SURPLUS VALUE THROUGH

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technische Hygiene & angewandte Mikrobiologie

CLINIC AND PRACTICE HYGIENE PREPARATION OF DENTAL UNITS RISK ASSESSMENTS HYGIENE CONCEPTS INDOOR AIR SYSTEMS

EXPERT OPINION ACCORDING TO VDI 6022 MOULD TREATMENTS

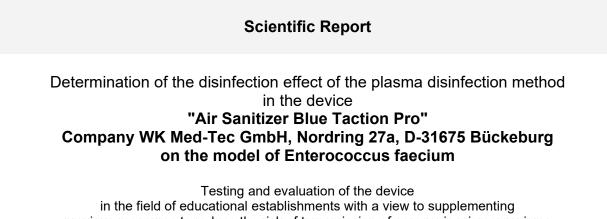
PLANT OPTIMIZATIONS

DRINKING WATER SYSTEMS V EXPERT OPINION ACCORDING TO VDI 6023

VDI 806-5

PLANT DISINFECTION RENOVATION CONCEPTS REPAIRS MAINTENANCE ACCORDING TO

> SWIMMING POOLS V HYGIENE CONCEPTS FORMATS



previous measures to reduce the risk of transmission of aerogenic microorganisms



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1.Introduction:

The subject of the study is the testing of the germinactivating effect of a plasma generator (Air Sanitizer "Blue Taction Pro")) with the aim of microbiological room air optimization and the interruption of aerogenic transmission paths.

In classrooms, there is the problem that microorganisms are introduced by students and teaching staff during their stay in the room. In particular, through respiration , aerogenically transmitted microorganisms enter the room air in this way. Many of these microorganisms originate from the human site flora, which is relatively constant between individuals. Consequently, such microorganisms are not relevant as infectious agents.

However, if a person suffers from respiratory infections (if necessary. Inapparent course or infection excretion before the appearance of clinical symptoms), this person can release the causative microorganicisms into the room air. This can be the beginning of an aerogenic infection, i.e., other people in the room can be infected by inhaling the contaminated room air.

Various infections can be transmitted aerogenically. In addition to certain bacteria (e.B pneumococci, Haemophilus influenzae), these include various viral pathogens, such as measles viruses (but here there is usually an immune collective, provided that the vaccination coverage rate is sufficient), as well as rhinoviruses, influenza viruses and also the new virus SARS-CoV-2.

In the form of a pandemic in 2020, the SARS-CoV-2 virus in particular is forcing special attention to the microbiological air conditions. Especially in the area of schools, it is necessary to achieve and ensure perfect indoor air conditions so that transmission of the SARS-CoV-2 virus in schools and thus the entry into parental households is avoided.

Since the beginning of the SARS-CoV-2 pandemic in March 2020, various forms of prevention have been recommended and implemented. Prevention is the implementation of risk reduction.

The most important measures are:

- Keep distance to limit the aerosol radius around a person
- Breathing masks to reduce aerosol release and inhalation of aerosols
- Everyday hygiene (e.B. sufficient hand washing) to reduce smear infections (with regard to SARS-CoV-2- transmission via smear infection is very unlikely, but everyday hygiene makes sense in moderation)
- Ventilation and air exchange of rooms to dilute introduced aerosols.

The measures can be understood figuratively as hurdles in the infection chain, which are opposed to the transmission of a pathogen. Each of these hurdles reduces the risk of infection transmission, but only the sum plus necessary supplements is effective.

However, it appears that despite the measures mentioned, the transfers continue to take place and not all measures can be implemented in all areas.

Especially in schools – and in many other educational institutions in the same way – distances cannot always be maintained. The number of students and the available

rooms are unchanged. A "contactless" school is a contradiction in terms, since in addition to the educational mission, the social aspect of adolescents must also be considered; especially if, conversely, it must be expected that the risk reduction measures must be implemented in the longer term.

