Method Article

Determination of hydrogen peroxide on N95 masks after sanitization using a colorimetric method

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ABSTRACT

Hydrogen peroxide is commonly used as a sterilizing agent for medical devices and its use has recently been extended to N95 masks during PPE shortages as a result of the COVID-19 pandemic. The hydrogen peroxide remaining on the masks after sterilization could potentially pose a health hazard to the mask users. In the present study a colorimetric method was optimized for the determination of hydrogen peroxide on N95 masks following chemical sanitizations. The developed analytical method demonstrated an overall recovery of 98% $\pm$ 7%. The limit of detection ranged from 0.16 to 0.25 mg/mask, depending on the type of mask. The expanded measurement uncertainty was 13% (at a 95% confidence interval). The sanitization process itself introduced a significant variation in hydrogen peroxide load between masks. The ozone used in the sanitization process had no significant impact on analytical performance. Stamped and printed marks on the mask surfaces could induce biased readings. Hydrogen peroxide decomposes quickly on the mask surfaces so timing of analysis is an important factor in method standardization.

- The validation data demonstrated that the in-house method is reliable and fit for the intended purpose, offering a sensitive, simple, rapid, and inexpensive method of residue monitoring.

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Specifications table

Subject Area:	Chemistry
More specific subject area:	Analytical chemistry
Method name:	Extraction and colorimetric determination of hydrogen peroxide on masks
Name and reference of original method:	10.1016/j.jhin.2021.02.018
Resource availability:	All reagents here were acquired from MilliporeSigma.

Background

Due to a shortage of the worldwide supply of N95 masks, the option to extend their useful life was considered by the World Health Organization [1], the U.S. Centers for Disease Control and Prevention [2], Health Canada [3] and the European Centre for Disease Prevention and Control [4]. Additionally the reuse of mask could reduce waste-burden created by PPEs [5]. Many physical and chemical sanitization approaches have been investigated for bactericidal and virucidal efficacy, filtration performance, and fit test as the masks may lose their filtration efficiency due to the sanitation procedures [6–10]. However these studies have thus far ignored the chemical residues left behind after chemical sterilization. Thus, residues of the chemicals used as ingredients in the sanitization are a topic of concern for the users, and knowledge on residues on N95 masks is limited.

Hydrogen peroxide is the most popular disinfectant for mask sanitization [11]. The residues of hydrogen peroxide on mask following chemical sanitization can potentially pose an inhalation and/or dermal exposure risk. Health effects depend on the concentration and quantity of hydrogen peroxide which may irritate the nose, throat and respiratory tract via inhalation and may cause whitening of the skin or severe burns when exposed to high concentrations [12].

Analytical methodologies to quantify hydrogen peroxide in household and cosmetic products, and in environmental samples, are primarily based on titrimetry [13], colorimetry and ultraviolet visible spectrometry (UV-Vis) [14–16], flow injection [17] or high-performance liquid chromatography with UV-Vis [16], fluorescence [18], chemiluminescence [19], and near-infrared detection [20].

In the sanitization system for N95 masks, hydrogen peroxide is utilized in a liquid or vapor form in relatively large quantities (3% to 50% w/w) to ensure high microbial efficacy. The remaining hydrogen peroxide after the sanitization process is absorbed or condensed on the surface of the masks [21]. The colorimetric method for hydrogen peroxide determination in air sample [16] was selected as a starting point in this study. Hydrogen peroxide from masks was extracted by water and reacted with titanium oxysulfate resulting in a yellow color solution. The 409 nm wavelength was used for quantification with a set of hydrogen peroxide standard solutions.

The method characteristics of hydrogen peroxide determination on masks were investigated using a single laboratory validation approach [22] and Eurachem guideline for uncertainty evaluation [23]. A colorimetric method between hydrogen peroxide with titanium oxysulfate was found to fit for use considering its reliability, sensitivity, simplicity, speed, and accessibility.

