Secure waste air filtration in laboratory autoclaves

Integrated water intrusion test to fulfil the requirements in the area of biotechnology

C. Grumbach, P. Czermak*

The thermal inactivation of natural or genetically modified micro-organisms is usually done in biological and medical laboratories in a steam sterilizer (autoclave) under defined parameters for pressure, temperature and time. The discharge of these organisms from the autoclave chamber - for example on the waste air or waste water path - must be prevented as a function of the risk group of the organisms [1, 2]. In particular in the course of the heating phase of the autoclaves, when the air is removed from the chamber and steam flows in, biological working substances can get into the workspace as aerosols. In practice, sterile filtration is often used for the inactivation of this waste air. For this purpose, the waste air is routed through hydrophobic membrane filters, completely removing its germ load from the waste air flow with proper handling. Sterile filtration can be associated with risks under certain conditions [3]. The filters have hitherto not been tested for their functionality (integrity), although this is technically basically possible and must be considered as necessary in terms of a risk assessment. On account of recent developments in the field of sub-statutory regulatory frameworks, changes are to be expected here [4]. The possible risks are hereinafter illustrated by means of practical example and the system design of a new test system, which was developed on the basis of the special requirements for integrity testing in laboratory autoclaves, will be presented.

Introduction

The methods for the inactivation of micro-organisms by means of saturated steam have been successfully carried out in laboratories of the pharmaceutical and medical industry in autoclaves for a long time. The sterilisation success of such processes causes complete removal of the air from the autoclave chamber as well as from the autoclaving material/items to be sterilised. The waste air removed from the autoclave in the process is normally discharged from the system into the environment. In biological laboratories, contaminated process waste air of autoclaves, from the protection and safety level 2 onwards, must be purified according to the Genetic Engineering Safety Ordinance (GenTSV) [2]. The removal stage of the air is followed by a downstream sterile filtration, in order to prevent around a discharge of airborne pathogenic germs into the environment. For this application, membrane filters are being used, through the narrow-pore membranes of which (as a rule 0.2 µm diameter) bacteria are reliably retained [5]. However, long-standing experience shows that sterile filtration can be associated with risks under certain conditions [3]. The filtrate is sterile for as long as the integrity (i.e. the intactness) of the filter is guaranteed. If bioaerosols are thrown up into the environment from the waste air path of the autoclaves in the event of a failure of the filter, this creates a significant risk to humans and the environment.

* Dipl.-Ing. Carsten Grumbach
Prof. Dr.-Ing. Peter Czermak
Technische Hochschule Mittelhessen
Institut für Bioverfahrenstechnik und
Pharmazeutische Technologie
www.thm.de/ibpt
peter.czermak@kmub.thm.de

The manufacturer specifications for the service lives of the filters ordinarily refer to ideal filtration conditions. The selection of a filter suitable for the process, as well as its coordination with the manufacturer-sided specifications on maximum flow rate, pressure load and operating temperature, are decisive for its integrity and service life [6]. Up to now, the functional capability of the autoclave waste air filters is not routinely checked in the areas mentioned above, although this is technically basically possible. Recent developments in the field of sub-statutory regulatory frameworks name the water intrusion test procedure as appropriate safety measure [4].

Risks of sterile filtration on autoclaves

The qualification of sterile filters is an essential task of the filter manufacturer. However, potential damage in transit, damage by improper handling or, for example, through steam sterilisation, can never be excluded completely [7]. Also, damage to the filter through the filtration process is possible. A great risk is already present with the installation of the sterile filter. If the O-ring seal necessary for the adaptation to the filter seat is damaged in the process, sterile filtration is no longer given for the subsequent sterilization cycles. Loss of the integrity of a sterile filter can already appear on account of microscopic damages or changes of the pore structure on the filter, which are macroscopically not to be identified. Therefore, verification is only possible by means of sensitive measuring methods, which are called filter integrity tests. With such integrity tests, a statement with regard to the filter integrity becomes possible with the aid of non-destructive technologies [7]. In addition to a verification of the filter integrity, an integrity test, besides, allows the inspection of the waste air filter system for possible leakages which can appear after its installation.