Even the breathing masks do not replace the effect of distance. The reduction performance ("filter performance") is not reliably reproducible (because it is strongly dependent on filter material, moisture penetration, wearing time, face shape and wearing shape of the mask). Even with correctly worn masks in the clinical environment (dental care), reduction rates of a maximum of 2 powers of ten = factor 100 are achieved. This means that even with correctly worn masks of 100 microorganisms, 1 microorganism breaks through.

Everyday hygiene is generally an important aspect, as many diseases are transmitted via the hands and contact. However, regarding SARS-CoV-2 as a primarily aerogenic (i.e., airborne) transmitted virus, direct transmission as a smear infection is rather unlikely.

Ventilation and air exchange are also very useful risk reduction measures. On the one hand, to dilute the aerosol in the room and on the other hand, to carry out the CO2 enrichment in the room. In the cold season, however, there are considerable restrictions on feasibility due to latent cooling of the interiors.

Hygiene as a field of prevention distinguishes two forms of prevention:

- Behavioral prevention
- Ratio prevention

Behavioral prevention is to be seen individually and in relation to a single person. Here, the acceptance and conviction of as many individuals as possible to implement prescribed hygiene measures is important. Behaviour prevention includes, for example:

- Keep your distance
- Wear a mask
- Everyday hygiene

Relationship prevention is related to the environment / setting. These include systemic and superordinate measures, such as .B.

- Air
- Microbiological room air optimization

The measures of behavioral prevention depend on the individual acceptance of the individuals. Therefore, unavoidable gaps in the implementation can arise again and again, e.B. lack of distance or non-wearing of masks.

Therefore, behavioral prevention measures must be supplemented by overarching proportional prevention measures in order to achieve a sufficient reduction in transmission risks. It is optimal if the measures interlock interlock.

The following becomes clear:

- Each measure alone must be supplemented by a further one in order to achieve a sufficient risk reduction
- The implementation of behavioral prevention varies from person to person and is therefore not reliably reproducible
- Supplementation with proportionate preventive measures is necessary in order to close gaps in behavioural prevention and at the same time to achieve an additional reduction in risk.

Therefore, special attention must be paid to measures of relationship prevention.

The ventilation measures can be automated in some buildings, provided that an airconditioning system is available. In an air-conditioning system, the recirculation function should be out of operation and the air exchange rates should meet the normative requirements. In these cases, a sufficient air exchange is achieved.

A large part of the school buildings, especially the school buildings, does not have an air conditioning system. In these cases, an air exchange can only be achieved by natural draft ventilation. The simplest and most effective form is window ventilation with the window open as shock ventilation or cross ventilation.

Ventilation can be carried out in the cold season only as a periodic measure since it is not possible to leave windows permanently open in classrooms. The air exchange is also dependent on the temperature difference inside / outside, as well as on the wind speed outside. Therefore, the actual air exchange is variable.

Between periodic aerations, aerosol accumulations with pathogenic microorganisms possibly contained in them may already occur. In addition, certain areas in the room may not be sufficiently ventilated, so aerosols can remain there.

Therefore, it makes sense to create a constant in air treatment. This could be achieved by microbiological room air optimization as room air disinfection.

Many of these measures are based on filtration. This requires large volume flows and correspondingly extensive devices (noise development / energy consumption). In addition, UV-C irradiation and / or activated carbon filtration may be installed. In addition to the problematic operation in the classroom, these devices require the entire volume of the room to be implemented by the device at a certain speed, since only by filtration or irradiation of the air the germs are eliminated.

Furthermore, the devices must be maintained and maintained.

Therefore, room air optimization is initially a sensible approach to supplement and shorten the ventilation maneuvers.

However, the devices used so far on the floor of filtration / irradiation of the air limit the use in classrooms for the aforementioned reasons.

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Therefore, the filtration of indoor air is not convincing as a safe, tried-and-tested, long-term implementable process in the area of medium-sized rooms in which no ventilation system has been installed so far.

However, the method of disinfection with atmospheric low-temperature plasmas can be a locally implementable approach to room air optimization and can usefully supplement the ventilation measures as a method of ratio prevention.