Material and method

Chemicals

30 % (w/w) hydrogen peroxide (PerhydrolTM), 25% (w/w) sulfuric acid (EMSURE®), and 15% (w/w) titanium oxysulfate were acquired from Millipore Sigma, Canada. Deionized water was produced in-house using Millipore Synergy UV-R, Canada. 30% (w/w) hydrogen peroxide was diluted to 30 mmol/L stock solution. Calibration of the hydrogen peroxide stock solution was carried out on a biweekly basis by titration with 20 mmol/L potassium permanganate solution (detailed in Supplementary Material).

Sample preparation

Six types of N95 masks were used for the method validation studies (Table S1 Supplementary Material). Two sanitization systems, employing either 3% hydrogen peroxide aerosol spray, ozone and UV-C or 50% hydrogen peroxide vaporization, were used in this study [18].

Sample extraction and measurement

A mask was weighed (W_1) before sanitization.

After sanitization, the mask was cut into a 2 cm x 2 cm piece and weighed (W_2) into a 50 mL polypropylene vial with a screw cap.

10 mL of deionized water (V) was pipetted into the vial, which was then tightly closed.

The vial was vigorously shaken for 2 min (manual shaking end-over-end, 120 to 150 times) or using a mechanical end-over-end shaker (at 30 rpm for 5 min) at room temperature. During the mixing it must be ensured that the test piece is always in the extraction solution as there is tendency to float.

5 mL of the extracted solution was pipetted into a 15 mL test tube.

0.4 mL of 7.5% (w/w) titanium oxysulfate and 2 mL of 25% w/w sulfuric acid were added into the 15 mL tube and gently mixed.

Approximately 2 mL of the test solution was transferred into a 10 mm cuvette.

Wavelength of 409 nm was used for the quantification in a UV-Vis spectrometer (Varian Easy 5000).

A set of hydrogen peroxide standard solutions was prepared according to Section 2.3.5 to 2.3.8.

Data processing

The concentration of hydrogen peroxide on the mask (mg/mask) was calculated using Eq. (1).

$$\text{Concentration } H_2O_2 \, (\text{mg/mask}) = \frac{[C] \text{ x MW x V x } W_1}{W_2 \text{ x } 1000} \tag{1}$$

Where [C] is the concentration of hydrogen peroxide in mmol/L in the test solution, MW is the molecular weight of hydrogen peroxide (34 g/mol), V is the extraction volume (10 mL). Weight of mask (W1) and a test piece (W2) in g as explained in Section 3.

Results

Limit of detection

The yellow color solution of peroxo-titanium complex, derived from the reaction between hydrogen peroxide and titanium oxysulfate, was scanned for the UV-Vis spectrum from 200 to 700 nm. The wavelength of 409 nm was selected for quantification of hydrogen peroxide (Fig. S1 Supplementary Material).

The absorbance at 409 nm was plotted against the concentrations of hydrogen peroxide from 0.015 to 0.765 mmol/L. The limit of detection (LOD) was calculated using a linear calibration equation and the LOD was 0.007 mmol/L (Table S2 Supplementary Material). The experimental LOD was determined and confirmed the LOD as 0.006 mmol/L, which is equivalent to 0.16 mg/mask for a thin fold-type mask, and 0.25 mg/mask for a thick cupped-type mask.

