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Quantitative assessment of the standard method for measuring steam composition in surface sterilisation

Francesco Tessarolo[1,2]✉ & Michela Masè[1]

Steam composition, defined as the fraction of non-condensable gases (NCGs) in the steam, is an essential parameter to ensure the efficacy and reliability of the steam sterilisation process. Standard EN 285:2015 + A1:2021 defines an operative method for measuring NCG fraction (NCG%) in the steriliser steam pipes, but lacks specifications about the process profile, timing and volume of condensate, and environmental conditions. This study aimed to quantify the effects of this lack of specifications on NCG% measured values to enable a more effective and informative testing procedure. Sterilisation processes, with different profiles and load amounts, were run in a medium-sized steam steriliser. NCGs and steam condensate volumes were measured every 30 s using the standard method, and NCG% values were calculated at different time points and condensate volumes. NCG% variations associated with environmental temperature and pressure were theoretically estimated. NCG% values and test outcomes showed dependence on the process profile, load, condensate collection timing and volume. Environmental conditions had a non-negligible impact on measurements. Overall, NCG measurement variability impacted standard method reliability to qualify the steriliser and to estimate steam composition in the steriliser chamber. Real-time quantitative monitoring of steam composition during every production process is advocated to improve steam sterilisation efficacy and quality.

Keywords Sterilisation efficacy, Steam composition, Non-condensable gases, Standards, Steam quality, Equipment qualification

Steam sterilisation is worldwide recommended for processing heat-resistant instruments in hospital settings, combining the nontoxicity of the sterilising agent with the reliability and cost-effectiveness of the process[1,2]. However, the efficacy and consistent quality of the steam sterilisation process are strongly related to the guarantee of sterilisation conditions at the load surface. The literature indicates time, temperature, and steam composition as the essential parameters for monitoring steam sterilisation conditions[3,4]. Appropriate combinations of time and temperature in the presence of saturated steam were identified by Perkins in 1956 and demonstrated to be able to inactivate heat-resistant microorganisms[5]. The Medical Research Council revised those time-temperature combinations by adding a safety margin to account for the inevitable presence of gases in the steriliser chamber that do not liquify at the conditions reached during the process[3]. These gases, typically part of air constituents, do not contribute to the effective and rapid heating of the load[6–9], since they are unable to transfer heat to the load surface by condensation[10,11]. For this reason, they are usually referred to as non-condensable gases (NCGs) in relevant standards[12–15] or, more properly, to non-condensing gases in the technical literature[16,17].

In the current standards for steam sterilisation[12,13,15], recommended time-temperature combinations are still those indicated by the Medical Research Council[3]. The steam composition is listed as one of the key physical parameters in relevant standards, and is considered part of the steam quality descriptors, together with dryness value, superheat, and pressure fluctuation, as well as chemical purity metrics like ions, conductivity, pH, endotoxins, and contaminants in the steam condensate[12,13,15]. Steam composition can be operationally defined as the amount of NCGs in the steam, and indications for the maximum acceptable amount of NCGs during the sterilisation phase are provided in the literature and standards. According to[3], the volume of NCGs should not exceed 5% of the steam volume (V_{NCG}/V_{Steam}) in any part of the load. In widely accepted standards, such as EN 285:2015 + A1:2021 and AAMI/ANSI ST79:2017, this recommendation was transposed into an upper threshold of 3.5 mL of NCGs in 100 mL of steam condensate (V_{NCG}/V_C)[12,13].

[1]Department of Industrial Engineering, University of Trento, via delle Regole, 101, Trento 38123, Italy. [2]Healthcare Research and Innovation Program, Bruno Kessler Foundation, Trento, Italy. ✉email: francesco.tessarolo@unitn.it

Several methods are reported in the current standards[12,13,15] and technical memoranda[14] to assess steam composition. However, only clause 21.1.1 of EN 285:2015 + A1:2021 provides a test method to obtain the NCGs amount in the steam, expressed in terms of mL of NCGs in 100 mL of condensate (V_{NCG}/V_C) and indicated as NCG%[12]. Although EN 285:2015 + A1:2021 clarifies that the method "*should not be regarded as measuring the exact level of non-condensable gases during normal use of the sterilizer*"[12], it remains the only standard test used in the current practice during the qualification and requalification of steam sterilisers. The method is considered essential to check the steriliser performance and to safely release the processed load[18].

An accurate evaluation of the test method specifications pointed out the lack of precise indications about the time when the steam condensate collection should be initiated and terminated, as well as the amount of the condensate to be collected. This lack of specifications leaves the setting of these parameters at the discretion of the professional performing the test. This gives rise to heterogeneous field practices, such as sampling of condensate during the conditioning phase or the exposure phase, or collecting variable condensate amounts. In addition, the test method indicates that steam composition measurements should be performed with an empty chamber, but no evidence is provided that measurements in this condition are representative of the composition of the steam generated when the load is present inside the chamber. Moreover, the standard method does not consider the steam destination of use, i.e. it does not differentiate time periods when the generated steam is injected into the chamber from those when the steam is used for other purposes. Finally, the environmental conditions at which the volume of condensate and the volume of NCGs should be determined are not specified in the standards, and only the maximum temperature for the condensate is indicated to avoid bubble generation and difficulty in reading the condensate volume.

This study aimed to quantify the effects of the lack of specifications in the test method of clause 21.1.1 of EN 285:2015+A1:2021 and of the consequent heterogeneity in field practices on NCG measurements and test outcomes, to enable a more effective and informative testing procedure. Specifically, systematic tests were performed to quantify the effects of the following factors: (i) the profile of the sterilization process, (ii) the amount and timing of the collected condensate, (iii) the presence of load in the chamber, (iv) the destination of use of the steam, (v) the environmental conditions during the test. We hypothesised that NCG measurement variability arising from the lack of specifications may impact the method's reliability to qualify the steriliser and to estimate steam composition in the steriliser chamber.

Materials and methods

Steam steriliser and supplies

A validated, commercially available steam autoclave (VS8/2, Steelco, Riese Pio X, Italy) with a chamber volume of 614 L was used. Reverse osmosis water (conductivity of 1.3 µS) was heated at 95 °C in the onboard 15 L steam-heated water degassing tank to remove most of the dissolved NCGs before admission into the electric steam generator. A liquid ring vacuum pump was used to achieve sub-atmospheric pressure in the steriliser chamber during the conditioning phase and the drying phase. To reduce the amount of NCGs in the steriliser chamber, the controller was programmed to avoid refilling water in the boiler when steam injection in the chamber was active during the conditioning phase.

In order to limit the possible sources of steam composition variability during the study, all working parameters, equipment settings, and external supplies were maintained, apart from the process profile and the load amount, where indicated. No changes in the settings of the chamber thermometric and pressure probes, the boiler level probes, the boiler operating temperature and pressures probes, the steam admission pathway, and the temperatures of the degassing tank were introduced.

