Safe work with fluid aspiration systems in cell culture

White-Paper

Safe and contamination-free handling of cell culture supernatants

vacuubrand

Technology for Vacuum Systems

SAFE AND CONTAMINATION-FREE HANDLING OF CELL CULTURE SUPERNATANTS

INTRODUCTION

The removal of supernatants from culture vessels is a typical and repetitive daily routine for many researchers. For small sample sizes, it can be a purely manual procedure using micropipettes. However, for larger sample volumes vacuum based techniques with the appropriate accessories are much more efficient and therefore provide a more effective workflow.

Depending on demand, or existing conditions, a vacuum source can comprise either a vacuum network or a decentralised single or multi-user solution. It is not uncommon for users to make their own makeshift solutions using a range of different components. It is important not to underestimate the risks with regards to biological safety posed by such non-standard equipment and to prevent the possible spread of contamination between cell cultures, in the laboratory and within the laboratory building.

In particular in laboratories with a low risk level, too little attention is usually given to this aspect. But even here there are sources for biological hazards and contamination. Ubiquitous germs often pave the way for pathogenic micro-organisms. The greater the occurrence of viable counts of micro-organisms in the laboratory, that is in the air, on surfaces of laboratory equipment, in the tube, on hands, etc., the more likely that this will need to be assessed critically. (3)

Class II biological safety cabinets protect the laboratory staff and the organisms in the work against contamination from germs and aerosols. For safe and contamination-free removal of cell culture supernatant, compact, semi-automatic vacuum systems are now the preferred method.

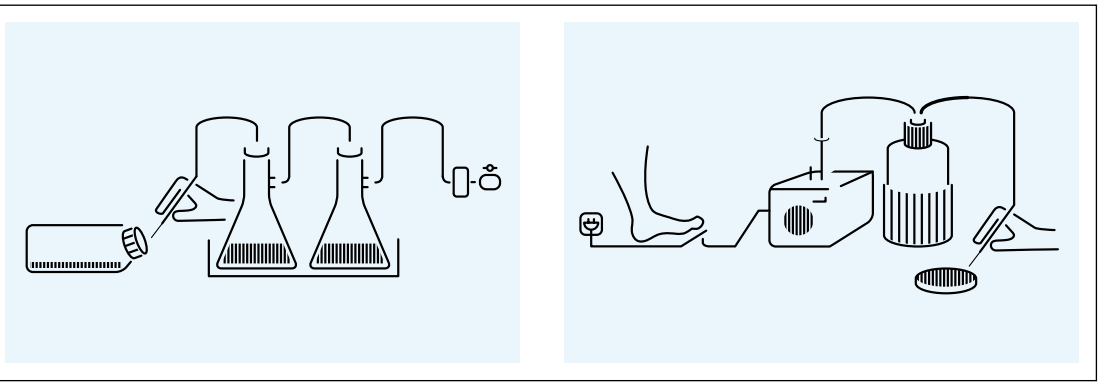
These systems should take into account all safety-related characteristics and as far as possible, allowing for no errors in the application.

1. TRADITIONAL PROCEDURES AND SYSTEM WEAKNESSES

In some cell culture laboratories, the supernatant fluid is simply emptied into a waste container, a glass beaker or even an old media flask. This is absolutely unacceptable from a sterilisation technique point of view, because tiny droplets and aerosol spray can go unnoticed. These aerosols are a completely unpredictable source of contamination, as it is the cell culture supernatant itself that can be contaminated with bacteria, yeasts or Mycoplasma. (1)

In the case of makeshift vacuum based solutions, which are often found in laboratories, there is a greater security risk associated with the use of such unsuitable, non-standard equipment and also from adhoc procedures regarding special hazards which are not coordinated, often comprise differing components as well as a lack of user-friendliness and the associated error-prone operation in the process related to the release of bioaerosols.

