การตรวจสอบความใช้ได้ของกระบวนการใช้ซ้ำของชุดวงจรช่วยหายใจ สำหรับเครื่องช่วยหายใจและเครื่องให้ออกซิเจนแบบไหลอัตราสูงในโรงพยาบาลแห่งหนึ่ง*

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บทคัดย่อ

ปัจจุบันโรงพยาบาลพระปกเกล้ามีการใช้ชุดวงจรช่วยหายใจสำหรับเครื่องช่วยหายใจและชุดวงจรให้ออกซิเจนอัตราการไหลสูงทางจมูก (HFNC) ทั้งชนิดใช้ซ้ำและชนิดใช้ครั้งเดียวที่นำกลับมาทำความสะอาดและทำให้ปราศจากเชื้อเพื่อนำกลับมาใช้ใหม่เป็นจำนวนมาก ทำให้เกิดความกังวลเกี่ยวกับความปลอดภัยและประสิทธิภาพของกระบวนการดังกล่าว การศึกษานี้ มีวัตถุประสงค์เพื่อตรวจสอบความใช้ได้ของกระบวนการนำกลับมาใช้ซ้ำ และประเมินประสิทธิภาพการทำงานของชุดวงจรช่วยหายใจสำหรับเครื่องช่วยหายใจและเครื่องให้ออกซิเจนไหลอัตราสูง โดยมุ่งเน้นประสิทธิผลของการทำความสะอาด การทำให้ปราศจากเชื้อ และความปลอดภัยของอุปกรณ์หลังการนำกลับมาใช้ซ้ำ

การวิจัยเป็นการศึกษาเชิงทดลองในห้องปฏิบัติการ กลุ่มตัวอย่างประกอบด้วยข้อต่อ (ชนิดใช้ครั้งเดียว) และท่อยืดหยุ่นซิลิโคน (ชนิดใช้ซ้ำ) อย่างละ 28 ตัวอย่าง สำหรับการตรวจสอบโปรตีนและคราบเลือดตกค้าง และอย่างละ 20 ตัวอย่าง สำหรับการทดสอบความปราศจากเชื้อและความเป็นพิษต่อเซลล์ ส่วนการทดสอบรั่วซึมใช้ชุดวงจรชนิดใช้ครั้งเดียวของเครื่องช่วยหายใจและ HFNC จาก 3 ยี่ห้อ ๆ ละ 1 ชุด ทดสอบ 10 รอบการใช้งาน เครื่องมือที่ใช้ประกอบด้วยชุดตรวจคราบโปรตีนและคราบเลือด การทดสอบความปราศจากเชื้อ ด้วยวิธี direct inoculation ตาม USP <71> การทดสอบความเป็นพิษต่อเซลล์ ด้วยวิธี MTT assay ตาม ISO 10993-5 และการทดสอบรั่วซึมตาม ISO 5367 วิเคราะห์ข้อมูลด้วยสถิติเชิงพรรณนา

ผลการวิจัย พบว่า ข้อต่อและท่อหลังการทำความสะอาดไม่พบคราบโปรตีนหรือเลือดตกค้าง (ผ่าน 100%) ไม่พบการเจริญของจุลชีพและไม่พบความเป็นพิษต่อเซลล์ (cell viability≥70%) การทดสอบรั่วซึมของชุดวงจรใช้ครั้งเดียวหลัง การนำกลับมาใช้ซ้ำ 10 รอบ พบว่า ค่าการรั่วเพิ่มขึ้นเล็กน้อยแต่ยังอยู่ในเกณฑ์มาตรฐาน (≤3 mbar/min) และสภาพอุปกรณ์ยังคงปกติ ผลการวิจัยนี้ยืนยันให้เห็นว่า ชุดวงจรช่วยหายใจทั้งชนิดใช้ซ้ำและชนิดใช้ครั้งเดียวที่ผ่านกระบวนการใช้ซ้ำตามวิธีที่ตรวจสอบแล้ว มีความสะอาด ปลอดเชื้อ ไม่ก่อให้เกิดความเป็นพิษต่อเซลล์ และยังคงรักษาสมรรถนะการทำงานได้ แม้ผ่านการใช้ซ้ำหลายรอบ จึงสามารถนำกลับมาใช้ซ้ำได้อย่างปลอดภัย ทั้งยังช่วยลดค่าใช้จ่ายและลดปริมาณขยะทางการแพทย์โดยไม่ส่งผลต่อความปลอดภัยของผู้ป่วย นอกจากนี้ ผลการวิจัยยังช่วยเพิ่มความมั่นใจให้กับหน่วยเครื่องมือแพทย์ของโรงพยาบาลในการนำชุดวงจรกลับมาใช้ซ้ำอย่างเป็นระบบตามขั้นตอนมาตรฐาน สนับสนุนการบริหารจัดการอุปกรณ์การแพทย์ที่มีประสิทธิภาพและยั่งยืนมากขึ้น ทั้งนี้ ควรมีการศึกษาวิจัยเพิ่มเติมในระดับคลินิกเพื่อยืนยันผลในระยะยาวต่อไป