Since the 1980s, atmospheric low-temperature plasmas have been used in various ways for the purpose of germ reduction:

- Plasma generators are used as a medical device for wound disinfection if chronic wounds with superinfection by antibiotic-resistant bacteria are present. In these cases, antibiotic therapy on the basis of secondary bacterial resistance is no longer sufficiently effective, so that such an infection can no longer be adequately treated even by maximum antibiotic therapy. Here the phenomenon of the antimicrobial activity of low-temperature plasmas can be observed, while tissues of the human body are not attacked (since the plasma reaction products that cause the antimicrobial effect are immediately catalytically degraded by higher, eukaryotic tissues, and therefore there is no adverse effect on tissues of higher organisms (plants, animals, humans).
- Furthermore, plasma generators are used in the food industry for residue-free disinfection of beverage bottles after rinsing before filling with the product.

So far, the need for room air optimization has only arisen in rare cases. The SARS-CoV-2 pandemic in 2020 is now forcing new challenges; one of them is the microbiological indoor air condition, which must be optimized by germ-reducing measures (dilution by ventilation, inactivation of the pathogens by e.B. low-temperature plasma products).

"Plasma" in the sense of physics is a state of an electrically conductive gas that is conductive to such an extent that a low current flow becomes possible. Two electrodes are positioned, which are positively and negatively polarized with a defined span. Between the electrodes is the gas in question.

By an ignition impulse, individual gas molecules decay into positively and negatively charged atoms (ions, respectively ionized gas), thereby making the gas conductive (the gas then acts as an ion conductor, i.e., there is a second-order conductor; according to the name "ions", these migrate in the electric field and, among other things, determine the current flow through the gas).

Once this has been done, an inverter limits the current flow through the gas to a small extent in the μ A range, at the same time reducing the voltage. The current flow is reduced to such an extent that a conductivity is just given, i.e., the state of the plasma is maintained between the electrode plates.

The flow of electricity through the conductive gas produces chemical-physical effects on the gas or the components of the gas mixture (air).

The plasma used is – as previously described – an atmospheric low-temperature plasma with a maximum potential difference of 1.75 kV. As a result, a chemical-physical effect is achieved that a proportion of the atmospheric oxygen decomposes into oxygen

radicals, which produce so-called hydroxyl radicals with other atmospheric oxygen molecules and water vapor.

Hydroxyl radicals are the disinfectant-active product of physical plasma.

Therefore, the entire volume of room air does not have to pass through the plasma generator in a certain time to achieve germ killing. Rather, the plasma generator emits active products into the room air, which are formed from oxygen and water molecules, spread in the room air, and destroy microorganisms located there.

High antimicrobial efficacy is observed regarding hydroxyl radicals. At the same time, the hydroxyl radicals are in no way detrimental to higher organisms and humans, but decay catalytically into water through contact with cells of higher organisms. Before an effect on higher organisms would be possible, the hydroxyl radicals have already decayed. Therefore, as already explained above, plasma generators are used to treat bacterial or virally infected wounds.

By limiting the potential difference of the charged plates in the plasma cell to approx. 1.75kV, it is achieved that mainly hydroxyl radicals are formed and the ocon formation is as low as possible. The plasma generator "Air Sanitizer Blue Taction Pro" used for the study generates < 10 μ g of ozone per hour. In electric machines with arc flashovers and interfering arcs (e.B carbon brushes on the commutator of an electric motor), a significantly higher ozone formation is already observed.

At the same time, by limiting the potential difference, it is also ensured that no nitrogen oxides are produced as a by-product of the plasma.

In fact, different products can be produced from the gas mixture of the air by electrochemical reactions. Up to approx. 1.75kV mainly hydroxyl radicals, between 2 and 3.5kV mainly ozone and at higher potential differences than 3.5kV increasingly nitrogen oxides.

Because the electrode potential is limited to 1.75kV, ozone formation is practically not observed as already described. The formation of nitrogen oxides is not possible at all. In addition, the electrodes are made of complex alloys, and the surfaces of the electrodes are specifically treated. The alloying of semiconductors such as boron, germanium and silicon further influences the boundary potential of the surfaces. Therefore, a voltage limitation on the one hand and a corresponding material composition and surface structure of the electrodes on the other hand can ensure that ozone formation is negligible and nitrogen oxide formation is completely excluded.

