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Field assessment of a novel sensor for measuring noncondensable gases in steam sterilizers

Francesco Tessarolo✉ & Michela Masè

Steam sterilization is widely used to process heat-resistant instruments in hospital settings. The presence of non-condensing gases (NCGs) in the steam may severely impact heat transfer and process efficacy. However, the current practice does not include routine NCG monitoring in the sterilizer chamber. This study evaluates the capability of a novel sensor to detect and quantify NCGs in the sterilizer chamber under different conditions occurring in the field. A commercially available steam sterilizer was equipped with the NCG sensor, and a range of sterilization processes were run. NCGs in the sterilizer chamber were obtained by inadequate vacuum during conditioning, or by introducing controlled air volumes during the conditioning or the exposure phase using different access points. Tests were performed with or without load into the chamber. NCG measurements were analysed by mixed-effects models. The sensor provided reproducible measurements of NCGs resulting from ineffective conditioning (marginal $R^2 = 0.88$) or introduced during the conditioning (marginal $R^2 = 0.97$) and exposure phase (marginal $R^2 = 0.96$), with negligible effects of access points or load amounts. The availability of a quantitative measure of steam composition, added to time and temperature, represents a relevant advancement in real-time monitoring of every sterilization process, toward the full-parametric release of steam sterilized loads.

Keywords Steam sterilization, Non-condensable gases, Sterilization conditions, Steam composition, Process monitoring

Steam sterilization is widely used to process heat-resistant instruments in hospital settings[1]. A typical sterilization process includes three main phases: conditioning, aimed at substituting the air with steam in the sterilizer chamber; exposure, when steam heats the load to a target temperature mainly by condensation heat transfer; and drying, required to remove condensate from the load and safely open the sterilizer door[2].

Sterilization conditions are achieved during the exposure phase, when steam at a specified temperature condenses on all the accessible load surfaces during a given time interval, releasing the water and energy necessary for protein coagulation and microorganisms' inactivation[2,3]. Combinations of time and temperature that are effective for steam inactivation of microorganisms were reported by Perkins several decades ago[4]. Some years later, the Working party on pressure-steam sterilization revised those time–temperature combinations to take into account the possible presence of a fraction of air in the sterilizer chamber[5], since air is not able to release thermal energy to the load by condensation. Indeed, in steam sterilization practice, a variable fraction of non-condensing gases, often referred to as non-condensable gases (NCGs), is mixed with water vapour. To guarantee that condensation is taking place, the Working party considered wise to ensure that during the exposure phase the NCGs content of the steam in any part of the load should not exceed 5% by volume[5]. However, even smaller fractions of NCGs in the free space of the sterilizer chamber can significantly impact the process of heat transfer from the steam to the load surface[6,7]. Steam composition is, therefore, the third essential parameter, together with temperature and time, to be monitored in a steam sterilization process[8]. Several studies provided evidence that every sterilization process is unique and steam composition varies in each process, stressing the importance of monitoring steam composition for every load and process[9,10].

Current standards[6] and technical memorandums[11] propose a variety of methods for checking steam composition, indicating different locations, timings, and frequencies for steam sampling. As part of the qualification and requalification of the sterilizer, it is recommended to perform steam quality tests, including the quantification of NCGs amount, in the piping system connecting the steam generator to the sterilizer chamber[6]. Steam ability to properly penetrate a daily Bowie and Dick (B&D) test[12] is also assumed as an indirect qualitative proof of sufficient steam quality in the sterilizer chamber for that specific production day[6], despite the variable

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performance and accuracy[13] of commercially available alternative B&D tests[14] and the low sensitivity to relevant NCGs amounts[15]. In addition to these methods, "air detector" devices have been proposed to qualitatively monitor air leakage in and air removal from the sterilizer chamber[11,16], but their frequency of use is not specified in the standards[6].

Among these methods and devices, only the approach specified in clause 21.1.1 of EN 285:2015 + A1:2021[6] aims to provide quantitative measurements of NCGs in the steam, expressed as mL of NCGs in 100 mL of condensate. Clause 13.3.1 of the same standard sets an upper limit for the consented NCGs amount of 3.5 mL of NCGs in 100 mL of steam condensate. However, the standard specifies that the proposed method "*should not be regarded as measuring the exact level of non-condensable gases during normal use of the sterilizer*"[6], which stresses the need for alternative methods for routine monitoring of the sterilization process.

A device able to monitor steam composition based on heat-flux measurements has been recently proposed to perform continuous measurements of NCGs amount in the sterilizer chamber during the exposure phase of each single sterilization process[7].

The present study aims to evaluate the capability of this sensor to identify and quantify NCGs in the sterilizer chamber under a broad spectrum of scenarios, which include different sources of NCGs, variable locations of the sources, and different timing when NCGs enter the sterilizer chamber. To assess the added value of this measurement technique in monitoring the variety of steam sterilization processes present nowadays in the field, experimental conditions included different process profiles and amounts of load in the sterilizer chamber.

Materials and methods

The study design included a set of experimental protocols, which reproduced different scenarios leading to the presence of NCGs in the sterilizer chamber. Specifically, we tested the capability of the sensor to detect and quantify NCGs associated with the following conditions relevant to the sterilization practice: (i) presence of NCGs in the sterilizer chamber due to inadequate vacuum in the conditioning phase (e.g., inefficient vacuum pump); (ii) admission of NCGs in the sterilizer chamber during sub-atmospheric phases (e.g., air admission from a leaking gasket); (iii) introduction of NCGs in the sterilizer chamber during super-atmospheric phases (e.g., air injection from a leaking pneumatic valve).

NCG sensor

A NCG sensor (Solid Too, Veldoven, The Nederlands) was used in the study. The sensor was composed of a L-shaped thermally-insulated stainless-steel pipe with a closed end and an open end. The open end was connected to the steam sterilizer chamber by a tri-clamp connector via a validation port (Fig. 1a). The closed