คำสำคัญ: กระบวนการใช้ซ้ำเครื่องมือแพทย์ วงจรช่วยหายใจ การทดสอบโปรตีนและคราบเลือดตกค้าง การทดสอบทางชีวภาพ การทดสอบการรั่วซึม

เลขที่จริยธรรมการวิจัย COA No. 075/67 ผ่านการตรวจไม่คัดลอกผลงาน พิจารณาโดยผู้ทรงคุณวุฒิ 3 ท่าน
วันที่รับบทความ 31 สิงหาคม 2568 วันที่แก้ไขบทความเสร็จ 15 ธันวาคม 2568 วันที่ตอบรับบทความ 17 ธันวาคม 2568

*งานวิจัยเรื่องนี้ได้รับการสนับสนุนจากกองทุนส่งเสริมวิทยาศาสตร์ วิจัยและนวัตกรรม โดยหน่วยบริหารและจัดการทุนด้านการพัฒนากำลังคน และทุนด้านการพัฒนาสถาบันอุดมศึกษา การวิจัยและการสร้างนวัตกรรม (บพค.) สัญญาเลขที่ B01F640054
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Validation of reprocessing process for breathing circuits used in ventilator and high-flow nasal cannula in a hospital*

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Abstract

At Phrapokklao Hospital, a large number of ventilator and high-flow nasal cannula (HFNC) breathing circuits, both reusable and single-use types are routinely cleaned and reprocessed for reuse. Concerns remain, however, regarding the safety and effectiveness of the current reprocessing workflow. To validate the reprocessing procedures and evaluate the functional and biological safety of reprocessed ventilator and HFNC breathing circuits used in the hospital, focusing on the effectiveness of cleaning, sterilization, and the safety of the equipment after reuse.

This laboratory experimental study included 28 samples each of disposable swivel elbows and reusable silicone tubes for residual protein and blood testing, and 20 samples each for sterility and cytotoxicity testing. Leakage tests were performed on single-use ventilator and HFNC circuit sets from three brands (one set per brand) through ten simulated reuse cycles. Instruments included Pyromol and Hemo Check-S kits for protein/blood detection, sterility testing by direct inoculation (USP <71>), cytotoxicity testing using the MTT assay (ISO 10993-5), and leakage testing per ISO 5367. Data were analyzed using descriptive statistics.

All reprocessed elbows and tubes tested negative for residual protein and blood (100% pass). No microbial growth was observed in sterility tests, and all samples were non-cytotoxic (cell viability ≥70%). Leakage testing showed a slight increase in mean leakage values after ten reprocessing cycles but remained within the ISO-specified limit (≤3 mbar/min). Visual inspection confirmed no physical damage to any circuit. The findings of this study confirm that both reusable and single-use breathing circuits, after undergoing the validated reprocessing procedures, demonstrated acceptable cleanliness, sterility, and absence of cytotoxicity, while maintaining functional integrity even after multiple reuse cycles. These results indicate that the circuits can be safely reprocessed and reused without compromising patient safety, while also reducing hospital costs and medical waste. In addition, the study enhances confidence among hospital medical device units in implementing systematic, standardized reprocessing practices, supporting more efficient and sustainable medical device management. Nonetheless, further clinical studies are recommended to verify the long-term outcomes and safety of repeated reuse in real clinical settings.

keywords: reprocessing; breathing circuit; residual protein and blood testing; biological safety; leakage testing

Ethical approval: COA No. 075/67, Plagiarism checked, 3 Reviewers.
Received 31 August 2025, Revised 15 December 2025, Accepted 17 December 2025

***This research was supported by The NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation (PMU-B) grant number B01F640054**
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Introduction

In recent years, the use of respiratory support devices such as ventilators and high-flow nasal cannula (HFNC) systems has markedly increased worldwide, particularly among patients with respiratory failure, chronic lung disease, or infectious conditions including COVID-19.[1] These devices require breathing circuits, either disposable or reusable to deliver oxygen and remove exhaled gases in both intensive care units and general wards. The reuse of single-use medical devices (SUDs) has become more common in many countries, as it is concerns about cost and environmental impact in healthcare, with studies reporting substantial savings and clinical outcomes comparable to new devices.[2-3] In Thailand, a national survey showed that 97.6% of hospitals reuse SUDs, with respiratory circuit being the most frequently reprocessed that 96.3%.[4] Inadequate cleaning or sterilization may lead to residual contamination or microbial regrowth, posing cross-infection risks.[5] Moreover, repeated chemical or thermal exposure may alter the material properties of medical devices, potentially affecting their functional performance.[6]

International guidelines from the World Health Organization (WHO),[7] the United States Food and Drug Administration (FDA),[8] and relevant ISO standards state that reprocessing should include validated procedures for cleaning, sterility assurance, cytotoxicity, and functional performance through standardized testing before devices are returned to clinical use.[9] However, there is still limited published evidence on how effective these reprocessing steps are for respiratory circuit systems in real hospital settings.

