EVALUATION OF AN AUTOCLAVE RESISTANT ANATOMIC NOSE MODEL FOR THE TESTING OF NASAL SWABS

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A nose model that allows for the comparison of different modes of sample acquisition as well as of nasal swab systems concerning their suitability to detect defined quantities of intranasal microorganisms, and further for training procedures of medical staff, was evaluated.

Based on an imprint of a human nose, a model made of a silicone elastomer was formed. Autoclave stability was assessed. Using an inoculation suspension containing *Staphylococcus aureus* and *Staphylococcus epidermidis*, the model was compared with standardized glass plate inoculations. Effects of inoculation time, mode of sampling, and sample storage time were assessed.

The model was stable to 20 autoclaving cycles. There were no differences regarding the optimum coverage from the nose and from glass plates. Optimum sampling time was 1 h after inoculation. Storage time after sampling was of minor relevance for the recovery. Rotating the swab around its own axis while circling the nasal cavity resulted in best sampling results.

The suitability of the assessed nose model for the comparison of sampling strategies and systems was confirmed. Without disadvantages in comparison with sampling from standardized glass plates, the model allows for the assessment of a correct sampling technique due to its anatomically correct shape.

Keywords: nose model, swab, *Staphylococcus aureus*, hygiene testing, autoclave resistant

Introduction

The nasal vestibulum is a typical locus that is often colonized by multidrug-resistant microorganisms like methicillin-resistant *Staphylococcus aureus* (MRSA) that can cause severe nosocomial infections. Particularly, the vestibular region of the nose is a common place from which this agent can be isolated.

Quality, reliability, and reproducibility of diagnostic results depend on pre-diagnostic features like the ideal sampling and transport of specimen. While great efforts have been made to standardize the transport of specimens, there is an alerting lack of studies dealing with the correct sampling techniques. However, optimal

sampling is crucial for reliable diagnostic results, especially if the bacterial density is low, e.g., in a mixed bacterial flora. Modern molecular diagnostic platforms have a limit of detection (LOD) as low as $<10^2$ colony forming units (cfu)/ml [1]. Broth culture should allow for the detection of even one single vital colony forming unit.

Accordingly, it is of great importance to acquire even small bacterial quantities by correct sampling techniques. We developed and evaluated a model that allows the comparison of different modes of sampling within a correct anatomical environment. Further, their suitability to detect defined quantities of intranasal microorganisms can be addressed.

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Materials and methods

Design of the nose model

An anatomically correct wax imprint of a human nose of a corpse was formed at the Institute for Anatomy, University Hospital Rostock. The imprint was then used as a matrix to form a model made of an addition-vulcanizing silicone at the Department of Prosthodontics and Material Science at the University Hospital Rostock (Germany) (Fig. 1a). An addition silicone (Dublisil 15; Dreve Dentamid, Unna, Germany) was chosen and processed according to manufacturer's specifications as it best resembles human nose vestibulum tissue with respect to surface texture and rigidity.

Further, posterior slots (Fig. 1b) for the connection to a vacuum pump were integrated into the model to allow for a use as a breathing model.

A consolidation of the material was performed by three times alternating autoclaving and heating to 90 °C in a pH-neutral tenside solution (a household scavenger).

Autoclave stability testing of the nose models

Autoclave stability of the silicone models was assessed by autoclaving for 30 times using the standard scheme for solid products (134 °C temperature, 1.1 bar air pressure, 3 min).

Definition of optimal bacterial inoculation for sampling experiments

To define the optimum bacterial inoculation dose of the nose models, the recovery of different inoculation densities of *S. aureus* 1 h after inoculation was assessed. Therefore, an overnight-culture of an *S. aureus* reference strain

