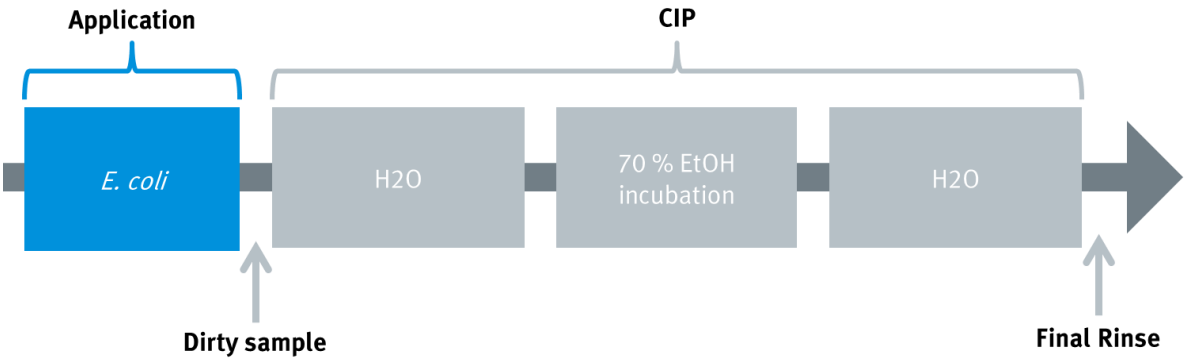


Fig. 1 Cleaning process for handling *E. coli*.

The success of the CIP process (Cleaning in Place) was evaluated based on criteria used in the pharmaceutical industry. For this purpose, the final rinse was subjected to the measurement of certain parameters as the last rinse step. To measure the total bacterial count, the final rinse volume was drawn through a membrane, which was incubated on agar plates for several days at the appropriate temperature. On different agar culture media, the final rinse was analyzed for aerobic bacteria - total aerobic microbial count (TAMC) and fungi and yeasts - total yeast and mold count (TYMC). Subsequently, the colony-forming units (CFU) grown on the plates were counted to determine the total bacterial count. Some bacteria have endotoxins in their cell walls, which can trigger fever and other undesirable physiological reactions in the human body even in very small quantities as a decomposition product. Due to these harmful effects, this parameter is also crucial for evaluating the success of

cleaning in the pharmaceutical industry. As another important measurement parameter, conductivity provides information about residual ionic components, such as salts in buffer solutions or alkaline and acidic cleaning substances.

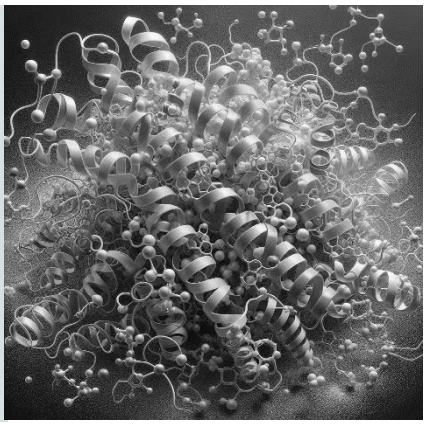
Test valves	Total bacterial count TAMC [CFU/100 ml]	Total bacterial count TYMC [CFU/100ml]	Endotoxins [EU/ml]	Conductivity [µS/cm]
VYKA 2/2	0	0	‹0,01	0,97
VYKA 3/2	0	0	0,04	1,07
VYKB F10 2/2	0	0	‹0,01	0,97
VYKB F10 3/2	0	0	0,2	1,06
VYKB F12 2/2	0	3	‹0,01	0,67
VYKC 2/2	2	1	‹0,01	0,96

The results of the total bacterial count for both TAMC and TYMC show no colonies for most of the test valves. The values for endotoxins and conductivity are also in a very low range for all valves. All results in the last rinse step, the final rinse, are below the official limits for water for injection (WFI), which is used in the pharmaceutical industry for drug production. With these very good results, it can be shown that the valves can be cleaned when handling bacteria such as E. coli.

To test the cleanability of organic molecules on a smaller scale than bacterial cells, the valves were exposed to other organic compounds and tested for cleanability.

3 Cleanability after contamination with proteins and other organic compounds

A conventional PCR kit containing a master mix of enzymes, nucleotides, buffer solutions and dyes was used for contamination at the protein level.



The organic compounds can be detected via the TOC value. The value is non-specific and an extremely important parameter, as it indicates the total amount of carbon compounds in a sample.

The PCR kit was rinsed into the valves and then cleaned using a purification process (Fig. 2). Sodium hydroxide (NaOH) and sodium hypochlorite (NaOCl) were used to clean the proteins, nucleotides and other organic substances in the PCR kit. The test valves were filled with the cleaning reagents and exposed to an incubation period.