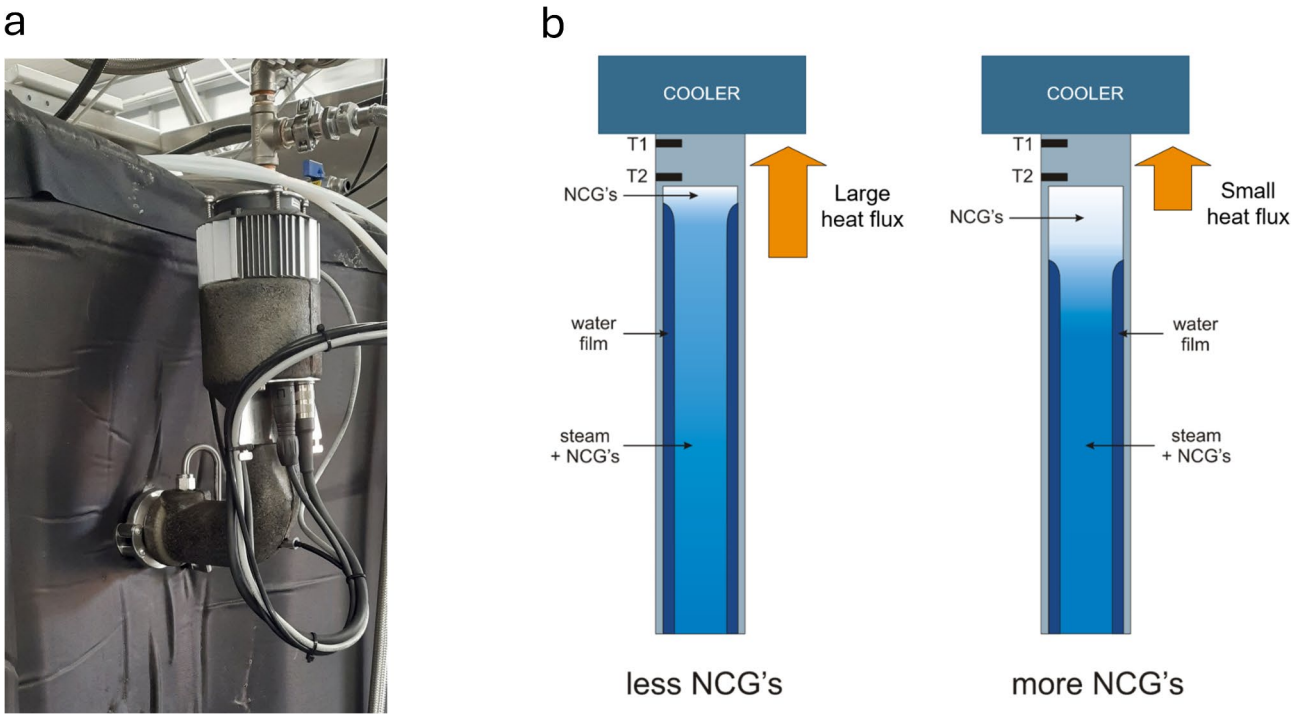

Fig. 1. Positioning and working principle of the NCG sensor. (**a**) NCG sensor installed at the lateral high validation port of the sterilizer chamber. (**b**) Schematic representation of the NCG sensor working principle in the presence of lower (left) or larger (right) amounts of NCGs in the sterilizer chamber. dT_{sink} is the difference between temperature measurements T2 and T1 and depends on the amount of NCGs in the steam. The lower the NCGs amount, the larger the heat flux and the larger the dT_{sink}. Adapted with permission from www.solidtoo.com.

end of the tube was in contact with the surface of a stainless-steel block, while the opposite face of the block was cooled and maintained at a predefined temperature (e.g., 30 °C) by a Peltier cell and a fan. The working principle is schematized in Fig. 1b. The heat flux from the hot to the cold face of the block was monitored by two thermometric probes located at increasing distances from the hot face. The sensor allowed the quantification of the NCGs in the steam of the sterilizer chamber during the exposure phase, based on the measurement of the temperature difference, dT_{sink}, between the two probes. The higher the NCGs in the sterilizer chamber, the lower the heat flux through the block and the dT_{sink} between the probes[17,18]. In other words, the sensor measured the ability of the steam to transfer heat to a cold surface. The higher was the steam efficiency in heating the surface (i.e., higher dT_{sink} values) the lower was the amount of NCGs in the steam.

The NCG sensor was calibrated by the manufacturer to allow the conversion of dT_{sink} measurements into percent of NCGs (mL of NCGs in 100 mL of steam condensate), in the range from approx. 0.5 to 7.5% during the exposure phase. Maximum and minimum values of NCGs during the exposure phase of each single process were provided in the sensor report at the end of each process. The NCG sensor was also equipped with a temperature and a pressure probe to monitor the sterilization chamber conditions independently from the steriliser. All probes provided measurements sampled at a 1 Hz frequency during the whole duration of the sterilization process. The temperature probes positioned in the NCG sensor block were Pt1000 resistance temperature detectors complying with IEC 60751:2022 class A or better as specified in the EN285:2015 + A1:2021[6]. All temperature probes were from the same manufacturer and production batch to grant an uncertainty in the differential temperature measurement (i.e., dT_{sink}) lower than 0.2 °C. The small uncertainty on dT_{sink} guaranteed that, around the NCG threshold value of 3.5%, the uncertainty on the derived NCG fractions was largely lower than that associated to the measurement method specified in clause 21.1.1 of EN 285:2015 + A1:2021[6] (see Supplementary Material for further details).

Steam steriliser

The sterilizer used for all the experiments was a validated, commercially available steam autoclave (VS8/2, Steelco, Riese Pio X, Italy). The chamber size (670 mm × 700 mm × 1310 mm, 614 l) fitted eight sterilization units. The sterilizer was equipped with an onboard water degassing system (15 l tank, 95 °C nominal working temperature) to remove most NCGs dissolved in the water before admission to the electric steam generator. The degasser tank was fed with reverse osmosis water with 1.3 µS conductivity, spilt from a 1000 l dedicated tank, and maintained at a temperature between 24 and 28 °C. To further reduce the amount of NCGs in the produced steam, water refill in the boiler was prevented when steam injection in the chamber was active during the conditioning phase.

Sub-atmospheric pressure into the sterilizer chamber during the conditioning phase and the drying phase was obtained using an onboard water-ring vacuum pump, cooled by softened water. The sterilizer chamber was equipped with two doors. In all the experiments, the frontal door was used for loading and unloading.

Validation ports were present on the chamber walls at three locations: (i) on the chamber roof close to the unloading door (1104 mm from the front door and 213 mm from the right wall), indicated as "*roof rear*" (*RR)* validation port; (ii) on the right lateral chamber wall (504 mm from the front door and 476 cm from the chamber floor) indicated as "*lateral high*" (*LH*) validation port; (iii) on the right lateral chamber wall (at 504 mm from the front door and 232 mm from the chamber floor) indicated as "*lateral low*" (*LL*) validation port.

Measurements of NCGs using the NCG sensor in the sterilizer chamber were performed at the *LH* validation port. The other validation ports were used to admit/inject known volumes of air into the chamber. Air admission was also possible through two access points: (i) one located in the steam pipe, between the steam admission valve and the chamber inlet point, referred to as "*steam inlet*" (*SI*) access point; (ii) the other made available in a secondary branch of the steam injection pipe and indicated as "*steam pipe*" (*SP*) access point. The *SP* access point was custom-designed to allow the evacuation of the branched line to 70 mbar pressure and the subsequent injection of known air volumes in the sterilizer chamber at a desired time. It allowed the admission of air before the steam sampling point typically used for steam quality tests according to the standard method[6].

Sterilization process

The experiments were realized by running processes with sub-atmospheric conditioning phases with four vacuum points. Vacuum and steam injection point pressure levels are specified in the description of the test protocols and summarized in Table 1. All processes were run with a target temperature of 134.5 °C and a pressure of 3080 mbar during the exposure phase. The exposure phase was maintained for 3.5 or 5 min according to the specific test protocol. The drying phase was shortened to a minimum to reduce experimental time.

Load amount, load pattern, and wrapping

Experiments were performed with an "*empty chamber*" (no load and no loading trolley in the sterilizer chamber) and with different amounts of metallic bulky (non-porous, non-hollow) load, corresponding to half and full nominal loads. The load was composed of stainless-steel disks (120 mm in diameter, 5 mm thick), oriented vertically, interspaced by 10 mm using a stainless-steel disk holder, and positioned in stainless-steel nets. The total weight of the disks, holder, and net was 15 kg. The "*half load*" comprised the loading trolley and four nets (60 kg of stainless-steel plus the trolley). The "*full load*" comprised the loading trolley and eight nets (120 kg of stainless-steel plus the trolley). The load was cooled down to environmental temperature before running the process. No wrapping was used in the experiments to avoid the potential uncontrolled generation of NCGs.