Test protocol and NCGs quantification

At the beginning of each experimental day, a Steam Quality Test Kit D (Dekon Solutions, Dunkeswell, UK) was connected to the steam piping system of the steriliser using a dedicated port, located in a horizontal pipe section before the steam access point to the steriliser chamber (Fig. 1). This NCG measurement equipment was claimed by the manufacturer to comply with the method specified in clause 21.1.1 of EN 285:2015+A1:2021. The test kit was calibrated by the manufacturer. The steriliser was warmed up, and an air leakage test followed by a Bowie&Dick (B&D) test was performed in accordance with ISO 17665:2024 recommendations for routine monitoring and control of the sterilisation process[15]. The leak test and the B&D test had to be passed before experimental activities could start. To implement NCG amount measurements according to the method specified in clause 21.1.1 of EN 285:2015+A1:2021, the Steam Quality Test Kit D was used following the manufacturer's instructions. The kit allowed for the separate collection and measurement of the volume of steam condensate and the corresponding volume of NCGs into two separate graduated glass tubes (Fig. 1b). The two volumes were measured over time with a resolution of 2 mL and 0.2 mL, respectively. The temperature of the steam condensate during all the experiments was maintained between 75 and 80 °C by adjusting the flow rate of the independent water cooling circuit integrated in the test kit.

The flow rate of the sampled steam was regulated to about 40 mL/min of steam condensate. Measurements were consistently initiated at the exact start of the conditioning phase of each sterilisation process, and the volumes of the steam condensate (V_C) and of NCGs (V_{NCG}) were recorded every 30 s. Measurements were terminated by the end of the exposure phase, since the steam composition in the drying phase is not relevant for sterilisation purposes.

Process profile and load amount

Two experimental sessions were performed. In the first session, the impact of the process profile on NCG measurements was investigated. A typical sterilisation process includes three consecutive phases, characterised by specific pressure and temperature values in the steriliser chamber that define the process profile (e.g. top

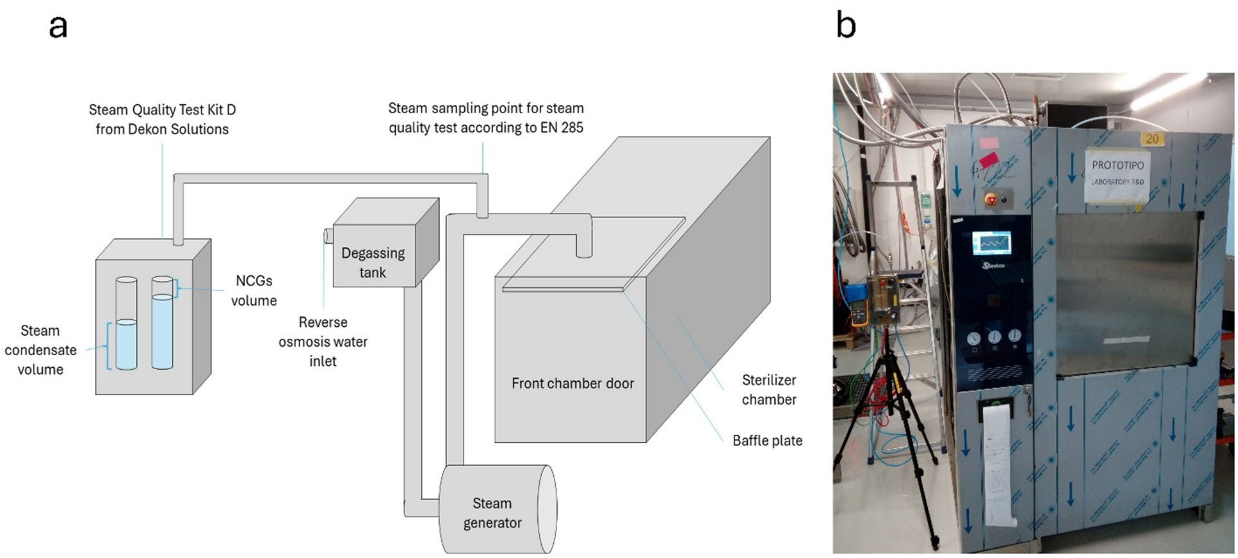

Fig. 1. Experimental set-up used in the study: schematic diagram (a) and real equipment (b). The steam quality Test Kit D (Dekon Solutions, Dunkeswell, UK) was used to measure steam composition. The water levels in the two glass pipes indicate the volumes of the steam condensate (left pipe) and of the NCGs (right pipe), respectively.

panels of Fig. 2). The three phases are: (i) a conditioning phase, aimed at substituting the air with steam in the sterilizer chamber; (ii) an exposure phase, when steam effectively heats the load to a target temperature mainly by condensation heat transfer; (iii) a drying phase required to dry the load and safely open the sterilizer door[19]. The duration of the overall process can vary, since only the duration of the exposure phase is specified, while the duration of the preconditioning and drying phases is dictated by the specific equipment, services[20], and load[21,22].

In this study, three different sterilisation process profiles, referred to as A, B, and C in the following, were performed. The processes were characterised by different conditioning phase profiles chosen among the variety of process profiles used in hospital practice. Process A included sub-atmospheric conditioning with four vacuum control points set at the target pressure of 7 kPa. The pressure of the steam injection control points was set at 80 kPa. Process B included a short-duration, trans-atmospheric conditioning phase with five steam injections at increasing control point pressures. Process C had a long-duration, trans-atmospheric conditioning phase, including two vacuum pulses at 7 kPa, seven steam injections at increasing control point pressures, and a five-minute holding time at 280 kPa. All the processes had the same target temperature of 134.5 °C and pressure of 308 kPa during the exposure phase. The exposure phase conditions were maintained for 3.5 min. All the processes of the first experimental session were run in an empty chamber (i.e., in the absence of load and loading trolley in the steriliser chamber) during a single experimental day.

In the second experimental session, tests were performed to evaluate the effect of load on NCG measurements. To this aim, the load amount was varied while running repetitions of the same process (process A). Three load amounts were considered: no load (i.e., empty chamber with no load or loading trolley), half, and full loads. The load was composed of stainless-steel bulky (non-porous, non-hollow) material in the shape of vertically-oriented disks (120 mm in diameter, 5 mm thick), spaced by 10 mm and positioned in stainless steel nets for a total weight of 15 kg for each net. The “half load” was composed of four nets (a total of 60 kg of stainless steel plus the trolley). The “full load” was composed of 8 nets (a total of 120 kg of stainless steel plus the trolley). The load was cooled down to environmental temperature before running the process. No wrapping was used. Experiments were performed in triplicate for each of the three load amounts, on two different experimental days. The load amount was randomised between different processes to avoid temporal correlation.

In both experimental sessions, the drying phase was shortened to a minimum to reduce experimental time. Environmental pressure and condensate temperature were monitored during each experimental session.