Linearity range

The assay demonstrated good linearity in the concentration range of 0.006 to 3.23 mmol/L as seen in Table S3 and Fig. S2 Supplementary Material. The average slope of the calibration curve, using 18 calibration data sets, was 0.5046 ± 0.0459, and the intercept of -0.0038 ± 0.0131 at a 95% confidence interval. The slope and intercept values, and associated standard deviations were used as a quality control parameter for the stability monitoring of standard solutions and UV-Vis spectrometer performance.
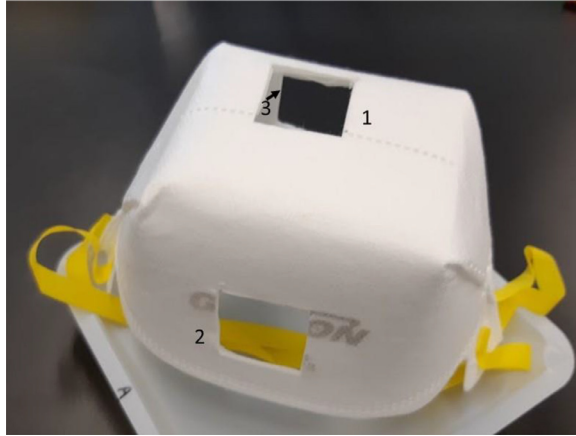
Fig. 1. Three sampling areas on mask: 1-top, 2-side with a stamped mark and 3-side without a stamped mark were extracted for hydrogen peroxide analysis.
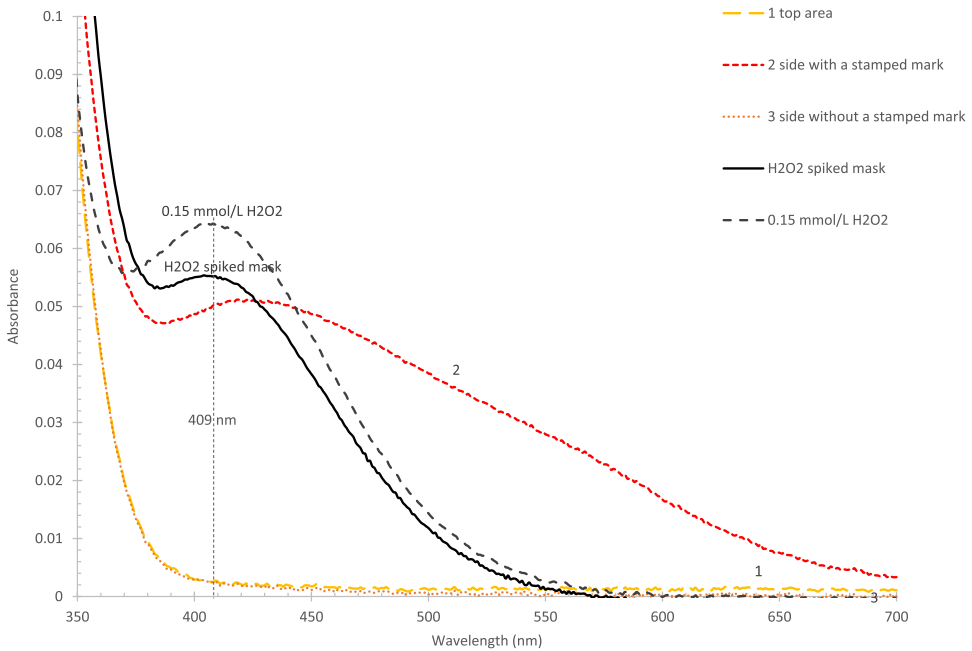

Fig. 2. Absorption profiles of various mask pieces sampled from: 1: top area, 2: side area with stamped and 3: side area without a stamped mark.