Complex regulations are often not complied with in practice

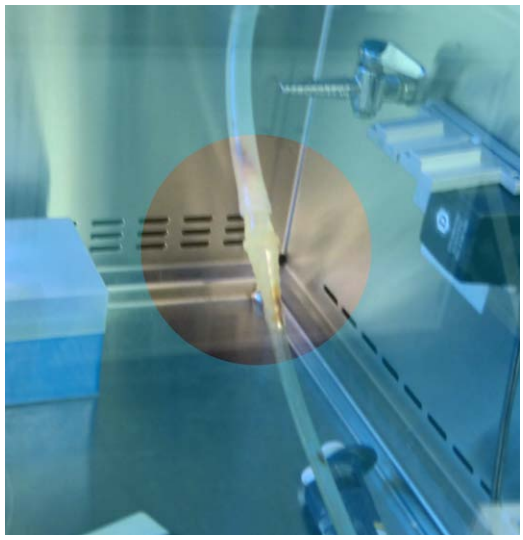


1.1 THE RELEASE AND SPREAD OF CONTAMINATION

Badly connected hoses and connectors can lead to leaks and microbial growth in the joints. The use of slightly ill-fitting connections in combination with a lack of awareness, ultimately results in a reduction of the general reliability of the process. Any leaking connections, as well as accidental faults can, especially in the case of biotechnology applications in the laboratory, lead to an uncontrolled spill of biologically critical material.

By not preventing the backflow of the waste media, cells or germs can spread between the individual samples. Where metal tip components are used in the aspiration system there is a risk to the internal parts from crusting and also to their stability from constant flaming - another possible failure in the workflow process.

In practice, these fragile receptacles without overflow protection and with a low stability often cause contamination of the work and surrounding area.



Microbial Growth on hose connections

Faulty construction: no filters, no overflow protection, no handle, no vacuum limitation

1.2 AEROSOL LOAD AT THE PROCESS EXHAUST

Inevitably in the course of the vacuum generation process air is emitted at the outlet of the vacuum pump.
In not having a safety protection filter between the collection containers and the vacuum source there is a very real risk that the aerosols from the extracted medium can enter into the vacuum system and as a result into the process exhaust air. In cell culture techniques, when working with microbial material this could lead to the release of potentially infectious or harmful substances into the ambient air, or also spreading it into the central vacuum system. Such possibilities represent an unacceptable biological hazard to both man and the environment. It is for this reason that the relevant rules and regulations state that all necessary steps should be taken to prevent the risk of aerosol process air escaping into the environment.

1.3 INAPPROPRIATE VACUUM SOURCE

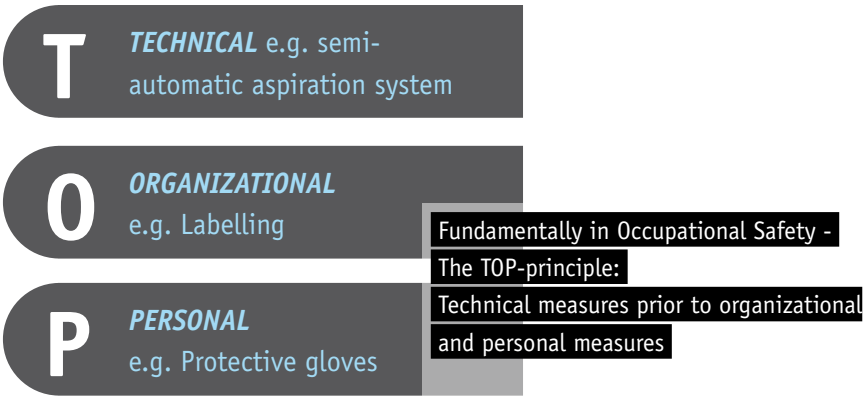
Vacuum sources used in the laboratory for specific evacuation tasks are often over- or undersized. This can result in a widely varying and difficult to control suction capacity.

In addition to these the ease of use constraints, these non-suitable vacuum sources can also have a negative effect on the economy and the environment. Too strong a vacuum can result in the partial or complete evaporation of the collected fluid. This vapour then enters the vacuum system, where it can condense. This condensate is an ideal breeding ground for contamination which is then disseminated into the air via the outlet of the vacuum system. In addition, inadequate, or lack of controls as well as inconsistent and complicated operations increases the risks for safety at work.

Demand oriented regulation of the vacuum level is therefore essential for secure and user-friendly handling of the process.

1.4 EXPENSIVE INSTALLATION AND LACK OF USER-FRIENDLINESS

These individually configured devices for extracting the media are often bulky and furthermore they can also be difficult to clean and disinfect. The individual components of these unwieldy installations are often not adequately replaced in the event of failure. Safety requirements in respect of the bio-containment protocol for liquid biological waste is then no longer observed. As such, the transfer process required to decontaminate the biological media or transport the waste fluid, results in an increased risk for contamination of the equipment and the surroundings. Especially in laboratories with increased hygiene levels, these individually configured devices do not meet the requirements or if so, only to a limited extent. The use of inappropriate, non-chemistry and non-disinfectant resistant components also leads to a total failure-prone system with higher maintenance and repair costs as well as increased downtime.