Condensate volume and timing of condensate collection

In the EN 285:2015 + A1:2021, NCG% is expressed as mL of NCGs per 100 mL of condensate according to Eq. (1):

$$\mathrm{NCG\%} \;:= \frac{V_{\mathrm{NCG}}}{V_{\mathrm{C}}} \cdot 100 \tag{1}$$

where V_{NCG} and V_C are the net volumes (expressed in mL) of the non-condensable gases and of the condensate, respectively. Since the standard does not specify the amount of condensate volume to be collected, the calculation of NCG% was performed according to different time intervals, which resulted in different condensate amounts. To evaluate the effects of time intervals on the estimated NCG% values, the calculation of NCG% was performed

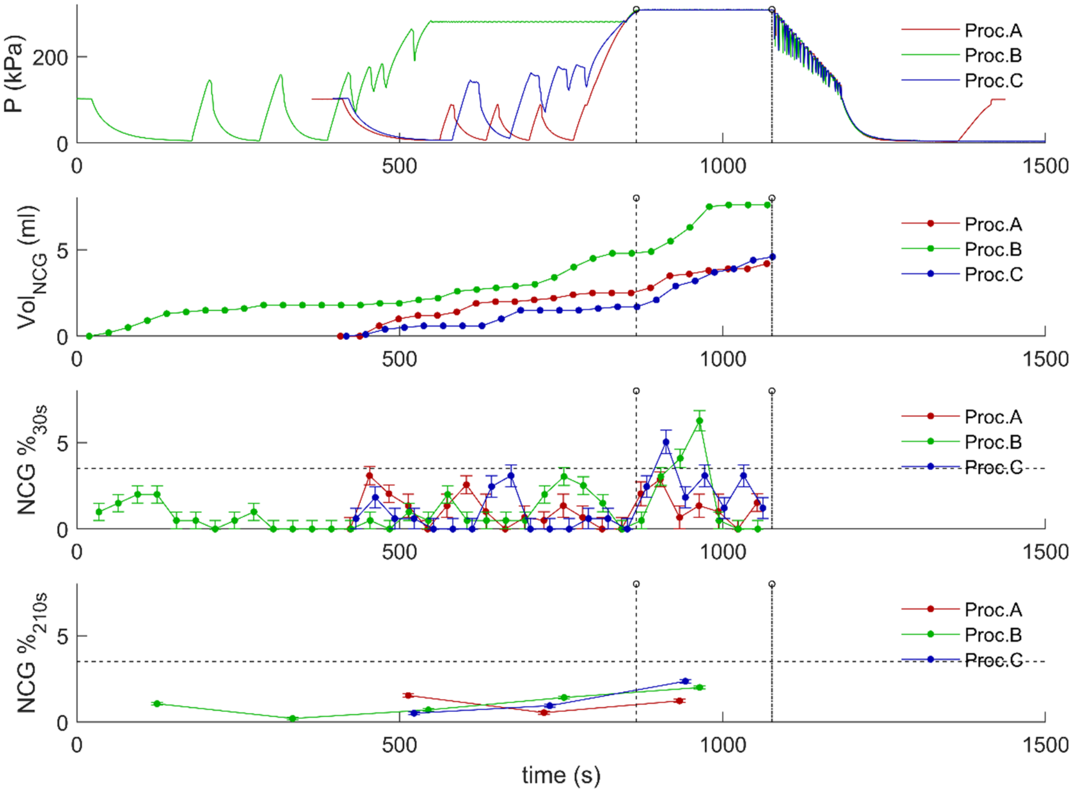

Fig. 2. Effects of the process profile on non-condensable gas quantification. Measurements of steam composition in the steam supply line according to the method specified in clause 21.1.1 of EN 285:2015 + A1:2021 for three different process profiles (A, B, and C) run in the first experimental session, with no load (empty chamber). Data referring to a specific process are consistently colour-coded in all the panels. The top panel shows the time course of the pressure in the steriliser chamber for each process. The start and end of the exposure phase are indicated by the dashed and dashed-dotted vertical lines, respectively. The second panel shows the volume of the accumulated NCGs, measured every 30 s. The third and fourth panels report the time course of NCG$\%_{30s}$ and NCG$\%_{210s}$ values, respectively. The error bars represent the total uncertainty (related to environmental and instrumental factors) associated to each NCG% measurement. The maximum amount of NCG% recommended by clause 13.3.1 of EN285:2015 + A1:2021 is indicated by the horizontal dashed line.

over two sliding windows of 30 s and 210 s. The first calculation yielded the time series NCG$\%_{30s}$ according to the formula:

$$\mathrm{NCG\%}\ _{30s} = \frac{\Delta V_{\mathrm{NCG}\ 30s}}{\Delta V_{\mathrm{C}\ 30s}} \cdot 100 \tag{2}$$

where $\Delta V_{NCG\ 30s}$ and $\Delta V_{C\ 30s}$ are the increments in the volume of the accumulated NCGs and in the volume of the collected steam condensate over a 30 s time window. The calculation on the 210 s sliding window yielded the time series NCG$\%_{210s}$ given by:

$$\mathrm{NCG\%}\ _{210s} = \frac{\Delta V_{\mathrm{NCG}\ 210s}}{\Delta V_{\mathrm{C}\ 210s}} \cdot 100 \tag{3}$$

where $\Delta V_{NCG\ 210s}$ and $\Delta V_{C\ 210s}$ are, respectively, the increments in the volume of the accumulated NCGs and of the collected steam condensate over a 210 s time window.

At the condensate flow rate of about 40 mL/min, measurements of NCG$\%_{30s}$ and NCG$\%_{210s}$ corresponded respectively to about 20 mL and 140 mL of condensate for each measurement. This range covers the condensate volumes typically used in practice during qualification activities.

To evaluate the impact of the starting point for the condensate collection in NCG% measurements, the time courses of both NCG$\%_{30s}$ and NCG$\%_{210s}$ were plotted, and individual values were compared to the maximum admissible NCG% threshold of 3.5%, adopted by both clause 13.3.1 of EN 285:2015 + A1:2021[12] and clause 3.3.3.2 of AAMI/ANSI ST79:2017[13].

Destination of use of the steam

To estimate the actual contribution of the steam composition measured in the piping system to that in the steriliser chamber, the process was divided into subphases in which the steam was actually injected into the steriliser chamber, and subphases in which the steam was addressed to other districts (e.g. chamber jacket, water degassing unit). Average and dispersion of NCG%$_{30s}$ values were separately calculated for: (i) steam injection subphases in the preconditioning phase (when the steam is injected into the sterilizer chamber), (ii) evacuation subphases in the preconditioning phase (when the steam is addressed to other sterilizer districts), and (iii) the exposure phase (when steam is again admitted into the sterilizer chamber). Different subphases in the preconditioning phase were automatically detected by measuring the first derivative of the pressure curves. For comparison, global average and dispersion of NCG%$_{30s}$ values were also calculated over the whole sterilisation process (i.e., conditioning phase plus exposure) for the different load amounts, considering triplicate data.