The device as a measure of relationship prevention is to be tested in classrooms as a supplement to the previously established measures (distance, wearing a mask, everyday hygiene, ventilation).

Specifically, the plasma generator in a classroom is operated under normal use (about 20 students and teaching staff, ventilation, wearing a mask) over a usual teaching morning parallel to the use.





After the lesson day is over, a determination of the number of airborne germs in the room is made.

Airborne bacterial counts are determined in a classroom comparable in terms of size, volume and number of people. Here, all measures are implemented with the exception of the use of the plasma generator.

In addition, a room in peace without use is examined with regard to the number of airborne germs. This room is heated exclusively, but ventilation or the stay of people does not take place.

Finally, the outside air bacterial count outside the building on the day of the measurement is determined.

Once this has been done, the germ count results are compared and evaluated after laboratory sampling and presentation of the colony numbers.

Presentation of sample numbers and sampling points:

01-SMZ-20201106 Outdoor air on 06.11.2020, Schwalmstadt 02-SMZ-20201106 Room without use, without ventilation (R029) 03-SMZ-20201106 Room with use, with ventilation, without plasma generator (R207) 04-SMZ-20201106 Room with use, with ventilation, with plasma generator (R126)

Dimensions of the rooms (with a deviation +/- 10% indicated):

L =	6.60 m	
W =	9.60 m	
H =	3.15 m	
V =	199,58m³	\rightarrow average envelope volume 200m ³



2.Methodology:

2.1 Material

- Basic equipment of a microbiological laboratory:
 - Air germ collector
 - Collecting head with mounting of the culture medium
 - Gas meter
 - Vacuum pump in the form of a diaphragm pump
 - Pipettes, sterile
 - Tweezers, sterile
 - o Vaccines
 - Drigalski spatula, sterile
 - Test tubes for dilution series, sterile
 - Test tube rack
 - Homogenizer (e.B. Vortex ®)
 - Laboratory burners
 - Incubator 36°C
 - Magnifying glass workstation with lighting for evaluation
 - o Sterilizer
 - Camera and color backgrounds (sound cardboard)
- Columbia thoroughbred breeding ground in Petri dishes
- Saline 0.9% sterile
- Air Sanitizer "Blue Taction Pro" as plasma generator test specimen

2.2 Methodology:

Sampling:

On the day of sampling, normal teaching is carried out in the rooms in use. In one of the two classrooms, the plasma generator is operated for 3.5 hours.

All previously established measures (distance, wearing a mask, everyday hygiene and ventilation) will be implemented as defined.

The room without use is not used for 24 hours and is not ventilated.

All rooms are normally heated (20 to 22°C; convector heating with radiators in the rooms).

After the lesson, an airborne count is determined as active impingement directly on the culture media. For this purpose, a special germ collector is used. This consists of an





inward-facing nozzle through which the air is sucked through when a vacuum is applied. The air is evenly distributed through a hole matrix.

The culture medium is placed under the hole matrix. In this way, the air is directed uniformly at right angles to the culture medium. Microorganisms contained in the air are bound to the culture medium surface and can later be cultivated in the laboratory. The air then flows sideways past the culture medium and is sucked out below the culture medium.



The extracted air is then passed through a gas meter to record the volume of air that has been enriched via the culture medium.

The air is sucked out by a commercially available diaphragm vacuum pump.

The enrichment takes place per sample over 10 minutes.

A Columbia whole blood agar culture medium is used as the nutrient medium. This is a microbiological universal medium for the cultivation of almost all aerobic microorganisms.

Incubation

After sampling, the plates are brought to the laboratory and incubated in the incubator for 48 hours at 36°C and water vapour saturation. Incubation is aerobic under air.

In this way, the aerobic, mesophilic colony number of the air is determined. Incubation at 36°C represents the mesophilic human bacteria, so that a human-emanating emission of microorganisms – which is representative of a virus release – can be represented.