Interferences

Markings on the mask surface

The mask was sanitized and dried for two hours. On the mask surface, there are stamped marks or emblems. Three areas on the mask (Fig. 1) were sampled (1: top area, 2: side area with a stamped mark, and 3: side area without a stamped mark). In Fig. 2, it is noted from the UV-Vis spectrum
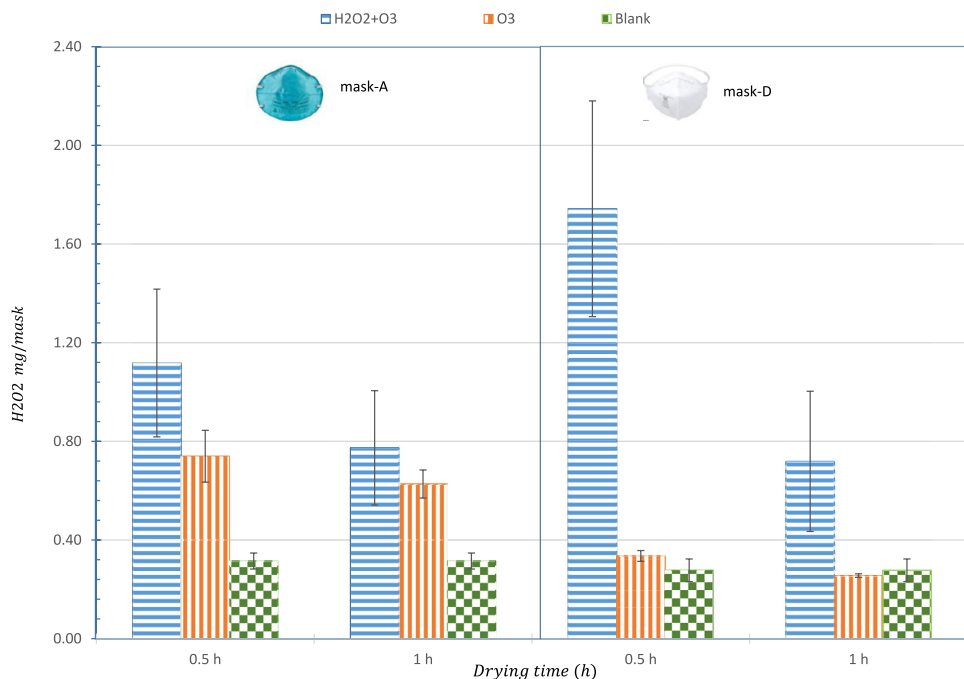
Fig. 3. The interference of ozone on hydrogen peroxide analysis on masks, the blue bar is from H_2O_2 and O_3 in a normal sanitization, the orange bar is only O_3, and a grey bar is untreated masks (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

that the absorbance of the test sample 2 (stamped area) was significantly higher (hydrogen peroxide reading equivalent to 2.02 ± 0.64 mg), showing a 15 times higher concentration than the unstamped areas. Thus the test piece sampled from the stamped mark or branded areas interfered with the analysis.

Presence of ozone

Ozone gas was often used in the sanitization processes along with hydrogen peroxide. Ozone is a strong oxidizer and could potentially react with titanium oxysulfate and result in a false positive hydrogen peroxide reading. To test this hypothesis, 8 mg/L ozone was applied in an aerosol sanitization system to the masks without any hydrogen peroxide. The mask was dried at room temperature before extraction.

As shown in Fig. 3, it appeared that the presence of a small amount of ozone slightly elevated hydrogen peroxide blank readings in mask-A (a thick cupped-type) but had no impact on mask-D (a thin folded-type). The concentration of hydrogen peroxide decreased after drying, as seen in both mask-A and mask-D. However, for masks treated with ozone only the concentrations seemed stable. It is possible that the ozone had a longer retention time and reacted with the reagent in a thick cupped-type. In normal sanitization operation, much higher concentrations of hydrogen peroxide (percent level) were used compared to the ozone (part per million level), it is unlikely that the ozone could interfere hydrogen peroxide analysis.