COMPONENTS OF THE SUCTION SYSTEM AND THEIR RISK POTENTIALS

VACUUM SYSTEM

- ▶ no suction power regulation and excessive vacuum
  - · leads to evaporation from the bottle and condensing in the pump or the vacuum or exhaust pipes
- ▶ low performance for multiple use
  - · risk of drop formation on the suction tip
- ▶ not resistant to disinfectants
  - · short lifetime, leaks
- ▶ possibility of failing to connect exhaust hose to the pump outlet
  - · unable to secure pipework for increased safety requirements
- ▶ increased noise level through continuous operation
  - · increased stress levels can lead to higher user error rates (4)
- ▶ footswitch
  - · impedes the cleaning of the laboratory floor

BOTTLE

- ▶ not vacuum-tight, nor shatter-proof
  - · risk of spread of contaminated liquid
- ▶ connections can be made incorrectly
  - · suck up the liquid into the vacuum pump
  - · damage to the pump
  - · liquid sprayed into the laboratory
- ▶ no inlet pipe for the media in the collection bottle
  - · increased aerosol formation/foaming in the collecting flask
- ▶ bottle with rubber stopper
  - · glass tubes can break when inserting
- ▶ risk of damage to the ports in the bottle
  - · medium can be partly directly drawn into the pump inlet
- ▶ lack of stability of the bottle
  - · bottle can tip over

FILTER

- ▶ not hydrophobic
  - · no pump protection from overfilling
- ▶ too large poresize
  - · insufficient protection against the spread of aerosols
- ▶ not directly at the collecting flask
  - · is not autoclaved, nor part of the bio-containment
- ▶ not autoclavable
  - · microbial growth on the filter surface

HAND CONTROLLER

- ▶ not used
  - · vacuum supply will not shut off
  - · risk of liquid droplets / dripping
  - · pump runs continuously or must be controlled via a foot switch
  - · unnecessary load on vacuum source
  - · tip attachment eg. Pasteur, can be difficult to change
- ▶ operated by way of a mechanical valve
  - · may leak, can stick, hard to clean
  - · seal must be lubricated

CONNECTING HOSES

- ▶ not autoclavable
  - · bacterial growth
- ▶ thin wall thickness
  - · collapses under vacuum
- ▶ different diameters
  - · bacteria build up around transition pieces/ adapters

INLET ADAPTER

- ▶ stainless steel tips
  - · possible crust build up resulting from flaming
- ▶ primitive use of disposable tips
  - · no safe fitting, tips can fall off
  - · uneven suction

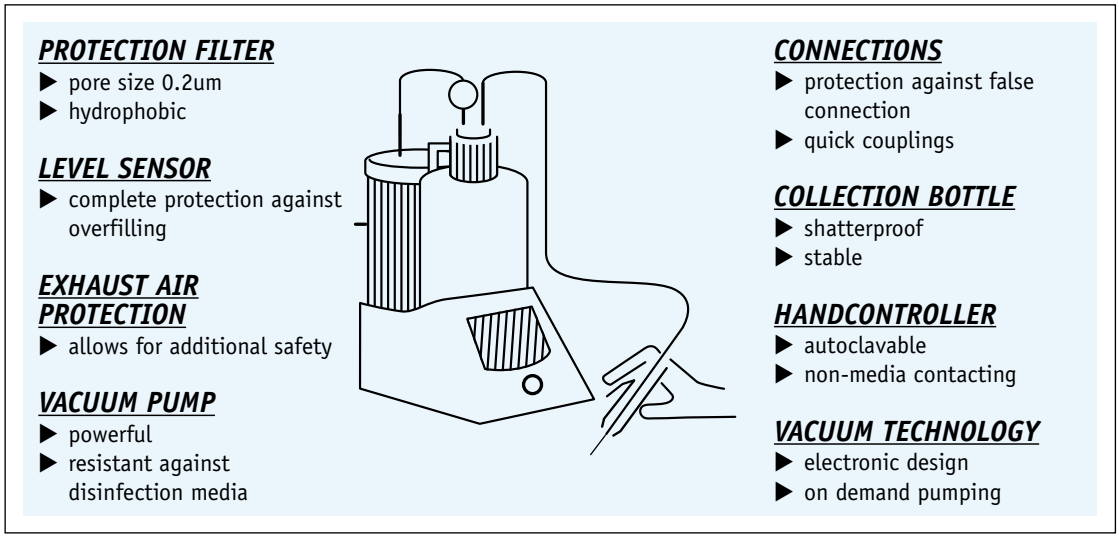
2. BIOCHEM-VACUUCENTER - THE SAFE SOLUTION