Test protocols

At the beginning of each experimental day, the test sterilizer was warmed up, and an air leakage test was performed in accordance with ISO 17665:2024 recommendations for routine monitoring and control of the

	Protocol #1	Protocol #2	Protocol #3
Process			
Type	134 °C sub-atmospheric	134 °C sub-atmospheric	134 °C sub-atmospheric
Number of vacuum points	4	4	4
Vacuum level control point pressure (mbar)	70, 80, 90, 100, 110, 130, 200	70	70
Steam injection level control point pressure (mbar)	800	800	800
Exposure phase duration (min)	3.5	3.5	5.0
Load			
Type and wrapping	N.A	Metallic, bulky, unwrapped	Metallic, bulky, unwrapped
Amount (Kg)	No load (0)	No load (0), half load (60), full load (120)	No load (0), half load (60)
NCGs			
Volume of air admitted into the chamber	N.A	0–10, 15, 50, 100, 180, 200, 300, 400, 500 mL (at environmental pressure and temperature	N.A
Volume of the air chamber used for injecting pressurized air	N.A	N.A	120, 240, 360 mL (at 7 bar, environmental temperature)
Air admission/injection timing	N.A	Start of come-up ramp	30 s after start of the exposure phase
Location of air injection	N.A	"Lateral low", "Rear roof" validation ports, "Steam inlet" and "Steam pipe" access points	"Lateral low", "Rear roof" validation ports

Table 1. Process, load, and NCG parameters for the three test protocols of the study. *N.A.* not applicable.

sterilization process[19]. The test had to be passed to continue with experimental activities. The NCG sensor was positioned at the *LH* validation port the day before and connected to its controller and power supply to allow temperature stabilization of the components overnight. The steam-operated degasser was activated with the sterilizer.

The following data were collected in real-time for each cycle: temperature (°C) of the sterilizer chamber (independently measured in the sterilizer drain and close to the NCG open end); pressure (mbar) in the sterilizer chamber (independently measured in the sterilizer drain and the NCG tube); dT_{sink} (°C) through the stainless-steel block of the NCG sensor. In addition, the following qualitative and quantitative information was recorded for each process: progressive cycle number, date of the process, process start time, process stop time, process duration, end of first vacuum pulse time, exposure phase start time, conditioning phase duration, pressure of the vacuum control points, pressure of the steam injection control points, load amount, location, volume, and pressure of the injected air, maximum (NCG_{max}) and minimum (NCG_{min}) values of NCGs during the exposure phase.

Following these common preparatory steps, specific test protocols corresponding to different scenarios were applied, as detailed below and summarized in Table 1. A flow diagram of the experimental activity from the set-up of the equipment to the analysis of the data and reporting of the results is showed in Fig. 2.

Test protocol 1: detection of air not removed during the conditioning phase

This protocol reproduced the scenario in which NCGs were present in the sterilizer chamber during the exposure phase due to an ineffective removal of residual air during the conditioning phase. Specifically, different amounts of residual air were obtained by modulating the vacuum control point pressure depth in the conditioning phase. A schematic representation of the equipment used in this scenario is presented in Fig. 3a, while the corresponding sterilization process is shown in Fig. 3b. The conditioning phase comprised four vacuum points sharing the same control point pressure, followed by three steam injection points at 800 mbar, and a final come-up ramp to reach the target pressure (3080 mbar) and temperature (134.5 °C) of the exposure phase. The exposure phase duration was set to 3.5 min. Experiments were repeated for vacuum point control values of 70, 80, 90, 100, 110, 130, 180, and 200 mbar. No load was present in the chamber. Experiments were performed in quadruplicate on different days.

Test protocol 2: detection of air introduced in the sterilizer chamber at the start of the come-up ramp

This protocol reproduced the scenario in which different amounts of NCGs were present in the sterilizer chamber during the exposure phase due to the intentional admission of a controlled volume of air at the beginning of the come-up ramp. A schematic representation of the equipment is presented in Fig. 3c, while the corresponding sterilization process is shown in Fig. 3d. Sub-atmospheric conditioning was composed of four vacuum points at 70 mbar, and steam injection control point pressure was set at 800 mbar (Fig. 3d). The volume of admitted air, measured at environmental pressure and temperature, ranged from 0 to 500 mL (refer to Table 1 for intermediate values). Air was admitted using a properly sized syringe to maximize air volume accuracy and perform injection in a single shot. Two sets of experiments were performed to evaluate the effects on NCG quantification of the location of the air admission points and the presence of loads, respectively. In the first set of experiments, the air admission point was moved among four different locations: *SI*, *LL*, *RR*, and *SP* (Fig. 3d). Due to the limited

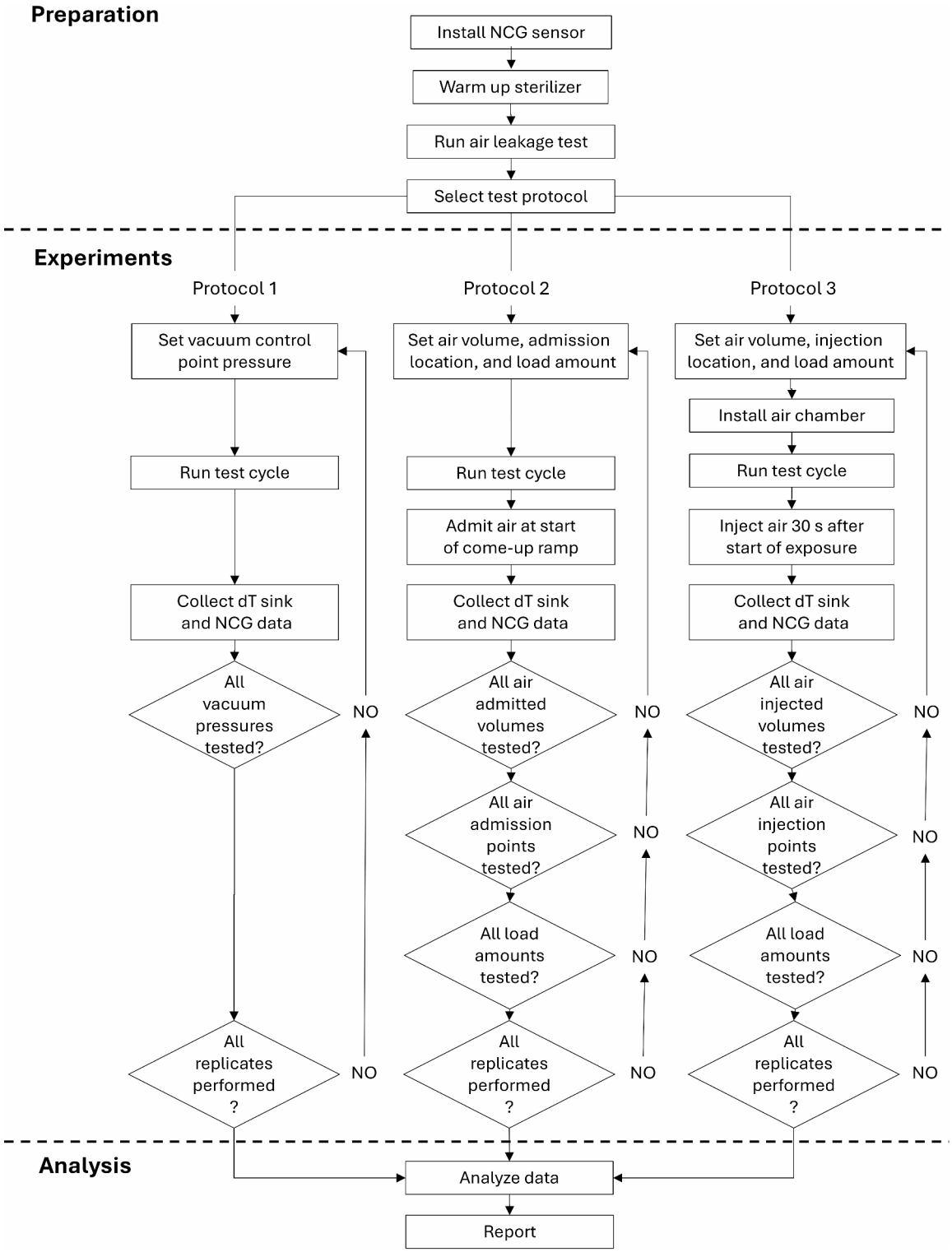

Fig. 2. Flow diagram of the experimental activity from the set-up of the equipment to the analysis of the data and the reporting of results. In the diagram, the key steps of the three experimental protocols (corresponding to the three diagram branches) are summarized.