Blank determination

Untreated masks were extracted (n=3) with water, and later the solution was reacted with titanium oxysulfate. The results (Fig. 4) showed that a green color mask (mask-A) gave the highest background reading corresponding to hydrogen peroxide of 0.37 ± 0.10 mg/mask, compared to white
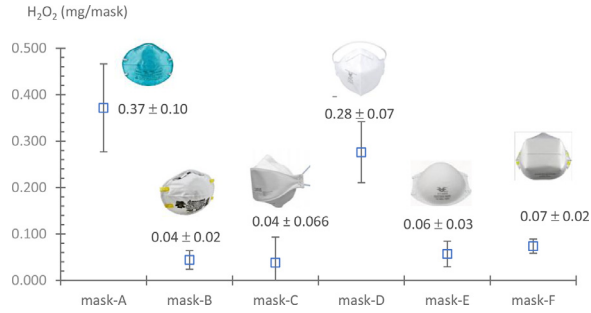
Fig. 4. The blank readings of various masks and the corresponding hydrogen peroxide concentrations (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.).
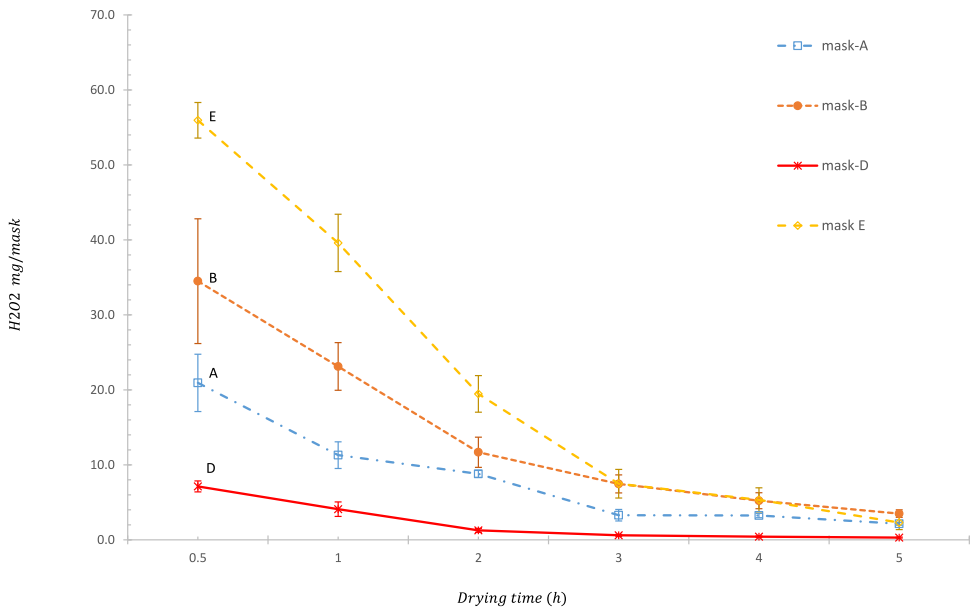

Fig. 5. Loss of hydrogen peroxide determination during room temperature storage.

color masks (0.04 to 0.28 mg/mask). This reading is likely the result of the low specificity of the assay, not the actual hydrogen peroxide presence on the mask. For trace level measurements, blank correction must be deducted from Eq. (1).

The effect of drying time

The masks, after sanitization using the 50% hydrogen peroxide vaporization system, were left in the laboratory at room temperature for five hours. Every hour, a 2 cm x 2 cm piece on the mask surface was cut and extracted. The stability of hydrogen peroxide concentrations on the mask was monitored. The concentrations of hydrogen peroxide rapidly decreased, as seen in Fig. 5. The rate of decrease is dependent on the mask types. Therefore, the determination of hydrogen peroxide on masks should be carried out as soon as possible. If an immediate analysis is not feasible, masks with hydrogen peroxide should be stored in a closed, non-absorbing container and kept in a refrigerator to slow down the decomposition of hydrogen peroxide prior to the analysis. However, stability in any of these storage conditions was outside the scope of this study.
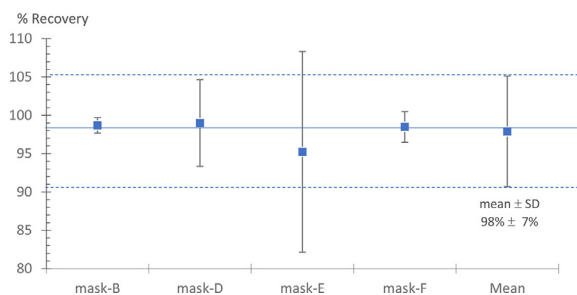
Fig. 6. Recovery (%) of hydrogen peroxide analysis on masks.

It is recommended that the time of analysis in relation to the mask sanitization should be recorded in the test report as this could be a significant variable. The method described here could be applied to risk evaluation studies as the demonstrated in a recent investigation of hydrogen peroxide residue on different types of masks, which showed the exponential decrease of the residue levels during the aeration at the room temperature [24].