Environmental conditions during measurement of NCG%

The effects of environmental factors on NCG measurements were theoretically evaluated. The variability of NCG% values due to temperature (T) and pressure (p) variations was estimated within the specifications provided by the standard test method.

The variability of NCG% related to temperature and pressure depends on the definition of NCG% in terms of the ratio between NCG and condensate volumes. Indeed, while the mass (m) of a substance depends solely on the number of molecules of that substance, the volume (V) and the density (ρ) of a gaseous or liquid substance are affected by temperature and pressure conditions:

$$V = V_{(T,\ p)}; \quad \rho = \rho_{(T,\ p)} = \frac{m}{V_{(T,\ p)}}; \tag{4}$$

Thus, for the mass of NCGs and steam condensate obtained according to the standard test method, both V_{NCG} and V_{C} are sensitive to T and p. Considering the volume of a given mass of NCGs, measured in two different measurement conditions characterized by temperature T_1 and T_2 and pressure p_1 and p_2, it held:

$$\frac{V_{\text{NCG}(T1,\ p1)}}{V_{\text{NCG}(T2,\ p2)}} = \frac{\rho_{\text{NCG}(T2,\ p2)}}{\rho_{\text{NCG}(T1,\ p1)}} \tag{5}$$

where $V_{NCG(Ti,\ pi)}$ and $\rho_{NCG(Ti,\ pi)}$ indicates respectively the volume and the density corresponding to a certain NCG mass at the thermodynamic condition i, defined by temperature T_i and pressure p_i.

The same relation holds for the volume $V_{C(Ti,\ pi)}$ and density $\rho_{C(Ti,\ pi)}$ of a mass of steam condensate (water) measured in two different conditions defined by temperature T_1 and T_2 and pressure p_1 and p_2:

$$\frac{V_{\text{C}(T1,\ p1)}}{V_{\text{C}(T2,\ p2)}} = \frac{\rho_{\text{C}(T2,\ p2)}}{\rho_{\text{C}(T1,\ p1)}} \tag{6}$$

Given Eqs. (1), (5) and (6), for a given mass of the NCGs and condensate obtained during a steam composition test, the NCG% values determined at two different environmental conditions, defined by temperatures T_1 and T_2 and pressures p_1 and p_2, vary according to:

$$\begin{aligned}\text{NCG\%}_{\ (T1,\ p1)} &:= \frac{V_{\text{NCG}(T1,\ p1)}}{V_{\text{C}(T1,\ p1)}} \cdot 100 \\ &= \frac{V_{\text{NCG}(T2,\ p2)} \cdot \rho_{\text{NCG}(T2,\ p2)} \cdot \rho_{\text{C}(T1,\ p1)}}{V_{\text{C}(T2,\ p2)} \cdot \rho_{\text{NCG}(T1,\ p1)} \cdot \rho_{\text{C}(T2,\ p2)}} \cdot 100 \\ &= \frac{\rho_{\text{NCG}(T2,\ p2)} \cdot \rho_{\text{C}(T1,\ p1)}}{\rho_{\text{NCG}(T1,\ p1)} \cdot \rho_{\text{C}(T2,\ p2)}} \cdot \text{NCG\%}_{\ (T2,\ p2)}\end{aligned} \tag{7}$$

The method specified in clause 21.1.1 of the EN 285:2015 + A1:2021[12] allows to operate with the condensate and the accumulated NCGs at temperatures ranging from 20 °C (temperature of condensing water at the start of measurements) to 75 °C (maximum temperature allowed by the standard test method for the collection of the condensate).

Volume measurements should be performed with the condensate and the accumulated NCGs at environmental pressure, which can be roughly estimated to range from 97.1 to 104.7 kPa for a central EU country (without considering the pressure change occurring in the case of measurements performed at a relevant altitude above sea level).

Based on these pressure and temperature ranges and assuming that the main contribution to the NCGs in the steam comes from air, an estimate of the variability in NCG% measurements was calculated considering two extreme cases: *condition 1*, characterized by $T_1 = 20$ °C, $p_1 = 104.7$ kPa, and *condition 2*, characterized by $T_2 = 75$ °C, $p_2 = 97.1$ kPa. Because the NCG volume was in direct contact with the condensate, NCG density was assumed equal to the density of the air at a 100% relative humidity.

Quantification of NCG measurement uncertainty

The uncertainty of NCG% measurements related to environmental factors was estimated using Eq. (7), considering the variability of condensate temperature and environmental pressure measured during each experimental session. All experiments were performed by collecting and measuring the condensate and

NCG volumes using the Steam Quality Test Kit D in a stationary temperature range, which limited variations of condensate temperature to less than 5 °C. This largely reduced the possible temperature excursion of the collected condensate with respect to the apparatus described in the standard EN 285, where the steam condensate temperature can vary from environmental temperature (around 20 °C) to 75 °C.

The uncertainty of NCG% related to the measuring procedure (instrumental uncertainty) was obtained by considering the uncertainties related to the measurements of NCG and steam condensate volumes and propagating these uncertainties by applying error propagation to Eq. (1).

The total uncertainty on NCG% measurements was finally obtained by the quadratic sum of the environmental and instrumental uncertainties.

Statistical analysis

$NCG\%_{30s}$ average and dispersion values on the whole process and in subphases were expressed as median and interquartile range values. The effects of different process subphases and loads on measured steam composition were quantitatively evaluated on pooled data from triplicate experiments, using the single $NCG\%_{30s}$ measurement as the statistical unit. Specifically, statistical differences between $NCG\%_{30s}$ measurements obtained in the two subphases of the conditioning phase (i.e. when the steam was injected into the chamber vs. elsewhere) were evaluated using the Mann-Whitney U test, which was applied separately to data for each load amount (empty, half, full). Differences among $NCG\%_{30s}$ measurements in the exposure phase among different load amounts were assessed using the Kruskal–Wallis test, followed by post-hoc multiple comparisons on all pairs of loads. Test results were adjusted for multiple hypothesis testing using Benjamini-Hochberg correction. All analyses used two-sided tests with a significance level of $p < 0.05$. Statistical analyses were performed using Matlab R2024b (The MathWorks, Inc., Natick, Massachusetts, US).

Results

Effects of the process profile on NCG quantification

Figure 2 presents the results obtained in the first experimental session, using different process profiles in the absence of load in the steriliser chamber. The pressure profiles of processes A, B, and C are shown in the top panel of the figure. The vertical dashed and dash-dotted lines indicate the start and the end of the exposure phase to facilitate the identification of the main process phases. To enhance comparability, data from the three processes were time-synchronised according to the start of the exposure phase. The second panel of Fig. 2 shows the measurements of NCG volume (V_{NCG}), performed every 30 s from the start of the process to the end of the exposure phase. The time courses of the calculated $NCG\%_{30s}$ and $NCG\%_{210s}$ values are shown in the third and fourth panels, respectively. In the first experimental session, condensate temperature and environmental pressure ranged from 75° to 80 °C and from 99.60 kPa to 99.78 kPa, respectively. According to Eq. (7), the uncertainty associated with environmental factors was less than 5% of measurements. Total uncertainty in NCG% values is indicated by error bars in Fig. 2.