(ATCC [American type culture collection] 25923) was grown on Columbia agar enriched with 5% sheep blood (BD, Heidelberg, Germany). An optical density of McFarland 0.4 was adjusted in physiological sterile 0.9% sodium chloride solution. Decadic logarithmic dilution steps were performed in 0.9% sodium chloride solution. Quantities of 10 µl were placed at a randomly chosen point in the nasal vestibulum using a 10-µl-inoculation loop and air-dried. Afterwards, the whole nasal vestibulum was thoroughly swabbed with a moist (moistened with 0.9% sterile sodium chloride solution) cotton swab (MEDIWOOD cotton swabs "wood," 15 cm long, 2.5 mm diameter, cotton front end 4 mm diameter, MEDIWOOD, London, UK). After swabbing, the swab was smoothed over the agar plates under rotation as it is typical for diagnostic microbiology. Quantitative cultural growth in 10²-decadic logarithmic dilution steps was added. Aims were the definition of the bacterial quantity; the application leads to a reproducible recovery of easily countable quantities of 10¹ to 10³ bacterial colonies from the noses. If the application led to the recovery of 10¹ to 10³ bacterial colonies, this dilution was defined as the standard suspension.

Thus, defined conditions for sample preparations were set up as a golden standard for subsequent analyses. For experiments with mixed colonization flora, a 100-fold higher concentration of the *Staphylococcus epidermidis* reference strain (DSM ("Deutsche Stammsammlung") 1798) was added.

If experimental settings were tested, which were highly likely to lead to reduced recovery rates, a 100-fold concentrated standard suspension (regarding both *S. aureus* and *S. epidermidis* concentrations) was used to ensure sufficient yields for an analysis. The 100-fold concentrated standard suspension was used for the following experiments: the comparison of altered sampling techniques, comparisons of different surfaces for swabbing and swabbing after more than 1 h after inoculation, as well as the comparison of different sample storage times.

Fig. 1. (a) Front view of the silicone elastomer nose model for standardized testing of swabs. (b) Posterior view. Posterior openings of the nostrils allow for a connection with a vacuum pump for simulated "inhalation" experiments with sedimentation of aerosolized pathogens

Definition of the ideal sampling time

The *S. aureus*/*S. epidermidis* working dilution was applied to the nostrils. Sampling with moist cotton swabs was performed 1, 6, and 12 h after inoculation. The cultural results were compared.

Comparison of inoculated nostrils and glass plates

After inoculation of a 100-fold concentrated *S. aureus*/*S. epidermidis* working dilution into the nostrils and on glass plates, sample acquisition was performed after 1 h with moist cotton swabs. Cultural results were compared.

Comparison of three different sampling techniques

After inoculation of a 100-fold concentrated *S. aureus*/*S. epidermidis* working dilution into the nostrils, sample acquisition was performed after 1 h with moist cotton swabs in three different ways: 1) one circle of the swab within the nasal vestibulum, 2) one circle of the swab within the nasal vestibulum with additional rotation around its own axis, and 3) one circle of the swab within the nasal vestibulum with additional rotation around its own axis and additional alternating inward–outward movement. Cultural results were compared.

Definition of the influence of storage time

After inoculation of a 100-fold concentrated *S. aureus*/*S. epidermidis* working dilution into the nostrils, sample acquisition was performed after 1 h, and storage of the swabs in sterile glass tubes under room temperature conditions was performed for 1 h, 2 h, 6 h, and 24 h, respectively. The numbers of recovered bacteria were compared.

Statistics

Test series were compared using Student's bidirectional *t*-test with unequal variances. Each test series had its own defined internal golden standard, i.e., the standard procedure with which altered procedures were compared, to avoid interpretative errors due to potentially lacking repro-

ducibility from own experimental preparation to another. $p < 0.05$ was considered as significant, $p < 0.01$ as very significant, and $p < 0.001$ as highly significant.

Results

Autoclave stability of the nose model

After the described consolidation steps of the silicone elastomere nose model, the shape close to the anatomic origin was preserved even after repeated autoclaving cycles. The model was stable for at least 20 autoclaving cycles. As subjectively assessed, the models had to be replaced due to a beginning loss of elasticity after more than 20 autoclaving cycles.

Definition of the ideal *S. aureus* working dilution for the inoculation of the nostrils and the ideal time for taking the swabs

An *S. aureus* suspension in sterile 0.9% sodium chloride was adjusted to an optical density of McFarland 0.4 and further diluted by 1:1000, 1:10,000, and 1:100,000 in sterile 0.9% sodium chloride. After preparing the charge of noses, moist cotton swabs were taken after 1 h, resulting in a linear dilution. For the 1:1000 dilution of McFarland 0.4, recovery was 356 colony forming units (cfu) $\pm$ 109 (standard deviation [SD], $n = 5$); for the 1:10,000 dilution, it was 31 cfu $\pm$ 12 (SD, $n = 5$); and for the 1:100,000 dilution, it was 4 cfu $\pm$ 1 (SD, $n = 5$) (Table 1).