At Phrapokklao Hospital, approximately 2,800 breathing circuit sets are used each month, resulting in high operational costs and increasing volumes of medical waste that challenge hospital sustainability. The hospital has implemented a controlled reprocessing program for both reusable silicone tubing and disposable plastic components of ventilator circuits, as well as disposable HFNC circuits. These devices are routinely cleaned with enzymatic detergents, disinfected, and sterilized using hydrogen peroxide plasma. However, concerns remain about the safety, sterility, and long-term functional performance of reprocessed devices. In the hospital's previous workflow, the brushing step during cleaning was performed manually using hand-held brushes, which was found to be insufficient for removing organic residues from narrow or curved sections of the circuits. To address this limitation, the Medical Metrology Laboratory at the National Institute of Metrology (Thailand) collaborated with the hospital to develop an automated brushing system equipped with interchangeable brush heads of various diameters. This improvement enhanced cleaning consistency and effectiveness across complex circuit geometries, thereby increasing the reproducibility and reliability of the cleaning process before sterilization.

However, the overall effectiveness and safety of this reprocessing process have not yet been formally evaluated. Therefore, this study was conducted to assess the reliability of the hospital's reprocessing workflow and to determine whether the reprocessed ventilator and HFNC breathing circuits remain sterile, non-toxic, and functionally safe for patient use. The findings of this study provide practical benefits for medical device reprocessing units by demonstrating that breathing circuits can be safely reused when validated procedures are followed, the results help build confidence among frontline staff and support safer decision-making in daily practice. In addition, the validated reprocessing process may contribute to reduced equipment costs and more sustainable management of medical devices within the hospital.

Research objectives

1. To validate the reprocessing process of both reusable and single-use breathing circuits used in ventilator and high-flow nasal cannula (HFNC) systems.

2. To evaluate the safety and effectiveness of the reprocessed circuits, including assessments of cleanliness, sterility, and cytotoxicity.

3. To examine the functional performance of reprocessed breathing circuits by determining leakage and physical integrity.

Research hypotheses

1. No protein or blood residues remain on sample of breathing circuit components after cleaning.

2. No microbial growth is detected after sterilization.

3. The cytotoxicity test shows a cell viability ≥70% after reprocessing.

4. Reprocessed ventilator and HFNC breathing circuits maintain leakage values within the acceptable limit (≤3 mbar/min) for up to ten reuse cycles.

Conceptual framework

This study was guided by the World Health Organization guidelines on decontamination and reprocessing of medical devices. The conceptual framework consists of three key validation stages: (1) cleaning effectiveness, (2) sterility and toxicity assurance, and (3) functional performance evaluation. Successful validation demonstrates that the reprocessed breathing circuits are safe, sterile, non-toxic, and functionally reliable for clinical reuse.

Material and methods

Research design

This study was a laboratory-based experimental quantitative study conducted to validate the reprocessing process and to evaluate the safety and functional performance of ventilator and high-flow nasal cannula (HFNC) breathing circuits. The study was conducted from August 2024 to October 2024.

All cleaning and reprocessing of ventilator and HFNC circuits were performed by trained staff in the Respiratory Maintenance Unit at Phrapokklao Hospital, following the hospital's standard operating procedures (SOPs). Each cleaning cycle was supervised and checked by technical staff to ensure that all steps and parameters were properly followed. Biological testing and result interpretation, including detection of residual protein and blood, sterility testing, and cytotoxicity, were carried out by researchers from the National Institute of Metrology (Thailand) who were trained in biomedical testing and ISO/IEC 17025 compliant analytical methods. The researchers were blinded to the sample group identity to prevent observer bias. Leakage testing and visual inspection were conducted by technical staff from the Respiratory Maintenance Unit, who had experience in functional performance evaluation of respiratory devices.

Where applicable, measurements were performed in triplicate. All instruments were calibrated and verified before use. The principal investigator independently reviewed and confirmed all results. Each operator was assigned specific responsibilities according to their expertise, and all data were recorded independently to minimize operator bias and to ensure accuracy and reproducibility of findings.

Sample size

All test samples were obtained with permission from the Respiratory Maintenance Unit, Phrapokklao Hospital, as part of an institutional collaborative project. Sample size determination was based on an a priori power analysis using G*Power software (version 3.1.9.7) for a one-way ANOVA (two-tailed) with effect size f=0.40, α=0.05, and statistical

power (1−β)=0.80 indicated a minimum of 20 samples per test group. The samples were categorized as follows:

1) Cleaning validation (residual protein and blood): Twenty-eight samples per item type of breathing circuit components were sampled randomly after cleaning, silicone tubing, and plastic swivel elbow connectors representing reusable and disposable medical devices, respectively. This number exceeded the powered minimum (n=20) to improve estimation precision, account for potential sample loss during processing, and provide broader coverage of device-to-device variability during the cleaning stage.