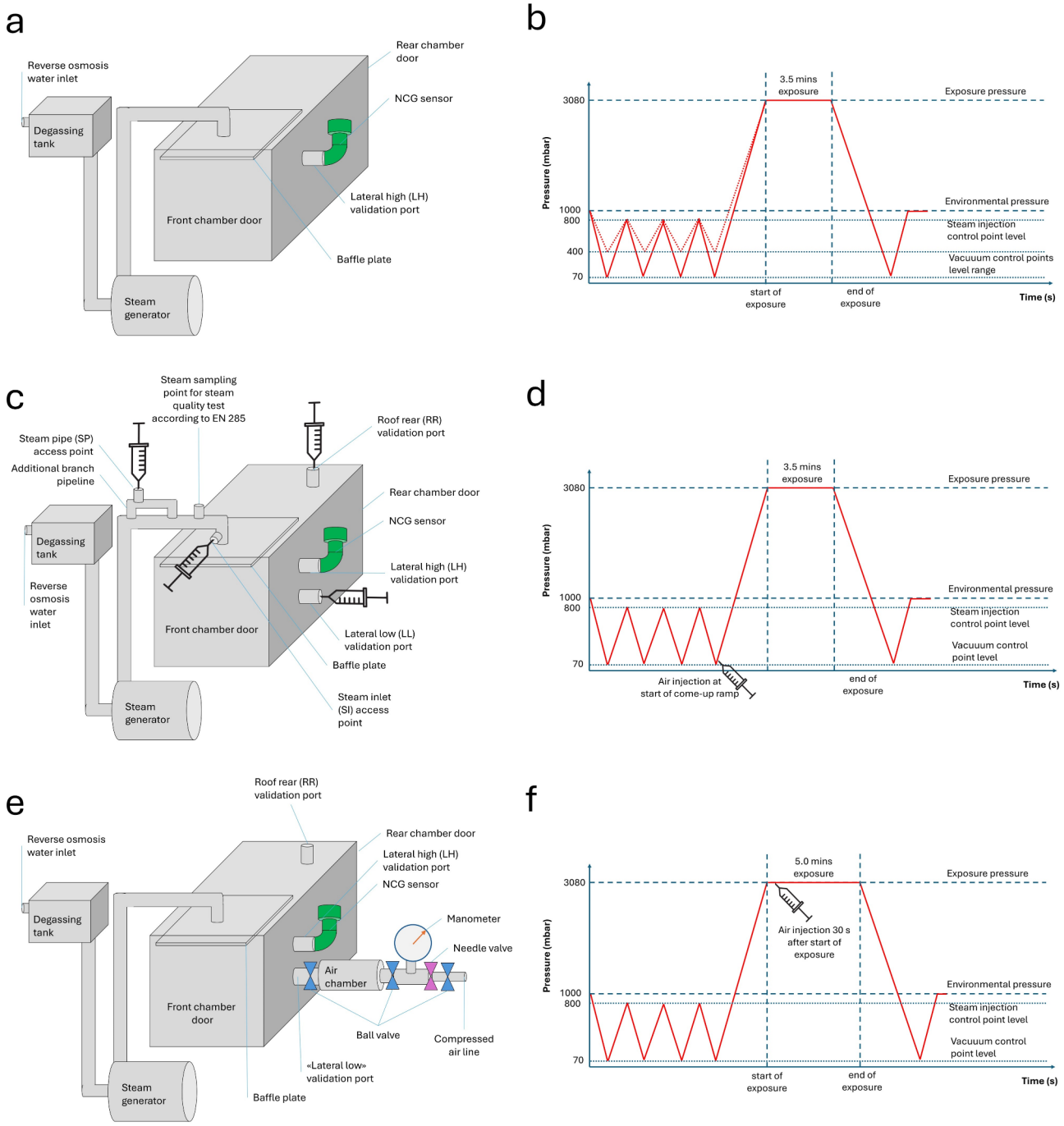

Fig. 3. Sketches of experimental set-ups (left panels) and corresponding diagrams of the 134 °C sub-atmospheric processes (right panels) performed according to test Protocol 1 (**a**, **b**), Protocol 2 (**c**, **d**) and Protocol 3 (**e**, **f**). The syringe icons indicate the locations and timings of air admission/injection into the sterilizer chamber.

volume of the secondary steam pipe branch, air volumes admitted through the *SI* access point were limited to the range of 0–100 mL. Experiments were performed in triplicate for each testing condition, with no load in the chamber.

In the second set of experiments, air volumes ranging from 0 to 500 mL were admitted to the chamber through the *SI* access point, and experiments were repeated with no load (empty chamber), half load, and full load in the sterilizer chamber.

Test protocol 3: detection of air injected in the sterilizer chamber during the exposure phase

This protocol reproduced the scenario in which different amounts of air were injected into the sterilizer chamber during the exposure phase. A schematic representation of the equipment is presented in Fig. 3e. The process

profile, shown in Fig. 3f, was the same as protocol 2, but the exposure phase was extended to 5 min to allow NCG measurement before and after air injection. Air injection into the pressurized sterilizer chamber (at a pressure of 3080 mbar) was accomplished by connecting a small air chamber to the *LL* (or *RR*) validation port via a manual ball valve (Fig. 2e). The air chamber was loaded using compressed air admitted via a needle valve. Once an absolute pressure of 7 bar was reached, the compressed air line was disconnected by closing a second manual ball valve, and chamber pressure was monitored via a manometer. Air was admitted to the sterilizer chamber 30 s after the start of the exposure phase (Fig. 3f) by opening the ball valve connected to the validation port. To introduce different amounts of air in the sterilization chamber, the volume of the small air chamber was varied among 120 mL, 240 mL, and 360 mL. Four sets of measurements were performed to evaluate: (i) the repeatability of NCG measurements in these experimental conditions; (ii) the impact of different injected air volumes on NCG measurements; (iii) the impact of the air injection point location (i.e., *LL* or *RR* validation port); (iv) the impact of load amount (i.e., no load or half load). dT_{sink} and corresponding NCG values were collected both at the start (status of the sterilizer chamber before air injection) and at the end of the exposure phase (status of the chamber after air injection).

Data analysis

dT_{sink} data corresponding to each process were exported for offline analysis to identify the values measured at the start of the exposure phase. NCG_{max} and NCG_{min} values during the exposure phase were obtained from the sensor report.

NCG_{min} data from Protocols 1 and 2 were fitted with linear mixed-effect models[20] to assess the relationship between measured NCG values and the air not removed or admitted in the sterilizer, as well as to quantify the impact of source variability.