The environmentally associated microorganisms are usually psychrophile and show a growth optimum between 10 and 25°C. These are only marginally recorded in the context of the study because the focus of interest is the release of human germs; this is mapped accordingly by mesophilic cultivation.





Evaluation

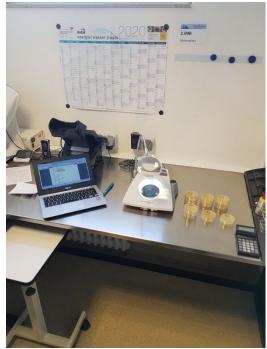
After 48 hours of incubation, the plates are morphologically quantitatively evaluated, i.e., the plates are viewed under normal observation and at 8 times magnification with the incident light microscope.

The colonies are counted and numerically indicated as CFU (colony-forming units).

Finally, taking into account the enrichment volume, the results are given as bacterial count density in CFU/m³.

A fine differentiation of the colonies does not take place at this point.

Subsequently, the results can be evaluated, evaluated and interpreted.



Calculation of microbiocidal efficacy (log. Reduction factor):

The logarithmic reduction factor of the measures is calculated from the colony number when comparing the logarithms of the germ counts.

Example:

No exposure = 12000 CFU (log 4.08) Nwith exposure = 280 CFU (log 2.45)

 $\log RF = \log_{Ninitial} - \log Nnach_{exposure} = 4.08 - 2.45 = 1.63$

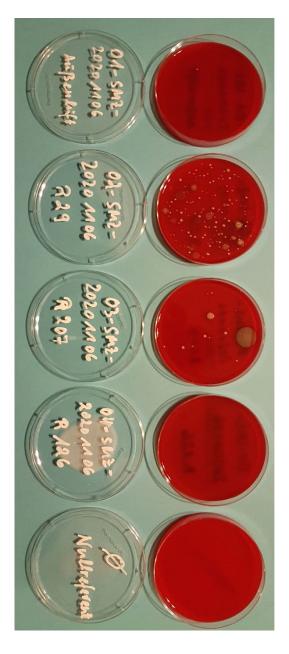
In this constellation, the following bacterial count reduction performance results:

 $^{101.63}$ = 43, i.e., a 43-fold bacterial count reduction, in the logarithmic scale a "two-digit bacterial count reduction", i.e., a bacterial count reduction of about 2 powers of ten is achieved.

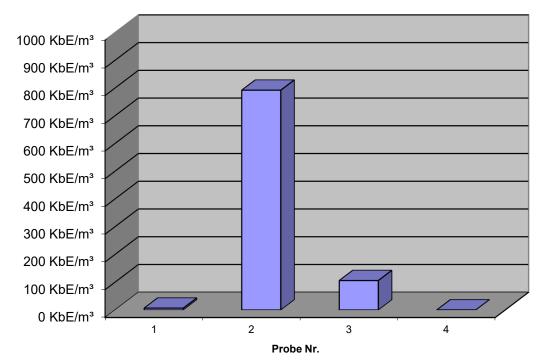


3.Results:

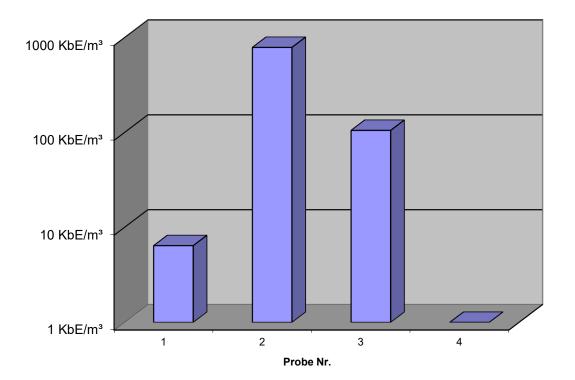
3.1Presentation of the microbiological findings / vegetation of the plates