2) Sterility testing: Twenty samples per item type (silicone tubing and plastic swivel elbow connectors) were used for sterility testing USP <71>,[10] matching the powered minimum and aligning with the national testing practice referenced for breathing tubes (TIS 2272),[11] which specifies testing of 20 units for sterility assessment. Twenty samples were sampled randomly after sterilization. Using n=20 ensured sufficient sensitivity to detect microbial growth while maintaining feasibility within laboratory resources.

3) Cytotoxicity testing: Twenty samples were sampled randomly after sterilization. Twenty samples per item type were used for cytotoxicity testing, consistent with the sterility testing group. Although ISO 10993-5[12] does not specify a fixed sample number, we adopted n=20 to mirror the sterility test sample size, maintain consistency across biological safety endpoints, and satisfy the same a priori power target.

4) Leakage and visual inspection testing: For leakage and visual inspection, three commercial brands of disposable ventilator and HFNC circuit sets were tested, with one set per brand (new set). These tests were performed as engineering validation to confirm functional performance and were reported descriptively, as statistical inference was not applicable due to the limited number of sets per brand.

Research instruments

The following instruments for data collection and validation were used:

1) Protein residue test kit (Pyromol Test, Borer Chemie AG, Switzerland): used for qualitative detection of residual protein; color change indicates contamination.

2) Blood residue test kit (HemoCheck-S, Borer Chemie AG, Switzerland): used for hemoglobin detection; quantitative range 0.1-10 µg Hb.

3) Sterility test system: direct inoculation method following USP <71> using fluid thioglycollate medium and soybean casein digest medium.

4) Cytotoxicity assay system: MTT assay following ISO 10993-5, using L929 fibroblast cells (ATCC, US) , optical density measured at 570 nm using a microplate spectrophotometer (BioTek Epoch, USA).

5) Leakage and physical integrity testing: The air pressure test setup following ISO 5367 specifications.[13] A calibrated vital sign simulator (ProSim 8, Fluke Biomedical, USA) was used as the internal pressure source. A gas flow analyzer (VT650, Fluke Biomedical, USA) was connected to monitor differential pressure and detect leakage. Three brands of ventilators and HFNC machines were used for operational testing. Visual inspection was performed after each reprocessing cycle under a clean, well-lit environment with a white background to ensure proper illumination. All samples were checked for any physical damage, discoloration, deformation, cracks, or surface changes.

All instruments were calibrated and verified before data collection. Pressure gauge and flow meter were calibrated using standard reference instruments traceable to the National Institute of Metrology (Thailand). All test kits were used within their validity periods.

Cleaning and sterilization process

The cleaning procedure for HFNC and ventilator breathing circuits in this study followed the actual workflow implemented by the hospital's CSSD and was aligned with

international recommendations for reprocessing of reusable medical devices.[7] Cleaning of breathing circuits, including the water trap, water chamber, flow sensor, expiratory valve, flexible tube, and breathing tube, shall be carried out as follows:

1) Soak the items in an enzymatic cleaning solution diluted at a ratio of 7 mL per 1 liter of water for 5 minutes. (Figure 1)

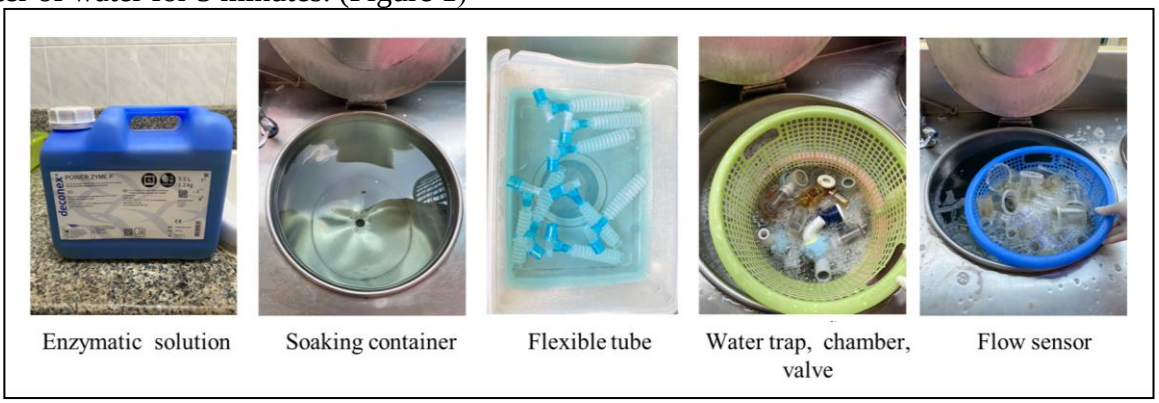


Figure 1 Soaking

2) Then gently scrub with a soft-bristled brush or sponge, followed by thorough rinsing with clean water. The flexible tube shall be brushed using a soft-bristled brush in combination with a cleaning machine, and rinse off the enzymatic solution thoroughly with clean water. (Figure 2)