Integrity testing

The integrity testing of sterile filters supplies the microbiological proof that a sterile filter delivers a sterile filtrate when undergoing exposure to a certain number of bacteria. It thus constitutes an essentially necessary quality assurance measure for sterile filtration. Only through a test will it be directly demonstrated that the test organisms used are reliably being retained by the filter [8]. However, this test kind concerns destructive testing, through which the filters become useless for their proper application. The filter elements are subjected, in addition, within the scope of a product / process specific validation, together with the respective product, to a bacterial load test under process conditions. According to the ASTM Guideline [9] a filter is only allowed to be classified as a sterile filter if it, upon bacterial expo-

Fig. 1: Waste air filter system of a laboratory autoclave with the measurement path of an adapted water intrusion test

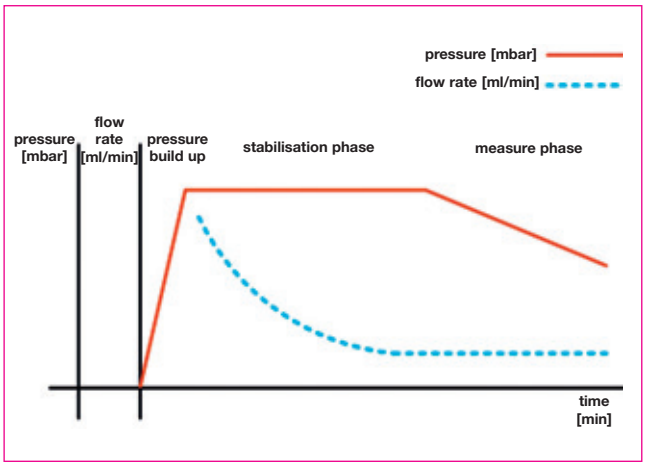

Fig. 2: Pressure drop method (schematically)

sure with 107 of a model organism of the species Brevundimonas diminuta, delivers a sterile filtrate per square centimetre of filter area. This test constitutes a "Worst-Case" scenario with an immensely high bacterial density and substantiates the high level of safety. The respective manufacturers of the filter elements are responsible within the scope of their product validation for the correlation of the test values with microbiological examinations. With the help of such destructive load testing, defined limit values are specified for the non-destructive integrity test by means of suitable model organisms [7].

Water intrusion test

To decontaminate the waste gas stream of laboratory autoclaves, gas-permeable hydrophobic membrane filter elements are usually used. The water intrusion test is a suitable integrity test method for testing hydrophobic membrane filters. Here, the hydrophobic filter element is flooded with water and a test pressure, which is below the penetration pressure of the filter, is applied to the measurement system. The water steam originating on the filter membrane by evaporation passes through the filter membrane. A measurement of the pressure drop provides the water intrusion rate (WIR), which can be correlated with the bacterial retention rate of the filter [10, 11]. A breakthrough or failure of the filter elements can be detected at an early stage in this way and be prevented.

The static pressure drop method is the technically simplest test method. The test run is divided into three phases (see Figure 2): a pressure build-up phase, which serves for the establishment and for the stabilisation of the critical constant pressure conditions necessary for the test. Only under these conditions can the intrusion flow to be measured be stabilised, via a stabilisation time typical for the filters, to a steady flow rate. By means of the subsequent measuring phase, the intrusion flow can be determined from the pressure drop [12].

Factors that influence the integrity

Beside the selection of a filter suitable for the process, the process terms are also decisive for its integrity and service life [6]. A rapid failure of the filter elements can appear in the autoclave when large amounts of steam are extracted via the vacuum pump under high temperatures and the filter, under these conditions, is subject to an increased differential pressure. The process parameters for temperature, autoclaving time and the amount of the drawn vacuum arise from the chosen autoclaving method and the contamination to be sterilised of the autoclave. Hence, the autoclaving method is of central significance for the filter integrity because the waste air filters are polluted through an evacuation of the autoclave chamber in connection with increased temperatures or through the permeation by means of saturated steam from the chamber. With regard to the mechanical stability of filter cartridges, it should be considered which maximum pressures and above all pressure fluctuations can appear in the running system. Extreme pressure pulses can be caused here, systems-specifically, by quick-closing valves or equipment units downstream of the filter (for example vacuum pumps).