When the swabs were taken from the nasal cavities after 6 h, the quantities of the recovered colonies were reduced by 90%. For the 1:1000 dilution of MacFarland 0.4, recovery was 32 cfu $\pm$ 3 (standard deviation SD, $n = 5$); for the 1:10,000 dilution, it was 2 cfu $\pm$ 1 (SD, $n = 5$); and for the 1:100,000 dilution, it was 0.4 cfu $\pm$ 0.5 (SD, $n = 5$) (Table 1).

Accordingly, optimum conditions for sample acquisition to achieve well-countable results were inoculation of the noses with a 1:10,000 dilution of an *S. aureus* suspension adjusted to McFarland 0.4 and sampling after 1 h air drying at room temperature. To simulate mixed colonization flora in later experiments, a 100-fold higher concentration of *S. epidermidis* was added. This combination is referred to as the standard suspension in the following.

Table 1. Identification of optimal *S. aureus* suspension concentration and sampling time from the nose models. Identified optimum conditions are highlighted in grey and bold typing

Dilution of an <i>S. aureus</i> suspension in 0.9% sodium chloride solution, initially adjusted to McFarland 0.4	1:1000	1:10,000	1:100,000
Recovered colony forming units (cfu, $\pm$ standard deviation [SD]) on agar in case of swabbing 1 h after inoculation ($n = 5$)	356 $\pm$ 109	31 $\pm$ 12	4 $\pm$ 1
Recovered colony forming units (cfu, $\pm$ standard deviation [SD]) on agar in case of swabbing 6 h after inoculation ($n = 5$)	32 $\pm$ 3	2 cfu $\pm$ 1	0.4 $\pm$ 0.5

Table 2. Identification of optimal sampling time from the nose models using the standard suspension. Identified optimum conditions are highlighted in grey and bold typing

Inoculation of the nose models with the standard suspension containing <i>S. aureus</i> and <i>S. epidermidis</i> . The <i>S. epidermidis</i> concentration was 100-fold higher than the <i>S. aureus</i> concentration.		
Recovery of	<i>S. aureus</i>	<i>S. epidermidis</i>
Recovered colony forming units (cfu, $\pm$ standard deviation [SD]) on agar in case of swabbing 1 h after inoculation ($n = 25$)	21 $\pm$ 5	1137 $\pm$ 102
Recovered colony forming units (cfu, $\pm$ standard deviation [SD]) on agar in case of swabbing 6 h after inoculation ($n = 20$)	0.7 $\pm$ 0.8	9 $\pm$ 8
Recovered colony forming units (cfu, $\pm$ standard deviation [SD]) on agar in case of swabbing 12 h after inoculation ($n = 20$)	0.6 $\pm$ 1	13 $\pm$ 14

Table 3. Comparison of inoculated nose models and glass plates regarding the recovery of *S. aureus* and *S. epidermidis* after swabbing

Inoculation of nose models and glass plates with a 100-fold concentrated standard suspension containing <i>S. aureus</i> and <i>S. epidermidis</i> . The <i>S. epidermidis</i> concentration was 100-fold higher than the <i>S. aureus</i> concentration.				
Inoculation site	Nose model		Glass plate	
Recovery of	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. aureus</i>	<i>S. epidermidis</i>
Recovered colony forming units (cfu, $\pm$ standard deviation [SD]) on agar in case of swabbing 1 h after inoculation ($n = 20$)	252 $\pm$ 46	3227 $\pm$ 546	258 $\pm$ 37	3319 $\pm$ 490
Recovered colony forming units (cfu, $\pm$ standard deviation [SD]) on agar in case of swabbing 6 h after inoculation ($n = 20$)	123 $\pm$ 39	844 $\pm$ 224	137 $\pm$ 11	2082 $\pm$ 289
Recovered colony forming units (cfu, $\pm$ standard deviation [SD]) on agar in case of swabbing 24 h after inoculation ($n = 20$)	85 $\pm$ 13	1341 $\pm$ 132	86 $\pm$ 13	1366 $\pm$ 115