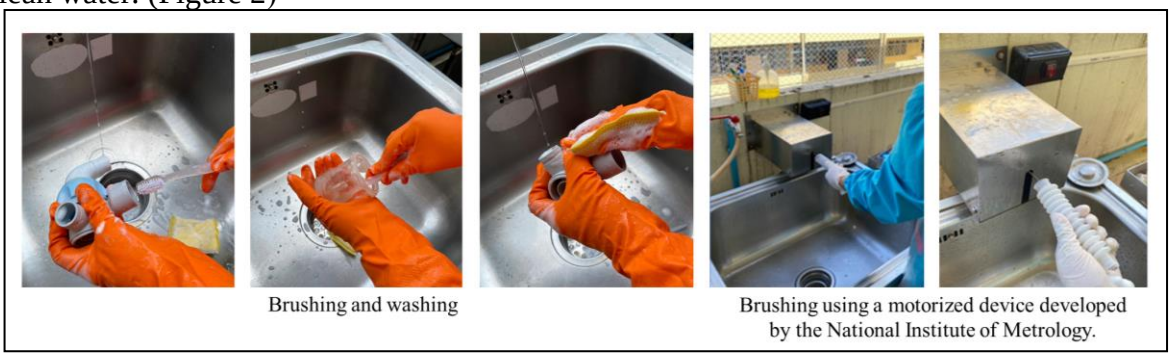


Figure 2 Brushing

3) Then immerse the equipment in a chemical high-level disinfectant solution, diluted at a ratio of 80 grams per 5 liters of water, for 5 minutes. (Figure 3)

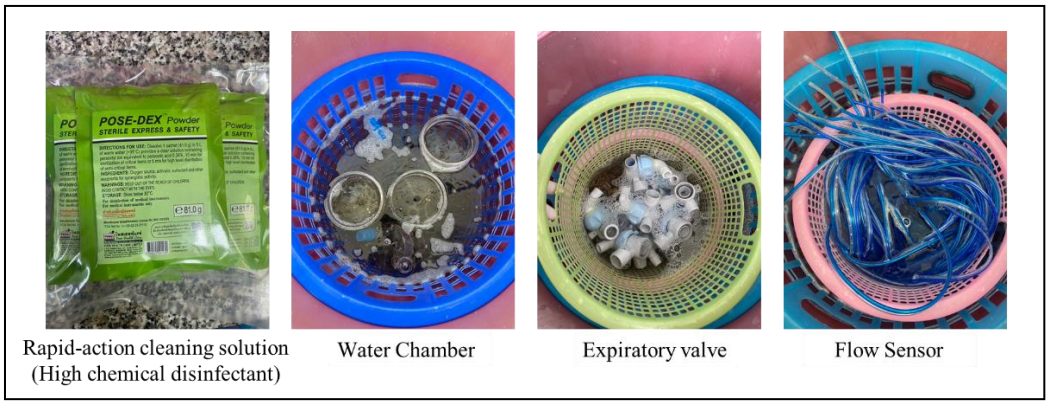


Figure 3 Immersing with disinfectant solution

4) The water chamber, water trap, expiratory valve, and flow sensor shall be rinsed with reverse osmosis (RO) water by allowing water to flow through for 5 minutes. The flexible tube shall be rinsed with clean water by allowing water to flow through for 2 minutes. (Figure 4)

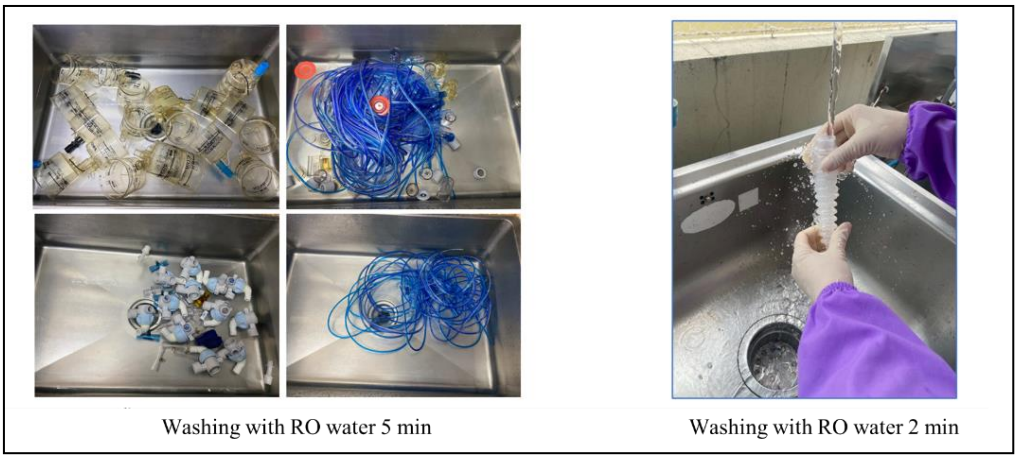


Figure 4 Washing

5) Dry the components using Med-Air. The procedure must be performed using aseptic technique. Place the accessory components in the hot air-drying cabinet (Prevention Team, PVT30K, Thailand) at a temperature of 70°C for 30 minutes. The cabinet can accommodate 100-120 components per cycle. (Figure 5)