In order to avoid the previously described safety deficiencies, these need to be addressed through a comprehensive and well-conceived technical solution that minimizes the risks and thus maximizes the process reliability. With regards to suction systems in laboratories, the main important aspects which play a decisive role in biological protection are bio-containment, hygiene and ergonomics.

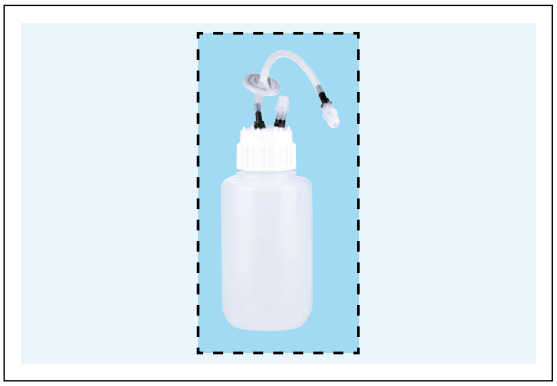
Semi-automatic suction systems have an integrated vacuum pump, which automatically turns on and off so that the vacuum in the suction flask and therefore the suction power is maintained within the desired range. Suction is activated by a hand controller via the suction line using a lever to release the vacuum. The hand controller can accept a wide variety of Pasteur or standard pipettes made either of glass or plastic.

The BVC Biochem-VacuuCenter from VACUUBRAND provides an optimized system for decentralized liquid handling. From a safety perspective (2) on the basis of specific criteria used, the BVC-systems offer maximum functionality, ease-of-use and optimal safety across a wide variety of usage.

Integrated Safety concept



BVC Safety-containment with collecting bottle, protection filter and optional quick couplings



2.1 HERMETICALLY SEALED

To prevent unintentional loss during the operation, transport, or disposal of contaminated liquids, the BVC-systems have a hermetic separation of media and the surrounding areas.

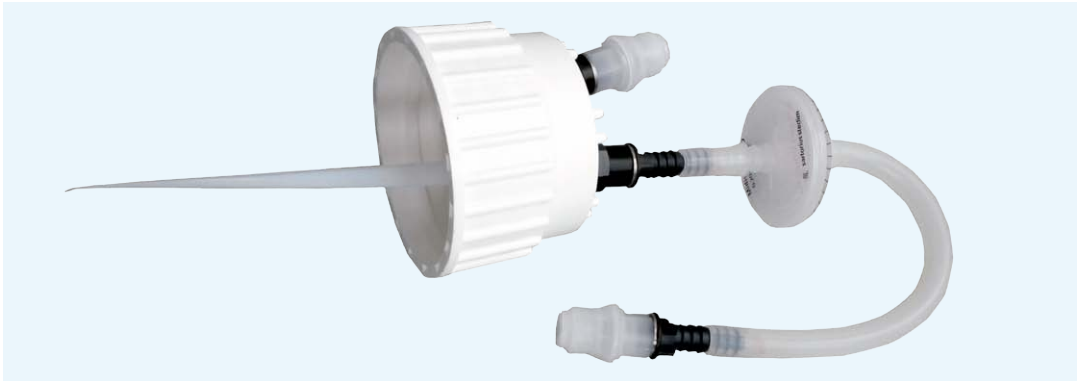
The connection between hand controller, collection bottle and vacuum supply is made via flexible tubing. The vacuum-tight ports are individually matched with each other to prevent incorrect connection. The connection of the pump and hand controller to the collection flask is made via quick couplings that prevent incorrect coupling and hermetically seal the bottle when disconnected. The collection containers are available in polypropylene (4L) or borosilicate glass (2L). Both vessels are break and shatterproof and are sealed with a tight screw top lid against unwanted spillage. The disconnection of the collection flask by the vacuum system and the suction line of the hand controller is done via quick connect couplings. The collection flask remains hermetically sealed for a safe transport for proper sterilization and disposal. Since the hydrophobic filter is fitted in the suction line between the collection flask and the pump, decoupling of the collection flask ensures that the filter is also removed for sterilizing and so no unwanted bacterial growth can occur.