Comparison on the influence of the sampling time after inoculation with the standard suspension

The standard suspension of *S. aureus* and *S. epidermidis* was applied to the nose models. Moist cotton swabs were taken after 1 h, 6 h, and 12 h. One hour after inoculation, 21 cfu *S. aureus* $\pm$ 5 (SD, $n = 25$) and 1137 cfu *S. epidermidis* $\pm$ 102 (SD, $n = 25$) were recovered (Table 2). After 6 h, it was a mean of 0.7 cfu *S. aureus* $\pm$ 0.8 (SD, $n = 20$) and 9 cfu *S. epidermidis* $\pm$ 8 (SD, $n = 20$); while after 12 h, it was 0.6 cfu *S. aureus* $\pm$ 1 (SD, $n = 20$) and 13 cfu *S. epidermidis* $\pm$ 14 (SD, $n = 20$), respectively (Table 2). For the comparisons of 1 h vs. 6 h and 1 h vs. 12 h, a significant difference ($p < 0.001$) was detected, while there was no significance for a difference between sampling 6 and 12 h after inoculation. One hour after, inoculation was defined to be the optimum standard for sampling.

Comparison of swab taking from inoculated noses and glass plates

A 100-fold concentrated standard suspension was used for the experiment. In direct comparison of moist cotton swabs from contaminated noses and glass plates, the re-

sults for *S. aureus* were 252 cfu $\pm$ 46 (SD, $n = 20$) vs. 258 cfu $\pm$ 37 (SD, $n = 20$) after 1 h, 123 cfu $\pm$ 39 (SD, $n = 20$) vs. 137 cfu $\pm$ 11 (SD, $n = 20$) after 6 h, and 85 cfu $\pm$ 13 (SD, $n = 20$) vs. 86 cfu $\pm$ 13 (SD, $n = 20$) after 24 h, respectively (Table 3). For *S. epidermidis*, the results were 3227 cfu $\pm$ 546 (SD, $n = 20$) vs. 3319 cfu $\pm$ 490 (SD, $n = 20$) after 1 h, 844 cfu $\pm$ 224 (SD, $n = 20$) vs. 2082 cfu $\pm$ 289 (SD, $n = 20$) after 6 h, and 1341 cfu $\pm$ 132 (SD, $n = 20$) vs. 1366 cfu $\pm$ 115 (SD, $n = 20$) after 24 h, respectively (Table 3). There was no significance for any difference between inoculated noses and glass plates regarding the recovery of both *S. aureus* and *S. epidermidis*.

Comparison of the sampling techniques from inoculated noses

A 100-fold concentrated standard suspension was used for the experiment. The compared sampling techniques comprised (1) one circle of the swab within the nasal vestibulum, (2) one circle of the swab within the nasal vestibulum with additional rotation around its own axis, and (3) one circle of the swab within the nasal vestibulum with additional rotation around its own axis and additional alternating inward–outward movement. For *S. aureus*, the results

Table 4. Identification of the optimal sampling technique from the nose models regarding the recovery of *S. aureus* and *S. epidermidis*

Inoculation of the nose models with a 100-fold concentrated standard suspension containing *S. aureus* and *S. epidermidis*. The *S. epidermidis* concentration was 100-fold higher than the *S. aureus* concentration. Swabbing was performed 1 h after inoculation.

Recovery of	<i>S. aureus</i>	<i>S. epidermidis</i>
Recovered colony forming units (cfu, $\pm$ standard deviation [SD]) on agar in case of swabbing by one circle of the swab within the nasal vestibulum ($n = 6$)	82 $\pm$ 27	304 $\pm$ 138
Recovered colony forming units (cfu, $\pm$ standard deviation [SD]) on agar in case of swabbing by one circle of the swab within the nasal vestibulum with additional rotation around its own axis ($n = 7$)	125 $\pm$ 32	381 $\pm$ 137
Recovered colony forming units (cfu, $\pm$ standard deviation [SD]) on agar in case of swabbing by one circle of the swab within the nasal vestibulum with additional rotation around its own axis and additional alternating inward-outward movement ($n = 7$)	172 $\pm$ 79	428 $\pm$ 81

Table 5. Influence of sample storage time after swabbing regarding the recovery of *S. aureus* and *S. epidermidis*

Inoculation of the nose models with a 100-fold concentrated standard suspension containing *S. aureus* and *S. epidermidis*. The *S. epidermidis* concentration was 100-fold higher than the *S. aureus* concentration.