In the case of Protocol 1, the linear mixed-effect model assumed as fixed effect the fourth power of the pressure depth of the vacuum points of the conditioning phase pressure. In contrast, random effects were associated with different replicate series (i.e., four groups indicated as Series A, B, C, and D in the following). The fourth-order dependence on the vacuum point pressure depth was based on a simple dilution model and the following assumptions: (i) the steam generator produced 100% water vapour (no NCG in the steam pipeline); (ii) the residual air and incoming water vapour were perfectly mixed inside the sterilizer chamber; (iii) the residual air fraction in the steam was determined solely by the serial air–steam dilutions occurring in the conditioning phase, according to the formula:

$$f_{\mathrm{NCGs}} = \left(\mathrm{p_v}/\mathrm{p_s}\right)^3 \left(\mathrm{p_v}/\mathrm{p_e}\right) \tag{1}$$

where p_v, p_s, and p_e are the set pressure values of the vacuum level control points, steam injection level control points, and exposure phase, respectively.

In the case of Protocol 2, two linear mixed-effect models were constructed. In both models, the fixed effect term was associated with the admitted air volume. Random effects were associated with the location of the injection site (i.e., four groups corresponding to "*SI*", "*LL*", "*RR*", and "*SP*" injection sites) in the first model, and with the presence of load (i.e., three groups corresponding to "Empty", "Half Load", and "Full Load") in the second model. The best model reproducing the data was identified as the one minimizing the Akaike Information Criterion. The marginal and conditional coefficients of determination (R^2) were used to quantify the proportion of variance explained by the fixed effect term and by the full model (i.e., fixed effects plus random effects)[21]. The partial R^2, defined as the difference between the conditional and marginal R^2, was used to quantify the contribution of random effects to predict data variability. The statistical significance of random effects was assessed through a likelihood ratio test. A p-value < 0.05 was considered statistically significant.

In the case of Protocol 3, NCG_{max} and NCG_{min} values were used to compute the variation of NCG amount (quantified as the difference between NCG_{max} and NCG_{min}). NCG variation was expressed as a function of air chamber volume and fitted with a linear model. The strength and statistical significance of the linear relationship between the two variables were quantified by Pearson's correlation coefficient (r) and the corresponding p-value. The goodness of fit was expressed in terms of amount of reproduced variability by R^2. dT_{sink} and NCG variation data collected for an air chamber volume of 120 mL, were expressed as mean and standard deviation over repetitions and compared between *RR* and *LL* injection sites and between empty and half load conditions.

All analyses were performed using Matlab R2024b (The MathWorks, Inc., Natick, Massachusetts, US).

Results

Quantification of NCGs in the sterilizer chamber due to ineffective conditioning phase (Protocol 1)

dT_{sink} measurements at the start of the exposure phase for the processes corresponding to Protocol 1 are shown in Fig. 4a, while NCG_{min} values during the exposure phase for the same processes are presented in Fig. 4b. Overall, the data showed the capability of the NCG sensor to detect and quantify NCGs present in the sterilizer chamber during the exposure phase, due to an insufficient vacuum in the conditioning phase. NCG_{min} values were in good agreement with the simple dilution model, although quadruplicate data collected on different experimental days (indicated as different series in Fig. 4) pointed out slight differences between repeated measurements. These observations were corroborated by the mixed-effect model results. The best model showed a marginal R^2 of 0.88, suggesting that the change in pressure depth explained most of the data variability. The addition of the random term associated with measurement variability over different days significantly ($p < 0.001$) improved the predictions leading to a conditional $R^2 = 0.99$ (partial $R^2 = 0.11$).

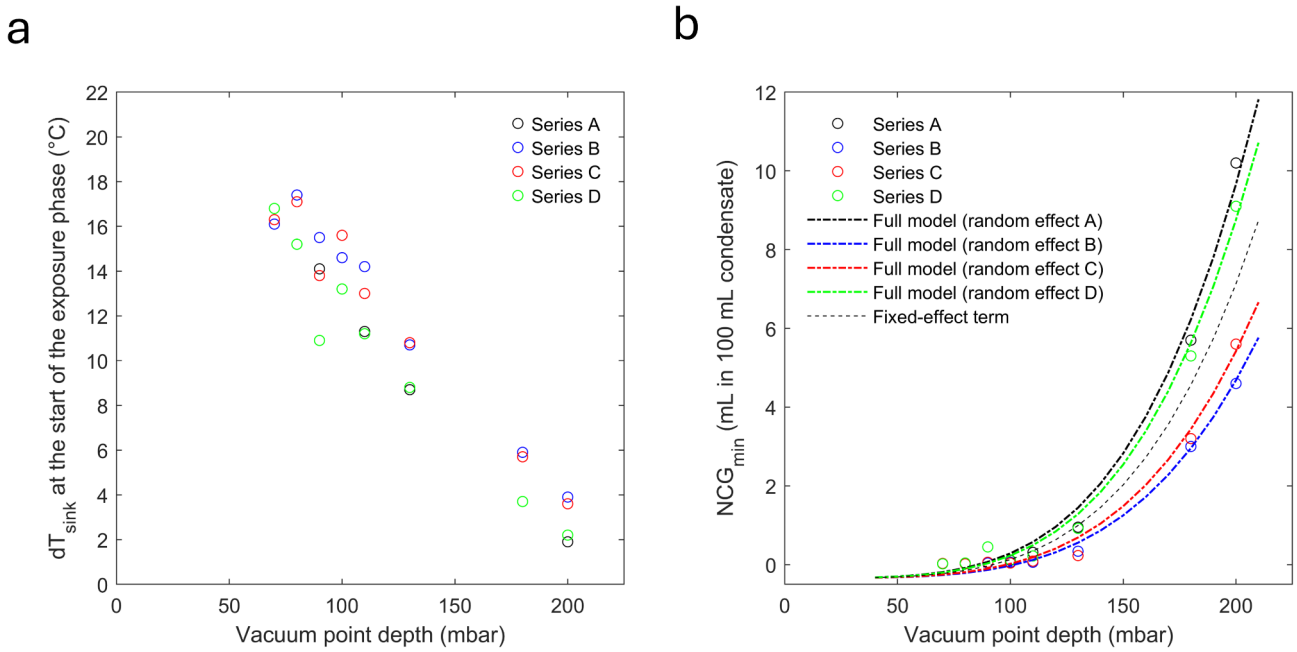

Fig. 4. Experimental values of dT_{sink} at the start of the exposure phase (left) and minimum NCG values (NCG_{min}) during the exposure phase (right), obtained from processes performed according to Protocol 1. Quadruplicated measurement series (Series A–D in the legend) were performed on different experimental days and are colour coded in both graphs. In the right panel, the dashed black line and the coloured lines indicate the fit by the fixed-effect term (fixed-effect variable: fourth power of the vacuum point depth) and the full mixed-effects model (random effect variables: day series A–D), respectively.

Quantification of NCGs due to air admission at the beginning of the come up ramp (Protocol 2)

dT_{sink} measurements at the start of the exposure phase for processes performed according to Protocol 2, without load in the chamber, are shown in Fig. 5a. The results showed the reproducibility of dT_{sink} values within 2 °C for admitted air volumes between 0 and 500 mL. The corresponding minimum values of NCGs measured during the exposure phase (Fig. 5b) testified the reproducibility of NCG_{min} measurements, irrespective of the air admission point location, with a good linear relation between injected air and measured NCG amount in the tested range from 0.01 to 5.0 mL of NCG in 100 mL of condensate. The mixed-effects model confirmed these observations. The marginal R^2 was equal to 0.97, and adding the random term did not significantly improve the prediction ($p = 0.84$, partial $R^2 = 0.003$, conditional $R^2 = 0.97$). Small deviations from linearity were possibly due to the variable and unpredictable amount of NCGs in the steam coming from the steam generator.