Data inspection pointed out that the accumulated NCG volume changed over time, showing stationary plateaux and abrupt increases, despite the constant increase of the condensate volume. This resulted in variable $NCG\%_{30s}$ values over time, where null values corresponded to stationary measurements of V_{NCG}, and peaks to abrupt V_{NCG} increases. Notably, $NCG\%_{30s}$ values were occasionally above the 3.5% standard threshold. In the three tested processes, $NCG\%_{30s}$ values exceeded the threshold during the exposure phase of processes B and C, while they remained below the 3.5% threshold during the conditioning phases of all three processes. Although no changes in the steriliser and in the feeding water were present, steam composition and conformity to clause 13.3.1 varied depending on the process profile, as summarised in Table 1.

Effects of condensate volume and steam collection starting point on NCG quantification

$NCG\%_{210s}$ measurements showed lower variability than $NCG\%_{30s}$ during the whole process, and values below the standard threshold in all three tested processes during both the conditioning and exposure phases. Table 1

Methodological option	NCG% below 3.5%? [a]		
	Process A	Process B	Process C
Collection of @20 mL of condensate during the conditioning phase	Pass	Pass	Pass
Collection of @140 mL of condensate during the conditioning phase	Pass	Pass	Pass
Collection of @20 mL of condensate during the exposure phase	Pass	Fail	Fail
Collection of @140 mL of condensate during the exposure phase	Pass	Pass	Pass

Table 1. Outcomes of the steam composition test according to the volume of the collected steam condensate (20 or 140 mL) and the sampling time (during the conditioning or the exposure phase) for three different process profiles (A, B, and C). [a]Compliant with clauses 13.3.1 of EN285:2015 + A1:2021 and clause 3.3.3.2 of AAMI/ANSI ST79:2017.

summarises the results of the conformity test to clause 13.3.1 when considering different condensate volumes (i.e., 30–210 s time windows) and steam collection starting points (i.e., conditioning versus exposure phase), which complied with clause 21.1.1 of EN 285:2015 + A1:2021. Considering the conformity requirement in clause 13.3.1, test outcomes were non-consistent in two out of the three tested processes.

Effects of the load amount on steam composition

Figure 3 presents the results of the second experimental session, where we tested the effects of variable load amounts in the steriliser chamber by repeating processes with profile A. During this session, the condensate temperature and environmental pressure ranged from 75° to 80 °C and from 101.55 kPa to 102.58 kPa, respectively, accounting for a variation in NCG% values of less than 6% of measurements. Total uncertainty in NCG% values is indicated by error bars in Fig. 3.

Data inspection showed that the composition of the generated steam was impacted by the presence of load. Major peaks in $NCG\%_{30s}$ were observed during the exposure phase of the processes with larger loads. Consistently, additional peaks, occurring during the conditioning phase, displayed higher amplitude in the presence of larger loads.

The comparison of data obtained from triplicate experiments performed with the same steriliser, process profile, and load amount evidenced additional variability in NCG% measurements. Major differences were observed in $NCG\%_{30s}$ values, both in terms of time course and absolute values, pointing out the uniqueness of every single process.

Table 2 summarises the outcome of the steam composition test with the 3.5% standard threshold. The outcome of the test and the conformity to the standard requirements showed a dependence on the collection timing and the amount of condensate considered in the NCG% calculation. In addition, the test results were strongly impacted by the load amount, and non-consistent results were present among process replicates.

Effects related to the steam destination of use

The detailed analysis of NCG% time course in relation to different subphases of the sterilisation process is exemplified in Fig. 4, which presents the process pressure profiles together with the time course of $NCG\%_{30s}$ corresponding to one exemplary process of each tested load amount. The exposure phase and the process subphases with a positive pressure gradient in the conditioning phase are highlighted by pink-shaded areas and correspond to temporal windows when the generated steam is injected into the steriliser chamber. Inspection of Fig. 4 clearly shows that, in the presence of larger load amounts, steam injection intervals in the conditioning phase became longer, due to the higher amount of condensate required to heat the load. The come-up ramp was characterised by a very low NCG amount in the steam, while markedly higher $NCG\%_{30s}$ measurements occurred at the beginning of the exposure phase. The temporal behaviour of NCG% was likely related to the steriliser controller program, which excluded refilling water in the generator when steam injection in the chamber was active during the conditioning phase.

Table 3 summarises the median and interquartile range of $NCG\%_{30s}$ values for the overall process (first row) and for different subphases (second to fourth rows). The comparison of NCG% values obtained in different process subphases pointed out lower NCG amounts in the subphases when the steam was injected into the chamber (second row) than elsewhere (third row), with differences reaching statistical significance in the case of half loads ($p = 0.0002$) and full loads ($p = 0.0000$). The inspection of NCG values during the exposure phase (fourth row) also confirmed the dependence of NCG amount on the load. Higher NCG amounts were observed for larger loads, with statistically-significant differences for empty vs. full loads ($p = 0.029$).

Effects of environmental factors on NCG% interpretation

Equations (3–6) were used to calculate the variations of NCG% at the extreme temperature/pressure conditions allowed by the standards (*condition 1*: $T_1 = 20$ °C, $p_1 = 104.7$ kPa; *condition 2*: $T_2 = 75$ °C, $p_2 = 97.1$ kPa). According to tabulated values, the densities of gas and condensate at the two conditions were: $\rho_{\mathrm{NCG}(T1,\ p1)} = 1.2337$ mg/mL, $\rho_{\mathrm{NCG}(T2,\ p2)} = 0.8258$ mg/mL, $\rho_{\mathrm{c}(T1,\ p1)} = 998$ mg/mL, $\rho_{\mathrm{C}(T2,\ p2)} = 975$ mg/mL. The substitution of these values in Eq. (7) gave the proportionality term between NCG% values in the two environmental conditions:

$$\mathrm{NCG\%}_{\ (T1,\ p1)} = 0.685 \cdot \mathrm{NCG\%}_{\ (T2,\ p2)} \tag{8}$$

Assuming a steam composition measurement, performed at T_2 and p_2, resulting in a value equal to the standard threshold of 3.5%, the same mass of NCGs and condensate, observed at the environmental conditions T_1 and p_1, would give an NCG% value of 2.4%, much lower than the threshold. Conversely, assuming a steam composition measurement, performed at T_1 and p_1, yielding a NCG% value of 3.5%, the same mass of NCGs and condensate, observed at the environmental conditions T_2 and p_2, would give a NCG% value of 5.1%, thus much higher than the threshold. Since no specification about environmental temperature and pressure is provided in the standards, NCG% measurements performed according to this method should be interpreted as follows: if the measured NCG% value is lower than 2.4% the steam quality is certainly better than the limit defined in clause 13.1.1; if the measured NCG% value is higher than 5.1%, the steam quality is certainly worse than the limit defined in clause 13.1.1; if the measured NCG% value is between 2.4% and 5.1%, the steam quality is equal to the limit in the standards within the uncertainty inherent to the measurement procedure described in the same standard.