Extraction procedures

The hydrogen peroxide was extracted using both 1) manual shaking and 2) mechanical end-over-end shaking. After sanitization masks were dried for 0.5 h, then test pieces (2 cm x 2 cm) were cut. Three pieces were extracted using manual shaking for 2 min (circa 120–150 times end-over-end shaking). Three test pieces were extracted using a mechanical end-over-end shaker at a speed of 30 rpm for 5 min.

The concentrations of hydrogen peroxide extracted by the two extraction procedures were compared, and data evaluated using One-way ANOVA (Tables S4 and S5 of Supplementary Material). The probability value of greater than 0.05 from the One-way ANOVA demonstrated no significant difference between manual and mechanical shaking.

Accuracy and precision

To demonstrate accuracy and precision of the analytical method, the recovery was assessed using spiked mask samples. On the test piece, 0.1 mL of 0.30 mmol/L hydrogen peroxide (equivalent to 1 µg/piece) was deposited. Then extraction was carried out followed by the determination of hydrogen peroxide. Percent recovery of the extraction ranged from 80 to 105%. The overall mean recovery associated and relative standard deviation was 98 % $\pm$ 7 % (Fig. 6) and Table S6 Supplementary Material). T-statistic detected no difference between the determined and "true" spike values for all mask types.

Precision within- and between-mask samples

Masks were sanitized using the two sanitization systems. For the within-mask study, each mask was cut into three test pieces to compare hydrogen peroxide levels (Table 1). The variation of results (as % RSD) by aerosol sanitization ranged from 2 to 11%, and by vaporization sanitization from 4 to 13%. All standard deviations were combined to be 7 %. The two sanitization systems gave different hydrogen peroxide loads, however the relative standard deviations were similar. It is apparent that the distribution of hydrogen peroxide was homogeneous on the mask surfaces.

The variation between masks (N = 3) were investigated using the two sanitization systems. The deviation (as% RSD) ranged from 6 to 28% by aerosol sanitization process, and from 2 to 21% by vaporization process (Table 2). It appears that the larger variation between mask samples is likely due to the actual sanitization equipment design, not the analytical procedure. However, when a monitoring

Table 1

Precision (% RSD) within-mask by two sanitization procedures.

| Mask	3% H_2O_2 aerosol sanitization (n = 3)			50% H_2O_2 vaporization sanitization (n = 3)		
	Mean	SD	RSD (%)	Mean	SD	RSD (%)
Mask-A	0.974	0.023	2	20.1	2.6	13
Mask-B	0.914	0.034	4	32.2	2.7	8
Mask-D	2.006	0.051	3	6.68	0.3	4
Mask-E	1.527	0.166	11	55.5	3.2	6
RSD_{pooled}	7 %					

RSD_{pooled} is calculated by $\sqrt{\frac{\sum (RSD_i^2 \ x \ (n_i-1))}{\sum (n_i-1)}}$, where RSD_i is a relative standard deviation from mask(i), $n_i =$ a number of analyses per mask(i). $n_i =$ a number of analyses per mask(i).

Table 2

Precision (% RSD) between mask samples by two sanitization procedures.

Mask	3% H_2O_2 aerosol sanitization (N = 3)			50% H_2O_2 vaporization sanitization (N = 3)		
	Mean	SD	RSD (%)	Mean	SD	RSD (%)
Mask-A	1.084	0.062	6	21.70	4.56	21
Mask-B	1.054	0.280	27	31.58	6.49	21
Mask-D	1.662	0.468	28	7.420	0.819	11
Mask-E	1.733	0.412	24	56.25	0.99	2
RSD_{pooled}	23 %			16 %		