Measurements of dT_{sink} at the start of the exposure phase obtained with air admission from the *SI* access point and different loads in the chamber are shown in Fig. 5c. In the tested air volume range, dT_{sink} measurements were reproducible for different load amounts. dT_{sink} data with half and full loads were almost superimposable to those collected with empty loads. The minimum values of NCGs obtained during the exposure phase for the same processes (Fig. 5d) supported the possibility to detect and quantify NCGs, and the reproducibility of measurements irrespective of the amount of load present in the chamber. In this case, the marginal and conditional R^2 of the mixed-effect model were both equal to 0.97 with no contribution of random effects associated with the load ($p = 1$, partial $R^2 = 0$).

Quantification of NCGs due to air injection during the exposure phase (Protocol 3)

Quadruplicate continuous measurements of dT_{sink}, obtained from Protocol 3 with air injection from a 120 mL pressurized air chamber and no load, are displayed in Fig. 6a. A qualitative inspection of the coloured solid curves, corresponding to experiment repetitions with air injection from the *LL* validation port, showed a reproducible time-course of dT_{sink} signal during the whole exposure phase, where dT_{sink} typically decreased after about 1 min from the start of the exposure phase (i.e., about 30 s after the air was injected in the sterilizer chamber). This time lag likely reflected the time required by the injected air to diffuse into the sterilizer chamber and to reach the NCG sensor from the air injection point, plus the time response of the NCG sensor. Measurements displayed general repeatability, considering possible variability in NCG amount in the steam produced by the steam generator. Inspection of the dashed curves, corresponding to air injection from the *RR* validation port (Fig. 6a) showed good superimposition with the *LL* solid curves, confirming that air injection location did not relevantly affect dT_{sink} and, consequently, NCG measurements in the sterilizer chamber.

Figure 6b displays dT_{sink} measurements obtained when varying the volume of the air-chamber, used to inject air 30 s after the start of the exposure phase, in the range from 0 to 360 mL. The progressive decrease of dT_{sink} values at increasing air volumes testified the capability of the sensor to track the progressive increase of NCGs

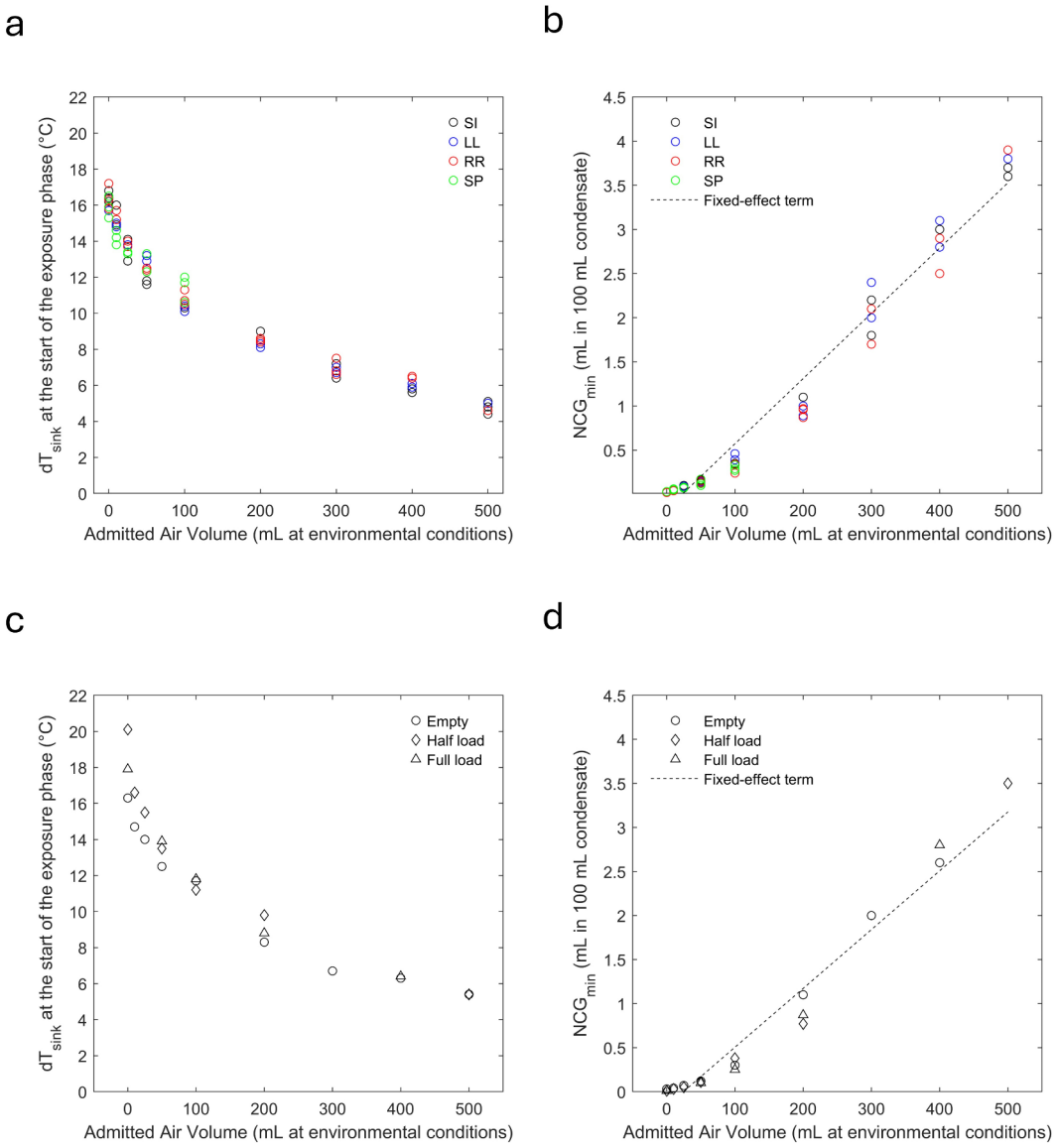

Fig. 5. Experimental values of dT_{sink} at the start of the exposure phase (left) and minimum NCG values (NCG_{min}) during the exposure phase (right), obtained from processes performed according to Protocol 2. Panels (**a**) and (**b**) show measurement series obtained with different air admission point locations (*SI*: "*steam inlet*" access point; *LL*: "*lateral low*" validation port; *RR*: "*roof rear*" validation port; SP: "steam pipe" access point). Panels (**c**) and (**d**) show measurement series obtained when different amounts of load were present in the sterilizer chamber ("*Empty*", "*Half load*", "*Full load*"). In panels (**b**) and (**c**), the dashed lines indicate the fit by the fixed-effect term of the model (fixed-effect variable: admitted air volume).

in the sterilizer chamber. Figure 6c reports duplicate dT_{sink} measurements obtained after air injection from a 120 mL air-chamber, when half load was present in the sterilizer. dT_{sink} values showed a marked decrease over time with respect to the condition of no air injection (in gray).