Storage time after swabbing	According to the experiment ($n = 20$)		Immediate cultural growth ($n = 5$)	
Recovery of	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. aureus</i>	<i>S. epidermidis</i>
Recovered colony forming units (cfu, $\pm$ standard deviation [SD]) on agar in case of storage for 1 h after swabbing	974 $\pm$ 401	15,550 $\pm$ 1521	2454 $\pm$ 362	22,534 $\pm$ 2375
Recovered colony forming units (cfu, $\pm$ standard deviation [SD]) on agar in case of storage for 2 h after swabbing	1329 $\pm$ 495	14,386 $\pm$ 3205	2367 $\pm$ 368	21,958 $\pm$ 2904
Recovered colony forming units (cfu, $\pm$ standard deviation [SD]) on agar in case of storage for 6 h after swabbing	984 $\pm$ 266	14,319 $\pm$ 3653	2237 $\pm$ 380	20,743 $\pm$ 3024
Recovered colony forming units (cfu, $\pm$ standard deviation [SD]) on agar in case of storage for 24 h after swabbing	934 $\pm$ 185	13,151 $\pm$ 2995	2232 $\pm$ 353	21,159 $\pm$ 2489

were 82 cfu $\pm$ 27 (SD, $n = 6$) vs. 125 cfu $\pm$ 32 (SD, $n = 7$) vs. 172 cfu $\pm$ 79 (SD, $n = 7$); for *S. epidermidis*, they were 304 cfu $\pm$ 138 (SD, $n = 6$) vs. 381 cfu $\pm$ 137 (SD, $n = 7$) vs. 428 cfu $\pm$ 81 (SD, $n = 7$), respectively (Table 4). The first technique was inferior to the second and third ones ($p < 0.05$), while there was no significant difference between techniques two and three regarding the recovery of *S. aureus*. With regard to the recovery of the *S. epidermidis*, there was a significant difference between the first and the third technique ($p < 0.05$), while no significant differences between the first and the second or the second and the third technique were observed.

Influence of sample storage time after sampling from the noses

A 100-fold concentrated standard suspension was used for the experiment. Sampling from the noses with moist cotton swabs was performed 1 h after artificial contamination. Storage in sterile glass tubes at room temperature was performed for 1, 2, 6, and 24 h.

After 1 h of storage compared to direct bacterial growth after sample acquisition, 974 cfu $\pm$ 401 (SD, $n = 20$) vs. 2454 cfu $\pm$ 362 (SD, $n = 5$) *S. aureus* was recovered ($p < 0.001$), i.e., a recovery rate of 39.7%. For *S. epidermidis*, it was 15,550 cfu $\pm$ 1521 (SD, $n = 20$) vs. 22,534 cfu $\pm$ 2375 (SD, $n = 5$), i.e., a recovery of 69.0% ($p < 0.01$) (Fig. 2, Table 5).

After 2 h of storage, 1329 cfu $\pm$ 495 (SD, $n = 20$) *S. aureus* was observed in comparison to 2367 cfu $\pm$ 368 (SD, $n = 5$) directly after sample acquisition ($p < 0.001$). Accordingly, the total recovery rate was 56.1%. For *S. epidermidis*, the difference was 14,386 cfu $\pm$ 3205 (SD, $n = 20$) vs. 21,958 cfu $\pm$ 2,904 (SD, $n = 5$), resulting in a recovery of 65.5% ($p < 0.01$) (Fig. 2, Table 5).