Discussion

This study, based on systematic experiments performed under controlled conditions, provided quantitative evidence that the standard method presented in clause 21.1.1 of EN 285:2015 + A1:2021 did not provide enough specifications to obtain consistent and reliable NCG% measurements, suitable for comparison with a threshold

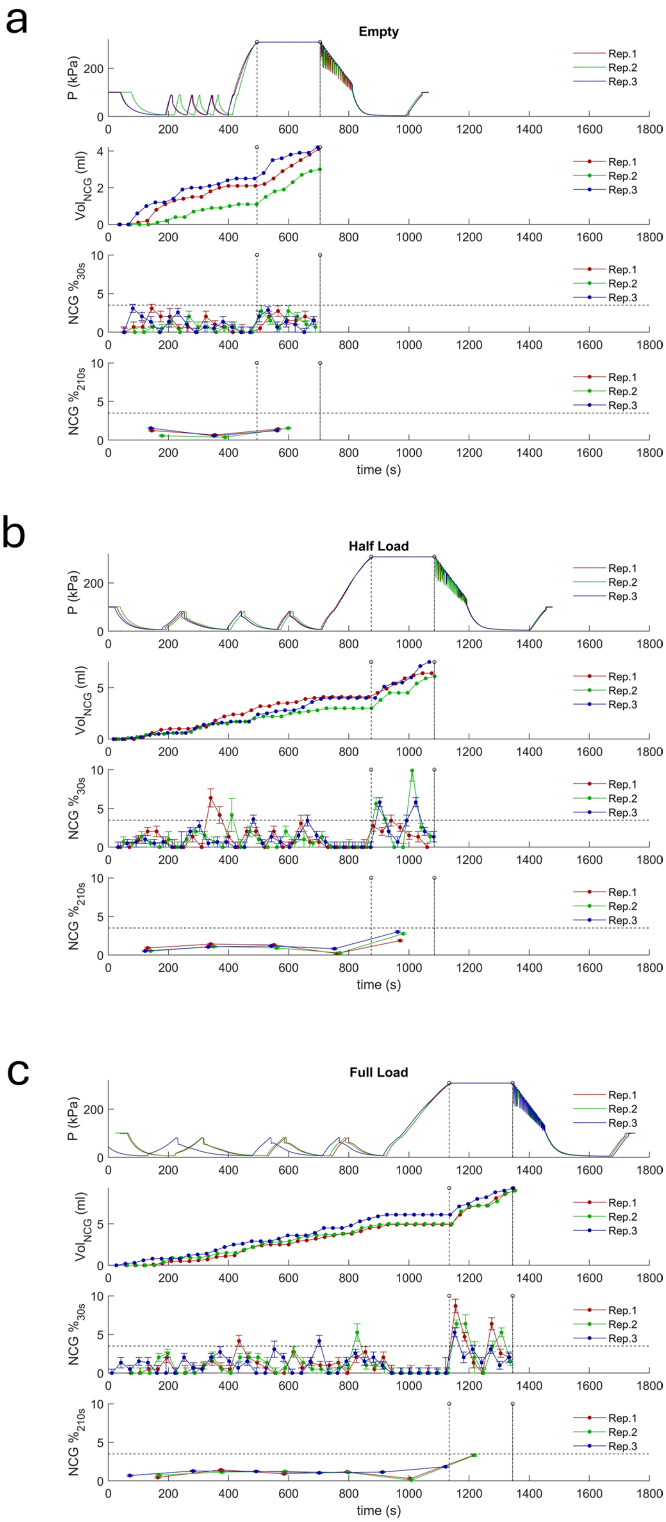

Fig. 3. Effects of the load on non-condensable gas quantification. Measurements of steam composition in the steam supply line according to the method specified in clause 21.1.1 of EN 285:2015 + A1:2021 for three different load amounts in the steriliser chamber: no load (a), half load (b), full load (c). Data refer to triplicate experiments performed in the second experimental session, running processes with profile A, where each replicate is consistently colour-coded in all the panels. The top panels show the time course of the pressure in the steriliser chamber for each process. The start and end of the exposure phase are indicated by the dashed and dashed-dotted vertical lines, respectively. The panels in the second row show the volume of the accumulated NCGs measured every 30 s. Panels in the third and fourth rows report the time course of NCG$\%_{30s}$ and NCG$\%_{210s}$ values, respectively. The errorbars represent the total uncertainty (related to environmental and instrumental factors) associated to each NCG% measurement. The maximum amount of NCG% recommended by clause 13.3.1 of EN285:2015 + A1:2021 is indicated by the horizontal dashed line.

Methodological option	NCG% below 3.5%? [a] Empty chamber	Half load	Full load
Collection of @20 mL of condensate during the conditioning phase	Pass/Pass/Pass	Fail/Fail/Fail	Fail/Fail/Fail
Collection of @140 mL of condensate during the conditioning phase	Pass/Pass/Pass	Pass/Pass/Pass	Pass/Pass/Pass
Collection of @20 mL of condensate during the exposure phase	Pass/Pass/Pass	Pass/Fail/Fail	Fail/Fail/Fail
Collection of @140 mL of condensate during the exposure phase	Pass/Pass/Pass	Pass/Pass/Pass	Pass/Pass/Pass

Table 2. Outcomes of the steam composition test according to the volume of the collected steam condensate (20 or 140 mL) and the sampling time (during the conditioning or the exposure phase) for three different load amounts (empty chamber, half load, full load). Results of triplicate experiments for each condition are presented. All tests were performed using process profile A. [a]Compliant with clauses 13.3.1 of EN285:2015 + A1:2021 and clause 3.3.3.2 of AAMI/ANSI ST79:2017.

limit. Consistent with our initial hypotheses, our results demonstrated that steam composition is affected by the process profile, the load amount, the timing and the amount of the condensate collection, and the environmental temperature and pressure conditions.

The results in Table 1, obtained with different process profiles, showed that the same steriliser complied or did not comply with the standard limit, depending on the process characteristics.