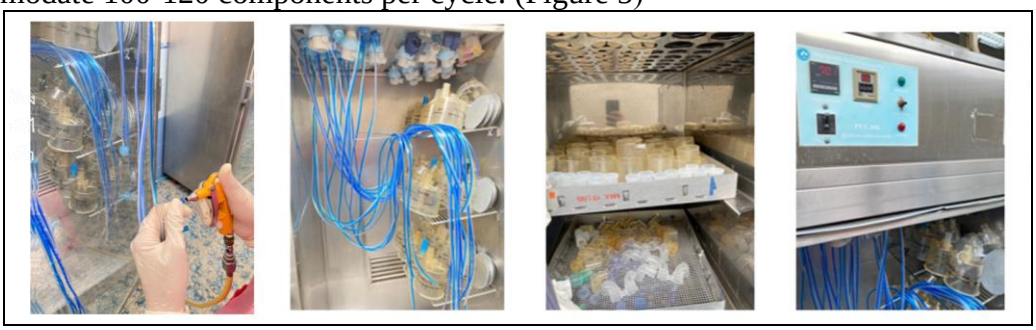


Figure 5 Drying

6) The water chamber, flow sensor, and expiratory valve shall be packaged and stored in a sterile area pending use, while the water trap and flexible tube shall be packaged for subsequent plasma sterilization. This process must be performed using aseptic technique. (Figure 6)



Figure 6 Packing

7) The water trap, flexible tube, and breathing tubing shall be arranged on a transport cart and properly covered with a sterile drape. The items shall then be sterilized using a plasma sterilizer at 55°C for 55 minutes (ASP STERRAD™ 100S, Philippines). Up to five sets of breathing tubing can be processed per cycle. (Figure 7)



Figure 7 Transfer to the Central Sterile Supply Department (CSSD) for sterilization

8) Cleaning process for breathing tubes and flexible tube components. Breathing tubes and flexible silicone tubes were cleaned using a washer-disinfector (Miele Professional, Germany). Each cleaning cycle consisted of six washing steps, with controlled temperature, washing time, and type of detergent as follows:

1. Pre-rinse: Tap water, 16 L at room temperature for 1 minute.
2. Main wash 1: Tap water, 16 L, with enzymatic detergent type 1 (Namzyme WD, Namwiwat Medical, Thailand) 100 mL at 55 °C for 5 minutes.
3. Main wash 2: Tap water, 16 L, with enzymatic detergent type 2 (Aniosyme synergy 5, ANIOS, France) 40 mL at room temperature for 2 minutes.
4. Rinse 1: Tap water, 16 L at room temperature for 1 minute.
5. Rinse 2: Tap water, 16 L at room temperature for 1 minute.
6. Final rinse: Reverse osmosis (RO) water, 16 L at 55°C for 2 minutes.

Each batch contained approximately 30 tubes. All components were washed both externally and internally using the automatic spray arms and internal rinse adapters of the washer-disinfector. After washing, the tubes were transferred to a hot air-drying cabinet (Prevention Team, model PVT100, Thailand) and dried at 70°C for 30 minutes. The cabinet has a capacity of 120-140 pieces per drying cycle. Dried tubes were then packaged together with the water trap (excluding the breathing air filter) for subsequent plasma sterilization as described in Section 6 and 7, respectively. (Figure 8)

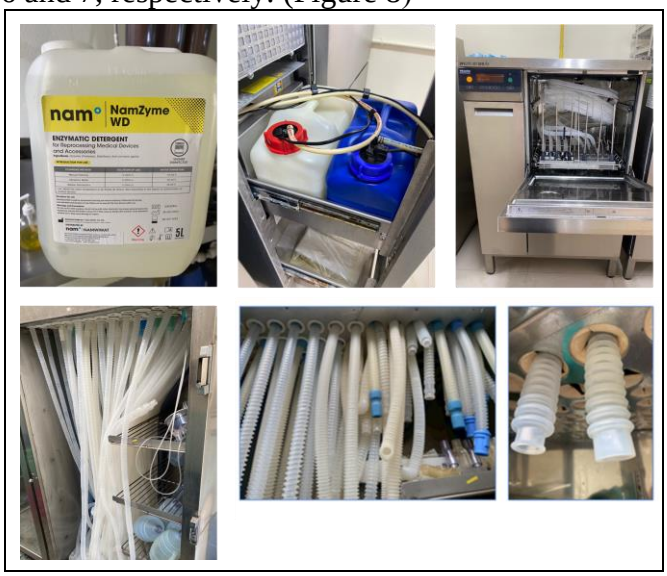


Figure 8 Cleaning of breathing tube with auto-washer and drying with cabinet

Experimental testing procedures

After sterilization, all samples were tested for the following methods:

1) Detection of residual protein and blood on reusable medical devices

Residual protein and blood contamination on breathing circuit components (swivel elbows and tubes) were evaluated using Pyromol and HemoCheck-S test kits.[8-9] All testing kits were verified using positive and negative control samples before analysis. Each sample measurement was performed in triplicate. Twenty-eight used samples that were contaminated with protein and blood were randomly sampled after cleaning. Swabs were applied thoroughly to joints, crevices, and visibly suspect areas, then incubated according to the manufacturer's instructions. A colour change to blue-green indicated the presence of protein or blood residues. The results provided routine monitoring and validation of cleaning efficacy. Data from the residual protein and blood tests were analyzed descriptively as pass/fail outcomes. The proportion of passed samples was calculated as a percentage of the total tested. Since all results were identical (100% pass rate), no inferential statistical test was required.

2) Sterility testing and cytotoxicity testing

Sterility testing was performed in accordance with United States Pharmacopeia (USP) <71> using the direct inoculation method using both Fluid Thioglycollate Medium (FTM) and Soybean-Casein Digest Medium (SCDM). Each test batch included positive and negative controls to confirm test validity.[12] For the positive control, the growth promotion capability of both media was verified by inoculating reference microorganisms specified in USP <71>: *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 9027), *Clostridium sporogenes* (ATCC 11437), *Candida albicans* (ATCC 10231), and *Aspergillus brasiliensis* (ATCC 16404). The presence of visible growth confirmed media suitability. The negative control consisted of uninoculated media incubated under identical conditions to ensure sterility. Positive and negative controls were included in every batch to confirm validity. Twenty reprocessed samples of swivel elbows and tubes were randomly examined after sterilization. Each sample was aseptically transferred into the culture media and incubated at 37°C for 14 days with periodic visual observation. Microbial growth in the test samples was assessed by comparing the turbidity of the media with that of the positive controls. A pass result (sterile) was defined as no visible turbidity or microbial growth observed in either Fluid Thioglycollate Medium (FTM) or Soybean–Casein Digest Medium (SCDM). A fail result (non-sterile) was defined as the presence of any turbidity, colony formation, or mycelial growth in either medium.

Cytotoxicity testing was performed in accordance with ISO 10993-5[14] to evaluate potential residuals on reprocessed medical devices. Cell culture incubators and pipettes were calibrated before use. A positive control (18 ppm zinc acetate, cell viability <20%) and a negative control (4 ppm zinc acetate, viability >80%) were included in each assay together with blank controls to verify test validity. Twenty reprocessed samples were individually extracted in Minimum Essential Medium (MEM) at 37°C for 24 h to obtain 100% (v/v) extracts. Each extract was then tested at four concentrations 100%, 75%, 50%, and 25% (v/v) together with a medium control. L-929 mouse fibroblast cells were tested by the MTT assay, measuring formazan absorbance at 540 nm. Each concentration from each sample was tested in triplicate wells, and the mean viability value of the three wells was considered the viability score for that sample. Cell viability was calculated as a percentage relative to the medium control. A cell viability more than 70% relative to the media control was interpreted as non-cytotoxic, while less than 70% indicated cytotoxicity. Qualitative evaluation was based on changes in cell morphology, where a grade greater than 2 was considered to indicate cytotoxicity, while quantitative evaluation was determined by a reduction in cell viability

greater than 30%, which was also regarded as a cytotoxic effect. Because the objective was to determine compliance with the ISO criterion, and because the extract from each sample represented an independent specimen rather than multiple treatment conditions, no inferential statistical testing was performed.

3) Leak testing and visual inspection of respiratory circuits

Leakage and physical integrity of breathing circuits were evaluated over 10 reprocessing cycles, following modified ISO 5367 specifications. One set each from three brands of new disposable ventilator circuits (Brand A, B, and C) and three brands of disposable HFNC circuits (Brand D, E, and F) were tested. Leakage was assessed by applying internal gas pressure (Vital sign simulator, ProSim 8, Fluke) at 50 ± 3 mbar (5 ± 0.3 kPa) for 1 min, repeated five times, and monitoring differential pressure with a gas flow analyzer (VT650, Fluke). Then, ventilator circuits and HFNC circuits were operated under controlled ventilation parameters at 50 L/min with 21% O_2 at 37 °C for 8 h. After each run, circuits were reprocessed by cleaning and sterilization, and repeated for 10 times. Visual inspections were also conducted to confirm physical integrity. Cycles 0 was a new set of both disposable circuits. For leakage data, descriptive statistics (mean ± SD) were calculated for each brand across 10 reprocessing cycles. As only one circuit set per brand was tested in this preliminary study, inferential statistical comparison among brands was not applicable. Instead, leakage trends over cycles were analyzed graphically to evaluate consistency and performance stability.