The temperature problem

Basic requirements for the reliable execution of a water intrusion tests are the availability of stable ambient conditions. Above all, the temperature stability is important because a temperature variation leads to a corresponding pressure fluctuation in the measurement system which is then falsely evaluated as water intrusion. The temperature problem gains further complexity still for the measurement in that the surface tension of the wetting liquid is also dependent on temperature, so that a temperature change can also have a direct influence on the flow rate to be measured [13].

Requirements description

Many test systems on the market are designed very generically for filter measurements and are on a par with the investment costs of laboratory autoclaves [14]. Such test systems are seldom suited for small plants, on account of the additional high qualification costs. Such costs can be reduced by the fact that a test is designed and qualified for only certain filters types and sizes. Besides, a test system should be able to be easily integrated into various plants.

Besides, different technologies have established themselves on the market in order to determine the flow rate across the filter membrane. A very inexpensive variation is the static pressure drop method which allows the determination of water intrusion from the measurement of an individual pressure drop within the measurement system. The water intrusion rate can be calculated from this, given knowledge of the exact boundary conditions. An essential role, here, is being played by the knowledge of the exact net volume of the gas quantity in the measuring system [12]. Many test systems automatically determine the net volume of the filter candle housing before the actual intrusion measurement. However, the basic test procedures can already be implemented by means of an individual pressure transducer and a fine pressure reducer [15], if one succeeds in allowing a reproducible setting of the test gas above the filter element. In this way, the relatively complex net volume determination can be avoided. Another important requirement is the already depicted temperature problem. If

the thermal radiation of the autoclave chamber has an influence on the thermodynamics of the test gas, this can lead to a considerable impairment of the measured results [8]. That is why special measuring equipment was developed which shields the test gas against thermal radiation from the outside. Long test runtimes and cooling periods can be avoided in this manner. Moreover, a defined gas volume in the measuring system can only be set through the filling process of the test system. For the measurement of filters of different sizes the measuring equipment can be designed and varied accordingly.

Besides the requirements for the test system, special demands are also to be made on the waste air filter system of the autoclave, to which the test is adapted. The essential test requirements include a successful condensate return from the waste air filter housing, as well as a successful inline sterilisation of the waste air filter. The sterilisation conditions prevailing in the autoclave chamber must also be transported into the waste air filter system for a successful sterilisation. Condensate occurring in the filter housing must be discharged into the autoclave chamber to avoid a condensate accumulation which would lead to a collapse of the sterilisation conditions [3].

The success of the sterilisation of the filter element has to be monitored here, so as not to contaminate the test water necessary for the integrity test, which is conducted after the test in the wastewater path. Moreover, care has to be taken during the installation of the waste air filter system to ensure that this is filled bubble-free for the test procedures and that there are therefore no dead spaces in the system which are not flooded during the filling. Otherwise, this would lead to a falsification of the total net volume of the gas volume. In particular, this concerns the measurement path of the measurement system. On account of these requirements, a special valve circuit was developed which eliminates dead spaces both in the test system and also in the exhaust air filter system of the target plants.

System description

The overall configuration (Figure 3) of the test system consists of three main components: Measuring equipment with pressure sensor for the measurement of the intrusion flow across the filter element. A valve system that switches the test system into the positions of the individual test phases. An exact pressure controller which regulates the test pressures necessary for the test and, furthermore, allows automated drying and draining of the test system. The valve system controls the steps necessary for the test execution like filling, pressure build-up, stabilisation measuring and final draining of the system.