A mean of 984 cfu $\pm$ 266 (SD, $n = 20$) *S. aureus* was identified after 6 h of storage, while 2237 cfu $\pm$ 380 (SD, $n = 5$) was observed if cultural growth was performed directly after sampling ($p < 0.001$). Thus, 44.0% could be recovered. In comparison, 14,319 cfu $\pm$ 3653 (SD, $n = 20$) vs. 20,743 cfu $\pm$ 3024 (SD, $n = 5$) *S. epidermidis* was grown on agar, i.e., a recovery of 69.0% ($p < 0.01$) (Fig. 2, Table 5).

Fig. 2. Effects of sample storage after sampling for up to 24 h. Pooled data are shown, comprising results of four independent experiments, each including 25 inoculated nose models. Inoculation of the nose models was performed with a 100-fold concentrated standard suspension containing *S. aureus* and *S. epidermidis*. The inoculated *S. epidermidis* concentration was 100-fold higher than the *S. aureus* concentration. The bars indicate mean recovery proportions (in percent, $\pm$ standard deviation [SD]) of *S. aureus* and *S. epidermidis* after storage for several hours ($n = 20$ per experiment) in comparison to immediate cultural growth after swabbing ($n = 5$ per experiment)

Finally, a difference of $934 \text{ recovered cfu} \pm 185 \text{ (SD, } n = 20)$ *S. aureus* after 24 h of storage vs. $2232 \text{ cfu} \pm 353 \text{ (SD, } n = 5)$ after growth without delay ($p < 0.001$) was shown, so 41.8% of the colonies were recovered after 1 day of storage. For *S. epidermidis*, this difference was $13,151 \text{ cfu} \pm 2995 \text{ (SD, } n = 20)$ vs. $21,159 \text{ cfu} \pm 2489 \text{ (SD, } n = 5)$, with a recovery of 62.2% ($p < 0.01$) (Fig. 2, Table 5).

Discussion

The developed nose model allows for the comparative testing of different modes of sample acquisition or swab systems, providing both an anatomical correct nasal vestibulum and the possibility of repeatable autoclaving cycles as recently demanded [2]. Infectious agents can be applied with standardized loop wires or pipettes. Charges with identical autoclaving cycle numbers are recommendable for comparative analyses, because each autoclaving cycle will slightly change the silicone elastomer effecting both elasticity and frictional coefficient. A prior standardization for each infectious agent appears necessary prior to each comparison in order to define optimal inoculation quantities for the microorganism of interest.

A study to characterize the model using an optimized suspension of *S. aureus* and *S. epidermidis* strains was performed after the definition of optimum concentrations. Mixed cultures relevantly limit the probability of positive results; therefore an *S. aureus*/*S. epidermidis* composition was used to assess various commercial test systems. It could be demonstrated that reliable coverage requires sampling about 1 h after the inoculation of the nostrils. Under such conditions, there is no difference between test-

ing with glass plates and the here described anatomic nose model. In contrast to the glass plate, however, the nose models allow for an assessment of the optimum sampling technique under realistic anatomic conditions. Especially rotation was shown to be of major importance for a good recovery of bacteria.

S. aureus is considered as a rather stable bacterium regarding its environmental resistance [3–6]. Nevertheless, sampling after 6 h and more after the inoculation of the nostrils led to relevant reduction of recovered bacteria. This finding is in line with previously described data. If stored at room temperature, 9% of *S. aureus* containing samples are negative after 24 h, 29% after 48 h; if stored at 4°C , the die-off rates increase to 47% and 49%, respectively [7]. Other authors even described a decrease of positive results by 30% after 2–5 h of storage at room temperature [8]. Therefore, only freshly inoculated nose models should be used. In contrast, the viability of staphylococci remained stable after sampling for 24 h.

Conclusion

In summary, we could show that the described nose model is suitable for the evaluation of swab systems and evaluation of respective pre-analytic conditions. It could also be used as a tool to compare differences in sampling habits before and after respective training programs to teach circular swabbing of the whole nasal vestibulum. If the wrong sampling technique is applied, resulting weak recovery of bacteria can be directly monitored as recently demonstrated [2]. Further future applications could include the testing of inhaled pathogenic microorganisms, sedimented after simulated “inhalation” us-

ing a vacuum pump connected to posterior openings of the nostrils.

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