Values of dT_{sink} measured at the end of the exposure phase for all processes performed according to Protocol 3 are displayed in Fig. 6d, while the corresponding increase in NCGs during the exposure phase (expressed as NCG_{max}–NCG_{min}) is plotted against the air-chamber volume in Fig. 6e. A strong, positive correlation ($r = 0.98$, $p < 0.0001$) was observed between the estimated NCG increase and the volume of injected air, supporting a linear response of the sensor also in this scenario (linear fit with $R^2 = 0.96$). In Fig. 6e, NCG measurements obtained with the air chamber volume of 120 mL at the *LL* validation port (black circles) are compared with those obtained with air chamber located at the *RR* validation port (red circles) in the absence of load. The good superimposition of the values (NCG_{max}—NCG_{min} = 0.82 ± 0.18 and 0.87 ± 0.09 at *LL* and *RR*, respectively) suggested that the air injection point location had no effects on NCG measurements. Comparison of values measured with half load versus no load showed higher dT_{sink} values (11.4 ± 0.1 °C VS 9.8 ± 0.6 °C, Fig. 6d) and lower NCG values (0.37 ± 0.01 VS 0.82 ± 0.18, Fig. 6e) in the presence of load. This could be related to a partial segregation of the injected air into the load, although additional data are required to confirm these

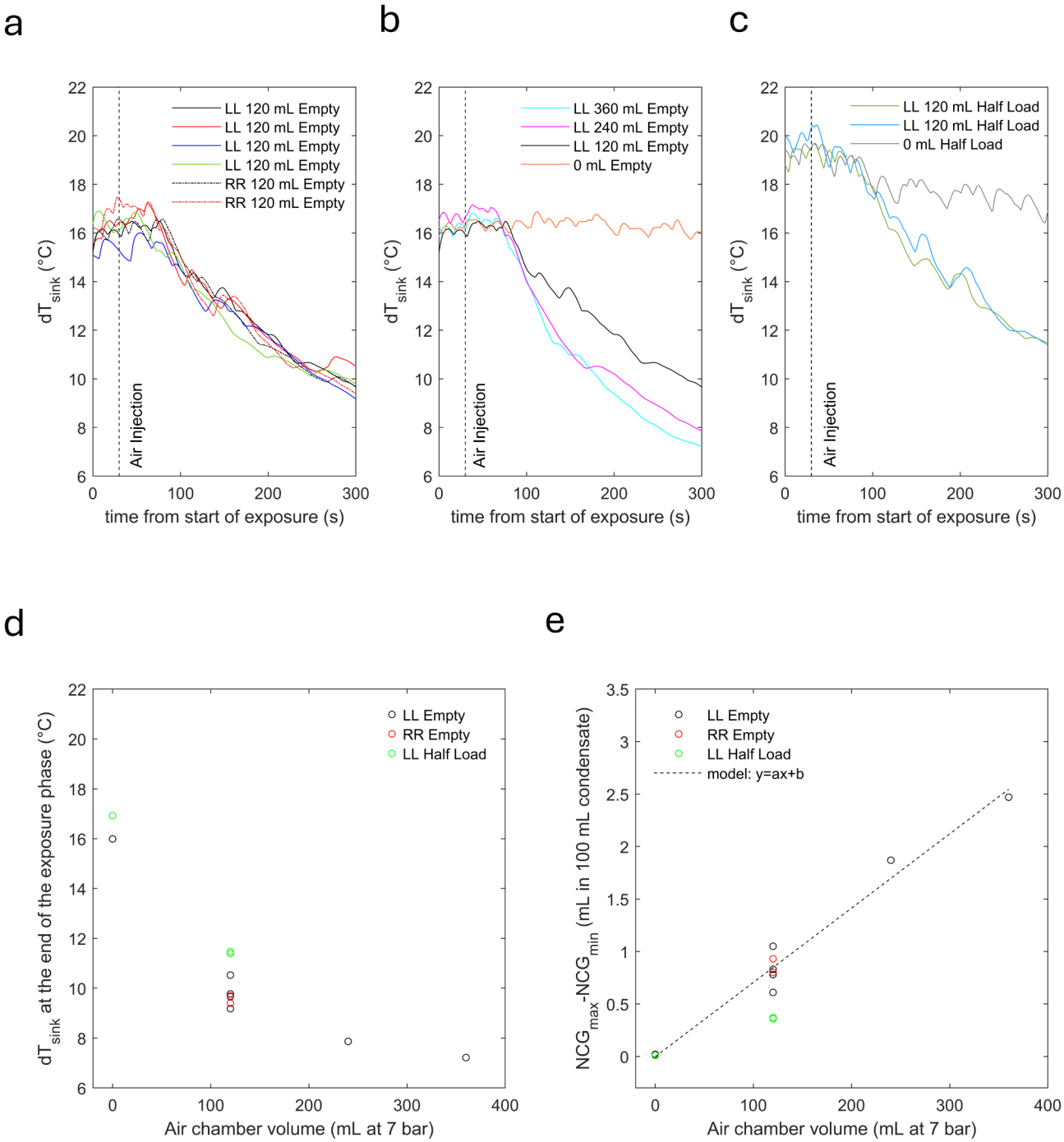

Fig. 6. Time course of dT_{sink} values (panels **a**–**c**), dT_{sink} at the end of the exposure phase (panel **d**), and NCG measurements (panel **e**), obtained from processes performed according to Protocol 3, with air injected 30 s after the start of the exposure phase (vertical dashed line). The time course of dT_{sink} measurements was monitored in the following conditions: (**a**) using an air chamber volume of 120 mL, at 7 bar, connected to the "*lateral low*" (LL) or the "*roof rear*" (RR) validation ports; (**b**) using different volumes of the air chamber connected to LL validation port; (**c**) running experiments with half load and different volumes of the air chamber connected to LL validation port. dT_{sink} measurements at the end of the exposure phase (**d**) and variations in the amount of the NCGs during the exposure phase (**e**) vs. volume of the air chamber. The dashed line in (**e**) indicates the linear regression line for NCGs vs. air chamber volume and highlights the presence of a strong positive correlation between the two variables ($r = 0.98$, $p < 0.0001$).

preliminary measurements. Overall, NCG measurements performed according to Protocol 3 documented that NCG detection and quantification in the sterilizer chamber were feasible using the NCG sensor, with a short time response in the quasi-stable thermodynamic conditions of the exposure phase and an approximately linear response of the NCG sensor, regardless of the location of air injection.

Discussion

Steam composition strongly affects the water vapour condensation process and the efficiency of latent heat energy transfer to exposed surfaces[17,22–26]. Already in 1929 Donald Otmer experimentally observed[27] and later Minkowikz and Sparrow theoretically proved[17] that the condensation heat transfer was reduced to less than 50% by NCG bulk mass fractions as small as 0.5%. Therefore, the presence of larger NCG amounts in the free space of the sterilizer chamber is associated with longer heat-up times. A low amount of NCGs should be preferred to speed up the process[2,28], maximise productivity, and minimise thermal ageing effects on the load[7].

Literature and standards also indicate that NCGs could accumulate locally, creating the so-called air "bubbles"[5] or "pockets"[28], impairing the repeatability of processes and impeding steam penetration in all the accessible districts of the load. The lower the fraction of NCGs in the sterilizer chamber, the smaller the steam composition variability across the sterilizer chamber, and the higher the repeatability of the process.