3.2. Evaluation of microbiological findings



	Α	В	С	D	E	F
1 Ergebnisdarstellung loxMed-Plasmagenerator im schulischen Umfeld, Proben vom 06.11.202						
2						
3						
4	Nr.:	Entnahmestelle:	KbE auf Platte	Luftvolumen:	KbE/m³	log. KbE
5						
6	1	Außenluft	1	0,155 m³	6 KbE/m ³	0,81
7	2	Raum ohne Nutzung, ohne Lüften (R029)	122	0,154 m³	792 KbE/m ³	2,90
8	3	Raum mit Nutzung, mit Lüften, ohne Plasmagenerator (R207)	16	0,151 m ³	106 KbE/m ³	2,03
9	4	Raum mit Nutzung, mit Lüften, mit Plasmagenerator (R126)	< 1	0,156 m ³	< 6 KbE/m ³	< 0,81
10						
11	11 ohne Nutzung = kein Aufenthalt von Personen zur Messung und 12h zuvor					
12	12 mit Nutzung = normaler Unterrichtsbetrieb, ca. 20 Schüler und Lehrpersonal sind zugegen					
13	mit Lüf	ten = Stoßlüftungen alle 20 bis 30 Minuten				
14	14 mit Plasmagenerator = zusätzlich war der Plasmagenerator über den Unterrichtstag im Raum aktivier					
15						
16	KbE = koloniebildende Einheiten = mindeste Zahl von kultvierungsfähigen Bakterien und Pilzen					
17	7 Luftvolumen = das auf dem Nährboden während der Probenahme angereichte Luftvolumen					
18	KbF/m	³ = Keimzahldichte, d.h. mindeste Zahl von kultivierungsfähiger	Bakterien und I	Pilzen pro m ³		

4.Interpretation and evaluation:

First, it should be noted that the outside air bacterial count (sample 01) is low at 6 CFU/m³, this is due to the cold weather on the day of sampling. Night frost and fog/ripening occurred. For this reason, the mentioned low bacterial count was determined.

The outside air bacterial count is a relevant measure, because it indicates the background germ load, which also occurs as a background germ load in the classrooms in natural train ventilation (window ventilation).

The bacterial count in the room air of the room without use and without ventilation (room 029; Sample 02) was found to be the highest indoor air bacterial count with 792 CFU/m³. This can be caused by particle-bound germs. Such particles (organic dust particles, e.B. natural or synthetic fibers; inorganic dust particles, e.B. sand particles; Skin and dandruff) carry microorganisms bound to the particle surface. By heating the room, the particles < 5µm typically circulate through the temperature-related air flow. Due to the fact that there is no ventilation of the room, the particles have accumulated in the indoor air.

In the room with regular use in the classroom (about 20 students and teaching staff; Room 207; Sample 03) and implementation of the previously established measures (distance, mask wearing, everyday hygiene and ventilation), but without plasma generator, a bacterial count density of 106 CFU/m³ was determined.

This first shows the benefit of regular ventilation. Compared to the room without use and without ventilation, the germ count density is about 1 ten-power lower due to regular ventilation – even under regular use – i.e., the bacterial count decreases by 90%.

In view of the low outside air bacterial count of 6 CFU/m³, the bacterial count density of 106 CFU/m³ in the classroom with regular use and ventilation is a power of ten higher. This in turn shows a significant germ entry into the room through use.

At the same time, this shows that the benefit of the masks alone is small. Reduction

rates of 2 powers of ten are given, i.e. 100-fold germ reduction. With sufficient distance, this is a useful addition. Regardless of the wearing of the masks, there is a significant release of microorganisms into the room air.

Therefore, in any case, in parallel, attention must continue to be paid to distance, as well as ventilation.

At the same time, ventilation leads to a significant reduction in germs by exchanging the room air with outside air. Nevertheless, the indoor air bacterial count has been determined ten times higher than the outside air bacterial count, which shows that despite ventilation and masks, a significant bacterial count density is introduced into the room air.

This is understandable, since ventilation in the cold season can not be carried out as a permanent (windows open, draught), but only as a periodic measure (shock ventilation). In the time intervals in which the windows are closed, germ enrichment inevitably occurs. Here, typical germs of the human site flora are detected in sample 03. If this is the case, pathogenic germs such as pneumococci, rhinoviruses or SARS-CoV-2 can also occur, provided that these pathogens are released by one or more people in the room.