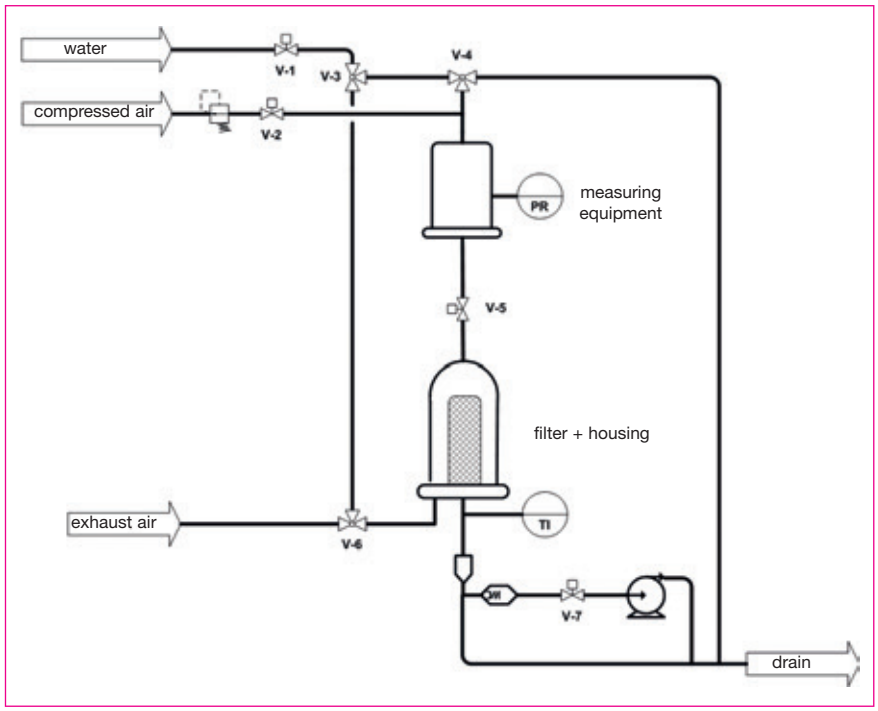

Fig. 3: Flow chart of the measurement system adapted to the waste air filter system of an autoclave

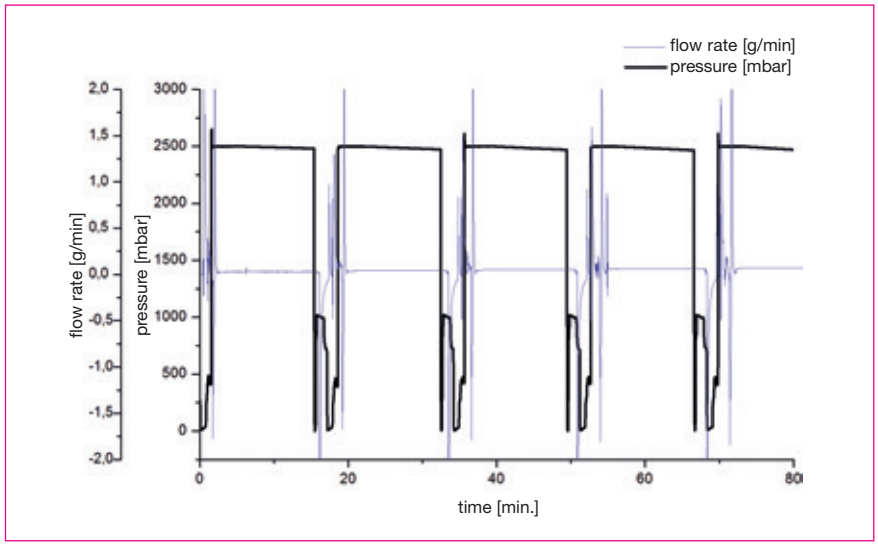

Fig. 4: Automated calibration sequence of the test system

Within the stabilisation phase, stabilisation of the intrusion flow takes place at exactly constant pressure conditions. The pressure drop generated as a result of the flow rate must be regulated in this phase to the exact test pressure via a fine pressure reducer. After the stabilisation phase, the measuring system is closed up and a pressure drop across the now stabilized flow is measured, which supplies the water intrusion rate.

Integration into the target systems

The test system (Figure 3) can be adapted to the waste air filter system of autoclaves via two lines. For this purpose, installation of a valve (V-6) is necessary in the waste air filter system of the autoclave. The inlet and outlet line of the test water to the test system is enabled via the valve V-5. The sterilisation of the filter element is monitored via a temperature sensor during the autoclaving. The control of all the components depicted, as well as the read-out of the sensors, is done via integrated controller hardware of the test system.