Despite the relevance of steam composition for monitoring the sterilization process, quantitative methods and tools for the quantification of NCGs are sparse, both in the standards and in the literature. The method specified in clause 21.1.1 of the EN 285:2015 + A1:2021[6] is currently the only quantitative method in the standards, which allows the quantification of NCGs in the steam sampled from the piping line connecting the steam generator to the sterilizer chamber[6]. However, this method does not grant that the measurements are representative of the steam composition in the sterilizer chamber. In fact, differences in the composition of the steam between the piping line and the sterilizer chamber are likely present due to the radically different thermodynamic conditions at the two locations[26]. Moreover, the method reported in the standards is likely incapable of detecting a change in steam composition after the sampling point. Differences between NCG content in the sterilizer chamber and at the standard sampling point may arise due to additional NCG sources, such as insufficient air removal during the conditioning phase, air leakage into the sterilizer chamber during the sub-atmospheric phases or from the valves operated with compressed air during the super-atmospheric phases, NCGs release or desorption from the load or the wrapping system. Even in the absence of these NCG sources, a progressive accumulation of NCGs in the sterilizer chamber can occur over time due to the condensation of the water vapour fraction on the load and the recall of new steam and a fraction of NCGs toward the condensing surface[28]. These differences concur to make the characterization of steam composition in the piping system not an adequate surrogate of the steam composition in the sterilizer chamber[6]. The tested NCG sensor complements the information from currently available standard methods, providing a quantitative index of the steam composition inside the sterilizer chamber during every sterilization process.

In the literature, only a limited number of technologies have been proposed to quantify NCGs in the steam for sterilization. One device was shown to have the potential for measuring NCG amount in the sterilizer chamber[18], but the commercial version (ETS, 3 M, Saint Paul, MN, USA), recently retired from the market, did not provide this measurement functionality. In 2013, a sensor based on the selective infrared absorption of the water vapour fraction in the steam was proposed for monitoring steam penetration in channelled loads by quantifying the water vapour fraction at the closed end of a tube[29]. Experiments performed using the engineered version of this sensor showed the possibility of identifying the worst case for steam penetration in channelled loads[30]. The currently available version of this infrared sensor (4D sensor, Miele, Gütersloh, Germany) is certified as an electronic B&D-like test and it does not provide a measure of the NCGs in the steam sterilizer chamber. Leiss-Holzinger and colleagues investigated the possibility of using infrared absorption to monitor steam composition, both inside the sterilizer chamber and at the end of a small cavity connected to it[31]. The set-up was prototypical and revealed limitations regarding the calibration procedure and the stability of measurements over time[31]. Using direct tuneable diode laser absorption spectroscopy, Pletzer et al. measured the mole fraction of water vapour at the end of a closed-end channel communicating with the sterilizer chamber [32]. Experimental results were in agreement with numerical simulations, but the complexity and cost of the measuring apparatus may hinder its routine use in the field.

The results of the present study showed that the quantification of NCGs in the sterilizer chamber is possible using the NCG sensor, irrespectively of the NCG source, the location and timing of NCG entrance into the sterilization chamber, and the amount of load in the chamber.

The results obtained in Protocol 1 showed that the sensor was able to detect the presence of NCGs resulting from an inefficient evacuation of the air during the conditioning phase, where NCG_{min} values were mainly determined by the pressure depth values according to a simple dilution model. However, the presence of statistically significant residual variance over measurement repetitions, which could be tracked by random effects, points out that residual air in the sterilizer chamber cannot be precisely predicted based on the sole pressure control point values, even in the absence of leaks, thus evidencing the need of monitoring steam composition in every single process[10]. Indeed, the variability of NCG measurements in Protocol 1 was likely related to differences between the set values of the vacuum and steam injection level control points and the actual pressure achieved in the sterilizer chamber before closing the steam injection valve or stopping the vacuum pump. However, other sources of variability could be present, including unpredictable changes in the quality of the steam coming from the steam generator and/or changes in the performance of the vacuum pump (e.g. difference in the vacuum gradient due to variation of the cooling water temperature of the vacuum pump) and the consequent variation of the conditioning phase duration[33].

The body of data collected using Protocol 2 supports the hypothesis that when air is present in the sterilizer chamber at the beginning of the come-up ramp, the turbulent steam–air mixing, generated by the large amount

of injected steam, produces an effective homogenization of the sterilizer chamber atmosphere, which allows the detection of possible air intake (e.g., due to an air-tight component leakage) from virtually any chamber location. According to the study results, this was true in the whole range of tested NCG concentrations, ranging from about 0.05% to 5% mL in 100 mL of condensate.

Besides the capability to detect variations in NCG amount during the conditioning, when steam–air mixing is favoured by forced convection, results from Protocol 3 showed the feasibility of tracking NCG changes also during the exposure phase, when the mixing due to convection is limited and time for air diffusion is needed. In this case, a time lag of about 30 s was observed before the air injected in the sterilizer chamber could be detected. The time interval was likely the result of the sum of the time needed for air diffusion from the injection point to the NCG sensor and the time response of the sensor, but the adopted testing methodology did not allow us to distinguish the contributions of the two components. Nevertheless, the detection time of the device is suitable for use in the sterilization field, where the typical duration of the exposure phase is longer than 300 s, and the conditioning phase is in the range of tenths of minutes.

NCG measurements collected with different load amounts in the sterilizer chamber indicated no significant impact of the load for detecting small volumes of air admitted into the sterilizer chamber during the come-up ramp. These findings also provide indirect evidence that no significant amount of air was segregated or confined within the load when admitted to the chamber in the conditioning phase. On the other hand, as reported in Fig. 6d-e, some differences in NCG measurements due to different load amounts were observed when air was injected in the sterilizer chamber during the exposure phase, but data are still preliminary and further confirmation is needed. Experimental results should also be carefully extended to loads different than unwrapped and bulky. Indeed, the presence of rigid or deformable sterile barrier systems, such as containers, pouches, or non-woven wrapping materials around the load, may affect NCG diffusion from the load surface to the free space of the sterilizer chamber and, therefore, to the NCG sensor. Similarly, NCGs inside a porous load or a long channel may require longer times to diffuse through the sterilizer chamber and a non-negligible amount of NCGs may remain confined in the load at the end of the exposure phase[30]. These aspects should be carefully evaluated during the validation of the process for the specific load or load family. While the presence of a good steam composition in the sterilizer chamber is a necessary condition for the sterilization of the load, it is per se not sufficient, since the reaching of all the accessible surface of the load by the steam needs also to be checked. The latter check is usually performed thermometrically during the validation, which remains an indispensable step. The NCG sensor is not designed nor intended to be a steam penetration test. However, monitoring the steam quality in the sterilizer chamber using the NCG sensor can provides quantitative evidence about the achievement of reproducible predetermined and validated conditions in every process.

Conclusions

Quantitative measurement of NCG amount in the sterilizer chamber during the exposure phase was feasible in a variety of processes by means of the tested sensor. Experiments performed in this study provided evidence that the NCG sensor could detect and quantify residual air due to an ineffective conditioning phase, as well as NCGs present in or admitted to the sterilizer chamber during the come-up ramp or the exposure phase. NCG measurements were reproducible irrespective of the location where air entered the sterilizer chamber and the load amount.

The availability of quantitative data about steam composition in the sterilizer chamber, combined with the measurements of temperature and time, represents a relevant step forward in the direction of a real-time monitoring of each single process and the load release based on a complete multiparametric assessment. The possible added value of this technology include the enablement of real-time monitoring of NCGs in every process, the improvement of quality control, sterilization processes, and regulatory compliance in terms of process monitoring, and finally the overall enhancement of hospital infection control measures.

Data availability

All main data are available in the text or figures. Complementary information on data used in the analysis is available upon reasonable request to the corresponding author.

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Author contributions

Conceptualization, F.T.; methodology, F.T. and M.M.; formal analysis, F.T. and M.M.; investigation, F.T. and M.M.; resources, F.T. and M.M.; data curation, F.T. and M.M.; writing—original draft preparation, F.T.; writing—review and editing, F.T. and M.M.; supervision, F.T.; funding acquisition, F.T. All authors have read and agreed to the final version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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