The performance of NCG%$_{30s}$ measurements pointed out high temporal variability of steam composition, suggesting that methods with higher temporal resolution should be used for monitoring NCG amount over the whole duration of the process (i.e., during both the conditioning and exposure phases). Clause 21.1.1 of the EN 285:2015 + A1:2021 reports that "*the content of non-condensable gases changes considerably* [in time]. *A peak which occurs for a few seconds can be sufficient to cause a fault during sterilization*"[12]. According to this statement, the sampling time of a system providing quantitative information on NCGs should be of the order of seconds. This is not the case for the method specified in clause 21.1.1 of the same standard, which requires collecting a certain volume of steam condensate by spilling steam from the pipeline "*to cause a small amount of steam hammer to be heard*"[12,18]. This process typically requires some minutes to obtain enough steam condensate in the measuring cylinder and complete the measurement procedure. Due to its low time resolution, the standard method may not be able to detect NCG peaks of short duration.

The results presented in Table 2 highlighted the influence of the load on the composition of the generated steam, in agreement with what is reported in the standards[12] and literature[21,22]. NCG% measurements, performed in an empty chamber, might not be representative of the steam composition generated when a load was present in the steriliser chamber. This makes questionable the value of the measurements performed with no load to assess steam composition during production.

As an additional source of variability, triplicated data collected in this study showed that steam composition in the supply line was variable over time and among processes, suggesting the necessity of monitoring steam composition in every cycle. This is consistent with several studies, which have provided evidence that every sterilisation process is unique[23] and that steam composition varies in each process, requiring steam composition monitoring, together with temperature and time, for every load[4]. Indeed, the result of a steam sterilisation process depends on the steriliser, the process, the load, the loading pattern, and the microbiological barrier used[21,22], which may all affect the steam composition[23].

The variability of the environmental conditions at the test site was also identified as a non-negligible source of uncertainty on NCG% measurements expressed as volume fraction. The pressure and temperature of the collected NCGs and condensate affect their volumes and thus the calculated NCG% values. To account for environmental effects, we proposed a more structured criterion for interpreting NCG% measurement conformity to clause 13.1.1. However, a more effective and direct approach to avoid the dependence on environmental conditions would be the quantification of NCG amount in terms of mass fraction (i.e., the mass of NCGs divided by the total mass of NCGs and water vapour together, $m_{\mathrm{NCG}}/m_{\mathrm{Steam}}$)[24]. As previously pointed out, the mass does not directly depend on the temperature and/or pressure of the gas mixture. For this reason, the description of steam composition using the mass fraction is typically adopted in physical models of steam condensation heat transfer in the presence of NCGs[10,16].

Besides the above-discussed limitations, the added value of NCG% measurements performed in the steam pipes to estimate steam composition inside the steriliser chamber remains unclear[24]. NCGs in the steriliser chamber (i.e. where steam sterilisation conditions should be obtained) can arise from different sources, including NCGs entering the chamber from the steam supply, an inadequate air removal stage in the conditioning phase, and air leakage into the chamber during sub-atmospheric phases[15,25]. Therefore, measuring the steam composition at the steam supply level may be poorly informative about the actual composition of steam in the steriliser chamber[24]. Even assuming that no other sources of NCGs were present beyond the steam generator, the results reported in Table 3 pointed out the necessity of considering the destination of use of the steam and

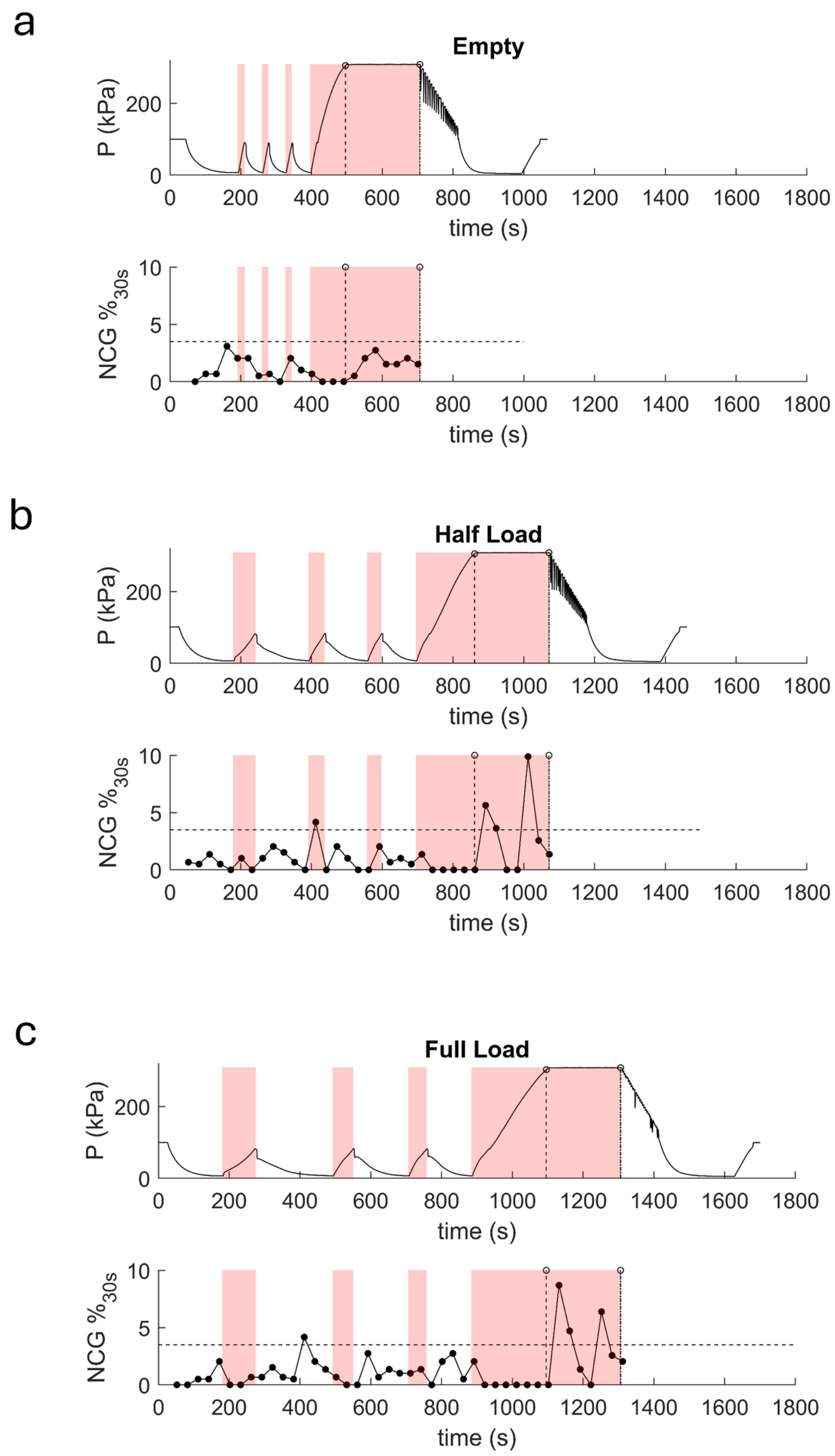

Fig. 4. Effects of the destination of use of the steam on non-condensable gas quantification. Time course of steam composition measurements in the steam supply line in relation to process phases/subphases. One exemplary process for each of the three different load amounts in the steriliser chamber is presented: no load (a), half load (b), and full load (c). Pink-shaded areas, including subphases in the preconditioning phase and the whole exposure phase, indicate process time windows in which steam is injected into the steriliser chamber. In the remaining process intervals, the generated steam does not enter the steriliser chamber. Data refers to experiments performed in the second experimental session, running processes with profile A. The top panels indicate the time course of the pressure in the steriliser chamber for each process. The start and end of the exposure phase are indicated by the dashed and dashed-dotted vertical lines, respectively. The lower panels show the volume of the accumulated NCGs measured every 30 s. The maximum amount of NCG% recommended by clause 13.3.1 of EN285:2015 + A1:2021 is indicated by the horizontal dashed line.