Calibration

The flow rate references required for calibration are usually generated by means of a needle valve and are recorded gravimetrically via laboratory scales. Alternatively, mass flow meters are also suitable for accurate determination of the flow. Moreover, these allow automation of

Tab. 1: Results of the integrity determination of a High Flow Tetpor of II sterile filter (Parker Domnick Hunter), effective filter area 0.1 m², pore size 0.2 µm, limit flow rate 0.06 ml/min

High Flow Tetpor II (Parker Domnick Hunter)			filter test device				
			Palltronic® XC			Sartocheck 4	new test system
filter type	ser. no.	filter loading	measure-ment 1 [ml/min]	measure-ment 2 [ml/min]	measure-ment 3 [ml/min]	measure-ment 4 [ml/min]	measurement 5 [ml/min]
ZHFT/BZ	A 358416	non-loaded	0,04	0,03	0,03	0,03	0,03
		after 1 autoclavation	0,05	0,05	0,05	0,04	0,03
		after 2 autoclavation	0,06	0,06	0,06	0,04	0,03
		after 3 autoclavation	0,06	0,06	0,06	0,03	0,03

Tab. 2: Results of the integrity determination of a High Flow Tetpor of II sterile filter (Parker Domnick Hunter), effective filter area 0.2 m², pore size 0.2 µm, limit flow rate 0.13 ml/min

High Flow Tetpor II (Parker Domnick Hunter)			filter test device				
			Palltronic® XC			Sartocheck 4	new test system
filter type	ser. no.	filter loading	measure-ment 1 [ml/min]	measure-ment 2 [ml/min]	measure-ment 3 [ml/min]	measure-ment 4 [ml/min]	measurement 5 [ml/min]
ZHFT/AZ	A 224757	non-loaded	0,05	0,04	0,05	0,04	0,03
		after 1 autoclavation	0,07	0,06	0,06	0,05	0,05
		after 2 autoclavation	0,08	0,05	0,05	0,05	0,04
		after 3 autoclavation	0,06	0,05	0,04	0,05	0,05

Tab. 3: Results of the integrity determination of a Sartofluor® GA Mini Cartridge (Sartorius Stedim Biotech), effective filter area 0.1 m², pore size 0.2 µm, limit flow rate 0.09 ml/min

Sartofluor® GA Mini Cartridge Sartorius Stedim Biotech			filter test device				
			Palltronic® XC			Sartocheck 4	new test system
filter type	ser. no.	filter loading	measure-ment 1 [ml/min]	measure-ment 2 [ml/min]	measure-ment 3 [ml/min]	measure-ment 4 [ml/min]	measurement 5 [ml/min]
518 15 07T 8	150005003 0087	non-loaded	0,04	0,03	0,03	0,03	0,03
		after 1 autoclavation	0,04	0,05	0,05	0,03	0,03
		after 2 autoclavation	0,05	0,04	0,04	0,04	0,03
		after 3 autoclavation	0,04	0,05	0,04	0,03	0,03

Tab. 4: Results of the integrity determination of a Sartofluor® GA Mini Cartridge (Sartorius Stedim Biotech), effective filter area 0.2 m², pore size 0.2 µm, limit flow rate 0.11 ml/min

Sartofluor® GA Mini Cartridge Sartorius Stedim Biotech			filter test device				
			Palltronic® XC			Sartocheck 4	new test system
filter type	ser. no.	filter loading	measure-ment 1 [ml/min]	measure-ment 2 [ml/min]	measure-ment 3 [ml/min]	measure-ment 4 [ml/min]	measurement 5 [ml/min]
518 15 07T 9	202006903 0517	non-loaded	0,07	0,06	0,07	0,05	0,05
		after 1 autoclavation	0,07	0,07	0,07	0,05	0,04
		after 2 autoclavation	0,06	0,07	0,07	0,04	0,04
		after 3 autoclavation	0,08	0,07	0,07	0,05	0,04

the calibration procedure, as these also allow precise control and monitoring of the flow. The following Figure 4 shows the calibration by means of a calibration kit specially developed for the new test system. The test system is filled once more with water for every single measurement and the desired target flow is set by means of a mass flowmeter/controller (Bronkhorst Mini Cori-Flow model: M12V14I-AGD-11-K-S). The system is afterwards stabilised, whereupon a determination of the flow rate is done via a pressure drop measurement. The test system is drained afterwards and once more filled, whereupon the next measurement can be done.