RSD_{pooled} is calculated by $\sqrt{\frac{\sum (RSD_i^2 \ x \ (N_i-1))}{\sum (N_i-1)}}$, where RSD_i is a relative standard deviation from mask(i), $N_i =$ a number of masks(i).
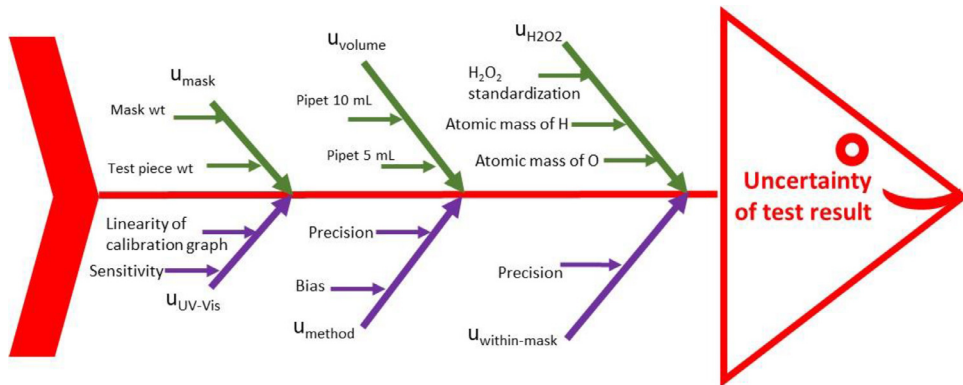
Fig. 7. Identify source of uncertainty.

regime is implemented, the mask to mask variation should be assessed, as this seems to be the single largest uncertainty component in this study.

Uncertainty of the measurement

Uncertainty (u) is a parameter associated with the measurement results and demonstrates the dispersion of the observations. The calculation of measurement uncertainty in this study followed the Eurachem approach on quantifying uncertainty in analytical measurement [23]. Sources of uncertainty were related to Eq. (1) and the method validation and identified in a fish-bone diagram (Fig. 7). Individual source was calculated to obtain % uncertainty distribution as shown in Fig. 8. All values used for calculation were from the validation data and the uncertainty calculation is illustrated in Table S7 Supplementary Material. All uncertainties were combined as in Eq. (2) and expanded to cover
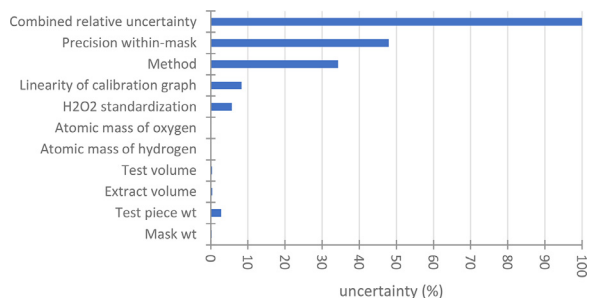
Fig. 8. Uncertainty profile of hydrogen peroxide determination on masks.

a 95% confidence interval. The expanded uncertainty (U) of the hydrogen peroxide determination on mask was 13%. It appears that two major sources of uncertainty were from within-sample precision and measurement method.

$$u = \sqrt{u_{mask}^2 + u_{volume}^2 + u_{H_2O_2}^2 + u_{UV-Vis}^2 + u_{method}^2 + u_{within\ mask}^2}, \tag{2}$$

Conclusion

The performance characteristics of an in-house method for the determination of hydrogen peroxide residue on sanitized masks were investigated along with the estimation of measurement uncertainty. The detection limit was 0.16 mg/mask (a folded-type) and 0.25 mg/mask (a cupped-type). The overall mean recovery and standard deviation was 98% ± 7%. Overall method uncertainty was 13 at a 95% confidence interval. Within-mask precision, followed by the measurement method, were the largest sources of uncertainty.

The interference study indicated that the printed marks on mask surfaces were giving false positive readings. A significant decrease of hydrogen peroxide concentrations was observed over time during sample preparation, so timing of analysis is crucial. Ozone showed no significant impact on the hydrogen peroxide analysis in this study. The engineering aspects of the sanitization process / equipment could have a significant impact on the mask to mask hydrogen peroxide load variation, so it should be investigated further. In this study, it resulted in variations of up to 28% RSD. Overall the validation data demonstrated that the in-house method is reliable and fit for the intended purpose, offering a sensitive, simple, rapid, and inexpensive method of residue monitoring in a production environment.

Acknowledgements

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10. 1016/j.mex.2021.101485.

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