Process phase/subphase used for the calculation of the average NCG%$_{30s}$ (steam destination of use)	**NCG%$_{30s}$ over the specified phase/subphase Median (25th percentile, 75th percentile), %**		
	Empty chamber	**Half load**	**Full load**
Precondition phase + Exposure phase (steriliser chamber + other districts)	1.52 (0.38; 2.04)	0 (0; 1.65)	0 (0; 1.52)
	0.67 (0.25; 1.58)	0.51 (0; 2.56)	0 (0; 1.65)
	1.01 (0.34; 1.44)	0.67 (0; 1.87)	0 (0; 1.35)
	1.01 (0.8; 2.04)	**0.67 (0; 2.04)**	**0 (0; 1.39)**
Steam injection subphases in the preconditioning phase (steriliser chamber)	0.34 (0; 2.04)	0 (0; 0)	0 (0; 0)
	0 (0; 0.67)	0 (0; 1.27)	0 (0; 0)
	0.67 (0; 1.10)	0 (0; 0.67)	0 (0; 0)
	0.34 (0; 0.84)	**0 (0; 0.67)**	**0 (0; 0)**
Evacuation subphases in the preconditioning phase (other districts)	0.67 (0.38; 1.27)	1.18 (0; 2.04)	1.01 (0.50; 2.04)
	0.67 (0; 0.84)	0.67 (0.38; 1.10)	1.01 (0.50; 2.04)
	1.01 (0; 2.04)	0.67 (0.50; 1.65)	1.52 (0.54; 2.04)
	0.67 (0; 1.35)	**0.67 (0.50; 2.04)**	**1.35 (0.50; 2.04)**
Exposure phase (steriliser chamber)	1.52 (1.52; 2.04)	2.04 (1.39; 2.70)	2.56 (1.52; 5.97)
	1.52 (0.76; 2.57)	2.56 (0.34; 5.13)	3.45 (1.65; 6.10)
	1.35 (0.76; 1.91)	2.04 (1.52; 5.23)	2.04 (1.52; 3.09)
	1.52 (0.93; 2.04)	**2.04 (1.35; 3.49)**	**2.56 (1.48; 5.26)**

Table 3. Effects of the destination of use of the steam and of load amount on non-condensable gas quantification. Medians (25th and 75th percentiles) of NCG%$_{30s}$ values were calculated over the whole phase/subphase of interest. Results are grouped according to load amount (empty chamber, half load, full load) and presented separately from each replicate experiment and overall on the triplicate pooled values (in bold format).

restricting NCG% quantification to subphases when steam is actually injected into the chamber for a better estimation of the steam composition in the sterilizer.

It is finally worth noticing that no calibration procedure is provided to verify the accuracy of the standard method, beyond the calibration of the volume of the graduated containers used for measuring NCG and condensate volumes. This crucial aspect suggests that the method should be considered as an indicative rather than a quantitative test method for measuring steam composition.

In summary, the demonstrated limitations and uncertainties associated with the current standard test method point out the urgency of developing alternative methods for a reliable and time-resolved quantification of NCGs. As specified in 21.1.1 of EN 285:2015 + A1:2021, "*An alternative procedure to the one described in 21.1 may be used, providing that it has been shown to give equivalent results to the methods specified in this European standard*". Based on the results of this study, alternative procedures should not only give equivalent results, but they should go beyond the current standard specifications, providing better time resolution, less sensitivity to environmental conditions, and, possibly, higher predictive power on the steam composition in the sterilizer, and the capability of monitoring every process during the sterilizer production. According to the findings of a recent field study[18], the reliable and accurate measurement of steam composition could also have the potential to indirectly monitor the water quality supplied to the steam generator, a relevant factor influencing medical device processing[26].

In the last decade, several technologies have been described in the literature to measure steam composition in the context of steam sterilisation. An electronic device intended to be used as an alternative Bowie and Dick test showed the potential for quantifying NCGs in the sterilizer chamber[27]. Some years ago, leveraging on the selective absorbance of infrared light by the water vapour fraction of steam, an infrared sensor was shown to allow monitoring steam penetration in channelled loads[28,29]. Similar approaches to detect the water vapour fraction inside the steriliser chamber[30], in small cavities connected to the steriliser chamber[31], or in long and narrow lumens[32] were developed at a prototype level, showing promising results and agreements with numerical models[31,32]. Recently, a sensor based on heat-flux measurements was released to the market to perform continuous measurements of the NCG amount in the steriliser chamber during each production process[6]. This sensor showed its capability to provide reproducible measurements of NCGs resulting from different sources, with negligible effects of the NCG source location and the load in the chamber[24]. A comprehensive study from the "Working Party on Improving Parametric Load Release for Steam Sterilisation" addressed the sustainability and costs associated with the use of this technique and pointed out that monitoring steam composition of every process was cost-effective and more environmentally sustainable than using other indicators[6]. Cost assessment assumed no changes in the safety of the sterilisation process. However, the literature indicates that the incidence of failed steam penetration tests performed in production processes ranges from 2 to 3%[6,33]. The opportunity to identify these failures may reduce the residual risk for patients and staff and lead to cost savings by reducing treatment of possible device-related infections[34].

In addition, the information provided by these innovative sensors, combined with the steriliser information about temperature and time, may radically improve the parametric monitoring of the sterilisation process[4,24], toward the full parametric release of the processed loads.

Conclusion

The current standard test method for measuring steam composition has severe limitations in terms of reproducibility, time resolution, and interpretation. The study findings showed that condensate collection timing, condensate volume, and environmental conditions determined a non-negligible NCG% variability, and the measured composition of the steam generated in a production process differed significantly from that of an empty load.

Overall, NCG measurement variability impacted standard method reliability to qualify the steriliser and to estimate steam composition in the steriliser chamber. New methodologies capable of real-time quantitative monitoring of steam composition in every process are needed to improve the safety and reliability of the steam sterilisation process.

Data availability

All main data are available in the text or figures. Complementary information on the 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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Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to F.T.