The flow rate values determined by the test system can be correlated with the flow values generated by the flow controller. Figure 5 confirms a correlation of both flow values and, in addition, confirms reproducibility of the volume setting via the newly developed measuring equipment.

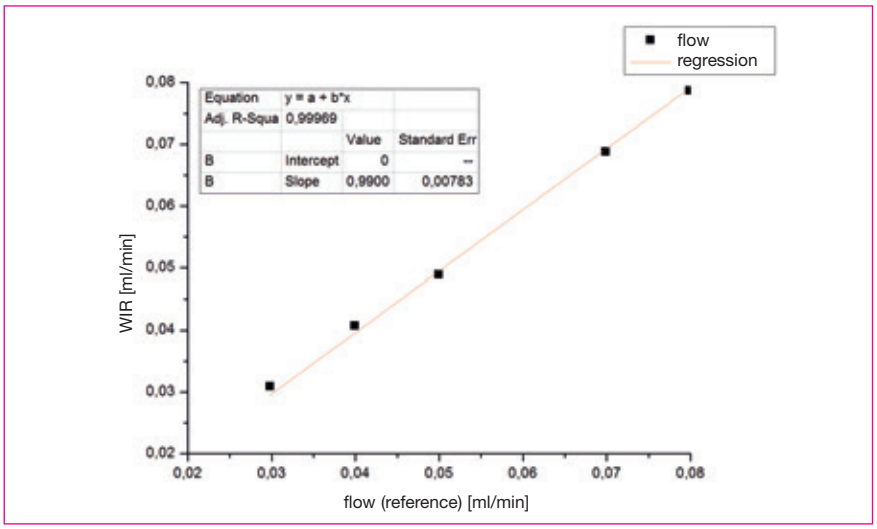

Fig. 5: Calibration curve (flow calibration)

Pre-treatment and post-treatment of the filter

A problem frequently appearing in practice is a collapse of the sterilisation conditions in the waste air filter systems of autoclaves during filter sterilisation [3]. To remove these condensate problems, drying the filter element after sterilisation has a supportive effect, in order to prevent water residues in the drainage layers of the filter hindering the sterilisation process of the filter. Also, for the pre-treatment of the test procedure, drying the filter can prevent a falsification of the measured results. If a filter still contains portions of water in its outer supporting layers after sterilisation, the test water can wet the actually hydrophobic membrane during a subsequent autoclave run, as a result of the increased temperature, and disturb the filter measurement. Hence, the new test system allows automated drying of the filter element before and after the water intrusion test. In addition, a cooling of the filter element via the test system was installed, which cools down the filter to the test temperature after the sterilisation.

Filter measurement in autoclaves

Figure 6 shows the entire sterilisation course (gravitational method) of the filter element of an ordinary laboratory autoclave (upright autoclave; chamber volume 85 L) with subsequent water intrusion test. The filter was sterilised in the function of a waste air filter by means of the sterilisation run of the autoclave and afterwards was tested by the test system. The software of the test system continuously records pressure values from the test system, as well as temperature values of the filter in the waste air filter system of the autoclave. The sterilisation run in its simplest form consists of a heating phase, the holding phase and a cooling phase. Only the holding phase is substantially decisive for the sterilisation success and it must hold the given temperature for a fixed period. The test run consists of drying, cooling, water intrusion test, draining and repeated drying of the filter element. After the end of the sterilisation, the test run begins with the drying of the filter element. The drying process uses the residual heat still available in the filter for the removal of condensate from the drainage layer of the membrane filter. The evaporating condensate leads to a strong temperature drop and works as an additional cooling for the filter element. The residual heat still available in the filter is discharged by the subsequent cooling step by means of water. The cooling for this is done in intervals in order to exploit the heat capacity of the water optimally. After the integrity test, draining and complete drying of the filter is done in order to prepare the filter for the next sterilisation cycle.