With a breath volume at rest of about 0.5 L in adolescents, an air volume of 4200 L is inhaled and exhaled over 30 minutes (time interval between the shock ventilations) with a breathing rate of 14 breaths per minute and 20 people in the room. This is about 2% of the air volume of 200m³ of the rooms examined.

If a strong germ release is carried out by an infection eliminator, this part of the air that has passed through the respiratory tract of the persons in the room can already be infectious.

In the overall view of the situation, it can be stated that the behavioural and relationship prevention measures implemented so far make sense with regard to risk reduction.

Regardless of this, there is a not insignificant residual infectiological risk, which is why additional treatment of the air is required, especially in the cold season, provided that the ventilation measures can no longer be implemented in every way.

The results of sample 04 are taken in an analogous classroom to sample 03 (here: room 126). The room is comparable in terms of room air volume, number of students and use of the room. The difference to Sample 03 is that a plasma generator (Air Sanitizer Blue Taction Pro) was operated in room 126 over 3.5 hours during the teaching sequence of the morning.

Based on the results under 3.1 and 3.2, it can be seen that the indoor air bacterial count could even be lowered below the outside air bacterial count. So, yet another power of ten of the indoor air germ count has been eliminated.

This means that the use of the plasma generator under consideration requires a clear and additional degree of safety. The plasma generator in addition to the previously established measures represents a useful addition and generates an effective risk reduction considering the results of this investigation.

The gap in transmission, which has existed so far independently of the previously established measures, can be closed by the plasma generator investigated.

Thus, the investigated plasma generator represents an additional and effective means of ratio prevention.

In this way, it is acceptable that deficits of ventilation (in severe cold outside or by a non-reproducible air exchange with a small temperature difference inside / outside) are compensated by permanent use of the plasma generator.

Due to the nature of the active products of the plasma generator, it is possible that such plasma generators can be operated constantly and parallel to the classroom.

As a result, a continuous disinfection of the room air is achieved, so that the previously described germ enrichment is omitted in the time intervals between the ventilation processes.

Overall, the plasma generator "Air Sanitizer Blue Taction Pro" is a very effective and useful method for indoor air treatment. In addition to the measures established so far, a rounding off of the prevention measures is achieved and the risk of aerogenic germ transmission is significantly reduced to a maximum low level.

The observation was exemplified in public space using the example of the setting school and teaching. In the same way, the results can be transferred to other public spaces, e.B offices, waiting rooms, treatment rooms, restaurants, hotels, congress centers and many more.

In a further assessment of the Air Sanitizer Pro device, the microbiocidal efficacy of the method against aerogenic Enteroccocus faecium bacteria was tested. For this purpose, Enterococcus faecium was introduced into a room in the form of an aerosol and the natural bacterial count density decrease was compared with the decrease in the bacterial count, which is present when the device is active.

The reduction performance determined in this present report on a real germ exposure is also proven in laboratory tests with Enterococcus faecium.



Enterococcus faecium is used as a test germ because explicit test germs for indoor air are not defined and Enteroccocus faecium is used to test the disinfection performance of industrial washing machines and dishwashers. The elimination of Enteroccocus faecium essentially involves the elimination of other important infectious bacteria and enveloped viruses (including the SARS-CoV-2 virus). Pathogen groups not covered by Enterococcus faecium are mycobacteria and unenveloped viruses, which are also not the subject of the question here.

The investigations for this opinion were already carried out in November / December 2020. The final report was written in May, as the assessor was waiting for official instructions / draft standards for further evaluation. Since these were not available by the end of quarter 1 / 2021, the report was then written in quarter 2 / 2021 at the end of quarter 1 / 2021.

If you have any questions, please contact the assessor at 0175 / 9150334.

Sincerely,

(Minich Polimet 2

Priv.-Doz. Dr. med. Ulrich F. Schmelz, 20.05.2021

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- Ms. Senior Director of Studies Marion Temme
- Mr. Director of Studies Ralf Lohse

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Mr. Mario Wagner

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