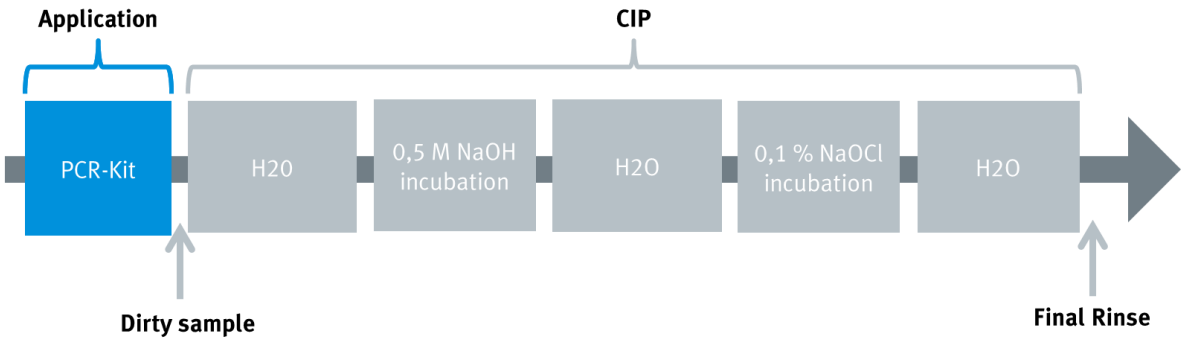
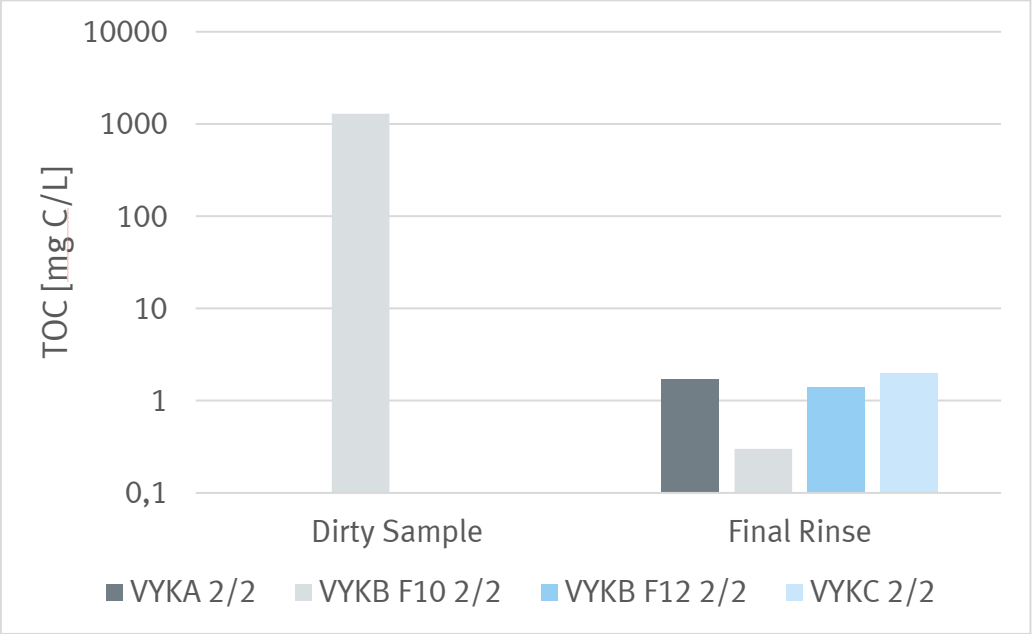


Fig. 2 Cleaning process for handling a commercially available PCR-Kit Ready Mix.

The success of the CIP process was evaluated based on criteria used in the pharmaceutical industry.

Using VYKB F10 2/2 as an example, at first a contaminated sample was taken as a reference and then the “final rinse” sample was taken after cleaning. The measured TOC value after the cleaning procedure shows a removal of organic compounds by a factor of around 1000

compared to the reference. Besides VYKB F10 2/2 also the other valves show very low TOC values around 1 mg C/l in the final rinse sample.



4 Cleanability in application with biological compounds

Due to the advantageous design without dead volumes and the media resistance of the components in contact with the product, very good results were achieved in the cleaning of biological substances such as bacteria and endotoxins as well as proteins and other organic compounds.

Thanks to evaluated cleaning processes, the components are generally suitable for use with cells, proteins, dyes and other organic substances and can be cleaned. However, it should be noted that successful cleaning is heavily dependent on the individually selected cleaning process of the corresponding assembly, which is significantly influenced by 4 basic factors: Time, mechanics, chemistry and temperature. Ultimately, cleaning processes must therefore always be tested and evaluated in the context of the actual application.