The BVC collection flasks are thermally stable and the hydrophobic filters can be autoclaved up to 20 times, thus allowing for sterilization of the liquid without the need to transfer it first. For autoclaving the pressure between the collecting flask and autoclave must be equalised. This is done by simply loosening the screw top lid. This will ensure that the contaminated media remains safely within the collection bottle and the risk of contamination during the entire working process is minimized.

2.2 AVOIDING AEROSOLS

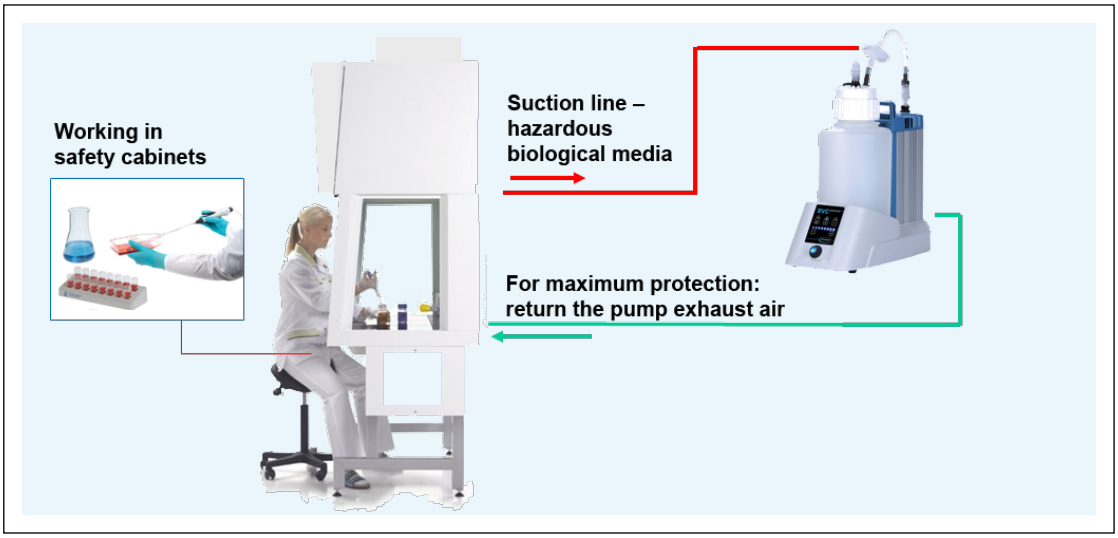
During the aspiration process, foaming of the media in the collection bottle should be avoided as far as possible. To this end, there is an anti-foaming device in the form of a discharge tube in the lid of the collection flask. However, the formation of aerosols cannot be fully eliminated.

Contamination of the vacuum unit or indeed the environment with potentially infectious biological media should be avoided. To do this, the exhaust air is passed through a hydrophobic protection filter before it is discharged into the environment. This hydrophobic membrane filter with a pore size of 0.2µm is used as an effective and safe barrier for bacteria, viruses, and other microbes. Together with the container, the filter is designed as far as possible to safely prevent the release of bio-aerosols into the environment. Despite the high mechanical and chemical resistance, 100% containment cannot be guaranteed. This residual risk is unacceptable for biological security areas with protection level 3 (S3). In this case, it is possible for the exhaust air to be discharged within a safety cabinet via a connector on the back of the BVC. In this way, the BVC-system can be used safely in conjunction with biologically and chemically critical substances.



Bottle lid with inlet tube, 0.2µm protection filter and quick couplings

Integrated Containment: ‚Aspiration system BVC in combination with a safety cabinet



2.3 HYGIENE AND CONTAMINATION PROTECTION

The basis of the compact design of the BVC-series is easy accessibility to all surfaces for easy cleaning and disinfection of the individual system components. In combination with the hermetically sealed vacuum lines, contamination of the device as well as a spread of wider contamination can be excluded.