Comparative measurements

For the evaluation of the new measurement system, it was compared to two commercially available measuring instruments (Palltronic® Flowstar XC and Sartocheck 4). The comparative measurements were done in four different sterile filter models of the manufacturers Parker Domnick Hunter (High Flow Tetpor II) and Sartorius Stedim Biotech (Sartofluor® GA Mini cartridge).

The filters were then inserted as waste air filters in the waste air filter system of a laboratory autoclave (upright autoclave; chamber volume 85 L) and exposed under normal process conditions (sterilisation temperature 121 °C, holding phase: 20 minutes, gravitational operation). The measurements of the individual filters were made both before and also after the stresses of the autoclaving process. The sterilisation procedure corresponds to the picture described in the previous section and the concluding test phase of the filter element was done as multiple determinations through the three test systems.

In Tables 1-4, the measured results of the comparative measurements are shown. With multiple determinations, the flow rates drop according to experience. The causes of this effect can be derived only from observations so far which show that, through the pressurisation, air bubbles on the filter surface dissolve in the water phase. This leads to a volume loss which is interpreted by the test system as a flow rate. With the pressure relief carried out at the end of the test, these bubbles are partially removed from the filter by ascending. However, the actual causes can be more complex, which makes it difficult to compare the flow values of measurement systems of different test systems. But the comparative measurements can be assessed under the criterion of whether an integrity test has been evaluated by the measurement system as having passed or as having failed. With all four filter models, no critical changes in the flow values can be observed through exceeding the limit flow due to exposure, something which can be confirmed with all three test systems. The integrity of the filter can be proved by the new test system without influence from external temperature.

Conclusion

The test procedures for integrity testing of membrane filters seem to work very simply at first glance metrologically. However, in the end, it is the interplay of many factors that leads to complexity in the matter and makes their development and integration into appropriate target systems anything but trivial. A hindrance also appears to be the low investment costs for such test systems that are necessary for small plants. However, with an accurate definition of requirements, these can be adapted to the corresponding target systems and to the filter elements to be tested. Under these conditions it is also possible to develop safe test systems for small plants, which, in addition to the easy detection of filter failure through to finding leaks in the system, also allow a supporting effect on the sterilisation method of the filter elements by means of their drying.

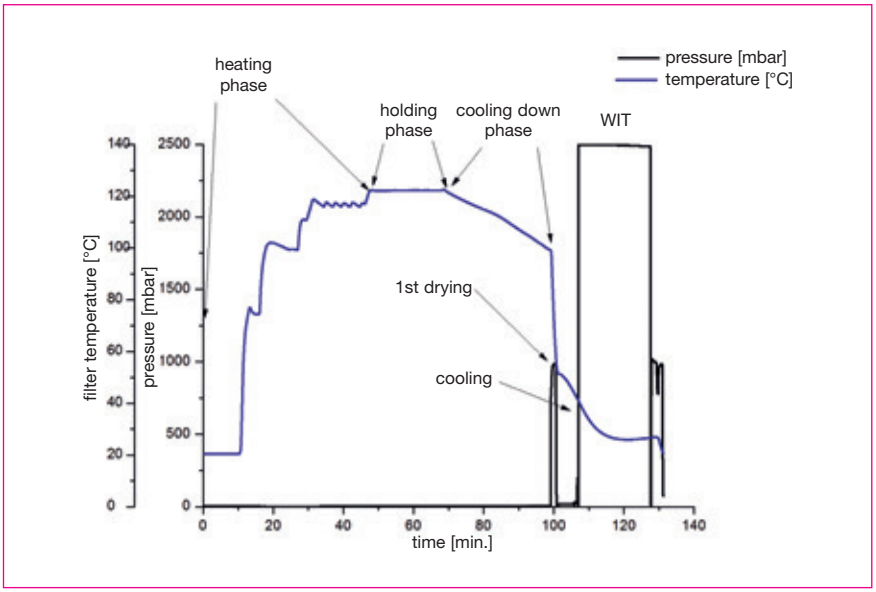

Fig. 6: Sterilisation procedure

Expression of thanks

We thank the Hessischen Ministerium für Wissenschaft und Kunst for financial support of our development project: Development of a modular, universally applicable Water intrusion test (WIT) for small plants. In addition, we thank our project partner biomedis Laborservice GmbH for the fruitful and trusting cooperation.