The suction systems should be in principle situated outside of the biological safety cabinet. If situated inside the safety cabinet, the devices can interfere with the air flow and thus affect the safety. The suction systems are typically placed below the safety cabinets. Easy accessibility and cleaning of the floor is made easier with an optional mobile trolley. In addition, if required the BVC-systems are equipped with an electronic level sensor, to prevent the overflow of the extracted liquids, which in turn also prevents the risk of contamination of the environment with potentially dangerous substances. The user will receive a visual and audible warning upon the safety-critical level being reached and the pump will automatically switch off to allow the user to empty the collection flask. The disinfection routine enables the user to exit the current step and temporarily restart the pump for the disinfection of the suction hose.

Safe and ergonomic work with the hand controller

2.4 THE HAND CONTROLLER - SAFE, ERGONOMIC AND FLEXIBLE

The new VHC^{pro} handcontroller has been designed with users in mind and takes into account the experience of many users in terms of functionality, ergonomics and safety as well as in the context of application testing. The result is a handcontroller which is very comfortable and easy to use and fits perfectly in your hand for continuous, fatigue-free working. The media carrying suction hose passes completely through the handle to the collection bottle. Therefore the hand controller itself does not come into contact with any of the aspirated media and cannot be contaminated. This also eliminates the risk of leaks.

On the plus side, this means that in terms of safety and contamination prevention, the complete handcontroller can also be autoclaved. The flexible tip adapters also means that standard Pasteur pipettes and disposable tips can be used safely and accurately, as well as other longer and relatively large standard or graduated pipettes used to aspirate from larger cell culture flasks.

The 8-way tip adapter is specially designed for the parallel extraction with 8 pipette tips from multi-well plates. Its special V-rings ensure uniform and accurate suction across all 8 of the disposable tips. The integrated tip ejection system allows for safe and simple removal of the tips.

The concept is completed with the inclusion of a simple wall bracket for safely holding the handcontroller during prolonged breaks or when not in use. There is also an optional benchtop stand to further support ergonomic routine working.

2.5 FLEXIBILITY AND COST-EFFECTIVENESS

The BVC-systems offer a flexible solution depending on whether your aspiration equipment is to be used with an existing vacuum supply or as complete all-in-one solution with built-in vacuum pump. All systems have the option to be used simultaneously by two users.

For systems used on existing vacuum supply, the optimum working pressure is controlled via an integrated mechanical pressure switch. For the systems with integrated chemical and bleach resistant diaphragm pumps, the electronic control system takes care of this, only turning the pump on as and when necessary.

Depending on the application use and the resulting fluid volume, users can choose between a 4l-Polypropylene and 2l-Glass bottle. The Glass bottle is especially suitable for use with highly corrosive disinfection media such as chlorine bleach.

By connecting a second handcontroller directly to the collection bottle, economic dual use of the devices is then possible.

The aspiration equipment depending on the requirements can be supplemented with various accessories and can be used safely in biological security restricted areas with the protection class 1 (S1) to 3 (S3).

Through the use of high-quality, corrosion-resistant materials, the BVC System can be used reliably and continuously with minimal maintenance requirements and a long service life.

BVC -
economic dual use

THE BIOCHEM-VACUUCENTER - WITH SAFETY FOR EVERY REQUIREMENT

The Biochem-VacuuCenter range from VACUUBRAND offers an innovative and modular solution for the extraction of cell culture media, which can be flexibly adapted to meet existing operational and work flow process requirements.

The BVC basic model provides an ideal complementary solution for laboratories with an existing vacuum supply and meets the safety requirements for activities in biological safety restricted areas with the protection class 1 (S1) and 2 (S2).

The BVC control and BVC professional versions offer an all-in-one solution with their integrated diaphragm pump for decentralized vacuum generation, which can be controlled via a user-friendly, multi-functional user interface. For the use in laboratories where the biological protection level 3 (S3) is required, the BVC Professional is most suitable since it offers additional safety features for enhanced hygiene requirements.

The BVC-systems are characterized by their compact and space-saving design, “plug and play” ease of use, as well as their low maintenance derived from the reliable chemistry diaphragm pump.

With the well-thought-out safety design concept, the BVC system represents an unrivalled alternative to traditional manual or semi-automatic biological waste suction processes.

List of references: ***White Paper „Safe and contamination-free handling of cell culture supernatants"***

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