Literature:

[1] Technische Regeln für biologische Arbeitsstoffe 100, Schutzmaßnahmen für gezielte und nicht gezielte Tätigkeiten mit biologischen Arbeitsstoffen in Laboratorien (Technical rules for biological agents 100, Preventive measures of specific and non-specific activities with biological agents in laboratories), 2006, ABAS.

[2] Verordnung über die Sicherheitsstufen und Sicherheitsmaßnahmen bei gentechnischen Arbeiten in gentechnischen Anlagen (Gentechniksicherheitsverordnung) (Ordinance about the safety stages and safety measures for genetic engineering operations in genetic engineering facilities (Genetic engineering safety ordinance)) in the version published on 14th March, 1995 (BGBl. I p. 2768).

[3] C.Grumbach, H. Schulte-Lünzum, P. Czermak (2013): Abluftfiltration unter der Lupe, Risiken bei der Sterilfiltration der Autoklavenabluft (Exhaust air filtration under the microscope and risks with the sterile filtration of autoclave exhaust air): Techno Pharm, in printing

[4] Einbauempfehlung für Neuanlagen, Nachrüstung oder Ergänzung, zur Wahl der Abluftbehandlung von Autoklaven (Recommended installation for new systems, retrofit or addition, for the selection of the exhaust air treatment of autoclaves), Resolution 3/2009 of ABAS, ELATEC.

[5] ASTM Standard F838-83, American Society For Testing Material (ASTM), ASTM 1983, amended 1988.

[6] U. Brendel-Thimmel, R. Jaenchen, F. Schlamp (2006), Chemie Ingenieur Technik (Chemical engineering) 78:11, 1655-1665

[7] J. Meyer, Einführung in die Integritätstestung von Membranfiltern (Introduction to the integrity testing of membrane filters), SPI8000-d11041, Sartorius Stedim Biotech, 2011.

[8] M.W. Jornitz, T. H. Meltzer, Filtration and Purification in the Biopharmaceutical Industry, Informa Healthcare USA, Inc., New York, 2008.

[9] Standard Test Method for Determing Bacterial Retention of Membrane Filters Utilized for Liquid Filtration, Committee D19.F838-05, ASTM, 2005.

[10] P. Czermak, G. Catapano (2003), PDA Pharmaceutical Science and Technology, 57:4, 277-286

[11] P. Czermak, G. Catapano (2000), European Journal of Parenteral Sciences 5:3, 59-63.

[12] M.W. Jornitz, T. H. Meltzer, Sterile Filtration a Practical Approach, Marcel Dekker, Inc., New York 2011.

[13] R. Jaenchen, J. Schubert, S. Jafari, A. West (1997), European Journal of Parenteral Sciences, 2:2, 39-45

[14] C. Grumbach, P. Grace, P. Czermak, T.Pillich, C. Rühl, K. Fey (2011): Einbauempfehlung für Neuanlagen, Sichere Überwachung der Abluftbehandlung von Autoklaven mittels Wasserintrusionstest mit hoher Temperaturstabilität (Recommended installation for new systems, Safe monitoring of exhaust air treatment of an autoclave by means of water intrusion test with high temperature stability); GIT Laborfachzeitschrift 55: 8, 540-542

[15] Filterelemente - Membranfilterelemente - Teil 12: Integritätsprüfung von hydrophoben Membranfilter?elementen mit Wasser (Wasserintrusionstest) (Filter elements - Membrane filter elements - Part 12: Integrity testing of hydrophobic membrane filter elements with water (Water Intrusion Test)), DIN 58356 – 12, NA 063 Normenausschuss Medizin (NaMed).

[16] Kradolfer M, R Jaenchen (2008), P&A Select Biotech 2: 28-30

Note:

This project (HA Project No.: 307/11-52)) is funded within the scope of Hessen Modell-Projekte from funds of the LOEWE State Offensive for the Development of Scientific and Economic Excellence, funding line 3: KMU Collaborative Project.