Data collection and analysis

Data were recorded in structured checklists and analyzed using Microsoft Excel 2016. Descriptive statistics (mean, SD, percentage) were used to summarize all findings.

Ethical consideration

The study has been reviewed by the Chanthaburi Research Ethics Committee/region 6 (COA No.075/67, approval date: 6 August 2024) for Human Research based on the Declaration of Helsinki and the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use-Good Clinical Practice Guidelines. The study strictly adhered to ethical principles of data confidentiality, non-identifiability, and non-patient involvement.

Definitions of technical terms

1) Breathing circuit: A set of components that connects a ventilator or high-flow nasal cannula (HFNC) system to the patient. It typically includes tubes, connectors, and related parts that allow oxygen and air to flow safely into and out of the lungs.

2) Reprocessing: A series of steps used to prepare previously used medical devices for safe reuse. This includes cleaning, removal of protein and blood residues, sterilization, and inspection of functional integrity before the device is used again.

3) Leak test: An assessment used to determine whether a breathing circuit allows excessive air leakage, which may affect the accuracy and safety of ventilation delivered to the patient.

4) Cytotoxicity test: A laboratory test used to evaluate whether any chemical residues remaining on a reprocessed device may be harmful to human cells.

5) Sterilization: A process that eliminates all forms of microbial life on a medical device to prevent transmission of infection to subsequent patients.

Results

Characteristics of test samples

Table 1 Characteristics of breathing circuit and component samples used for reprocessing validation

No.	Sample	Type	Brand code	Quantity Tested	Intended Test
1	Swivel elbow (plastic)	Disposable	-	28 samples 20 samples	Protein, blood residual Sterility, Cytotoxicity
2	Flexible tube (silicone)	Reusable	-	28 samples 20 samples	Protein, blood residual Sterility, Cytotoxicity
3	Ventilator circuit	Disposable	A	1 set	Leakage, Visual
4	Ventilator circuit	Disposable	B	1 set	Leakage, Visual
5	Ventilator circuit	Disposable	C	1 set	Leakage, Visual
6	HFNC circuit	Disposable	D	1 set	Leakage, Visual
7	HFNC circuit	Disposable	E	1 set	Leakage, Visual
8	HFNC circuit	Disposable	F	1 set	Leakage, Visual

Validation of residual protein and blood

To validate the cleaning efficacy of reprocessed breathing circuit components, a total sample was analyzed, consisting of twenty-eight swivel elbows (disposable) and twenty-eight flexible silicone tubes (reusable). Each sample was tested using Pyromol and HemoCheck-S colorimetric kits. Positive and negative controls confirmed the validity of each test run. No color change was observed in any sample, indicating the absence of residual protein and blood contamination after reprocessing. Therefore, all samples passed the test (100%). Because all data showed identical results, only descriptive statistics (frequency and percentage) were applied; no inferential analysis was required.

Validation of the sterilization process

The microbiological sterility testing of the reprocessed devices was performed to validate the effectiveness of the sterilization process and confirm the feasibility and safety of reprocessing. After sterilization, twenty swivel elbows and twenty flexible tubes were randomly sampled for testing. No visible turbidity or microbial growth was detected in either culture medium for all tested samples (100% passed), as summarized in Table 2. Because all samples yielded identical outcomes (pass), results were analyzed using descriptive statistics only (frequency and percentage).

Table 2 Test results of sterility on swivel elbow and flexible tube (n=20/each type)

Device type	n	Positive growth (n, %)	No growth (n, %)	Interpretation
Swivel Elbow (Disposable)	20	0 (0%)	20 (100%)	Passed
Flexible Tube (Reusable)	20	0 (0%)	20 (100%)	Passed

Validation of cytotoxicity

To ensure the safety of reusable medical devices. Cytotoxicity testing was used to assess the toxicity of a medical device and a material by extracting devices in a cell culture medium. Morphology grade based on ISO 10993-5 scoring system (0 = no reactivity; 1 = slight reactivity). Data are presented as mean ± SD. For each device type, twenty reprocessed samples were individually extracted and tested at each concentration. Each concentration per sample was tested in triplicate wells, and the mean viability per sample was used for analysis. All tested extracts from reprocessed swivel elbows and flexible tubes showed cell viability above 70% (Mean ± SD = 75-88%) and morphological grades ≤1,

indicating non-cytotoxicity according to ISO 10993-5. Both positive and negative controls responded as expected, confirming test validity (Table 3).

Table 3 Test results of cytotoxicity on swivel elbow and flexible tube (n=20/each type)

Device type	Extract Concentration (%v/v)	Qualitative (Grade)	Quantitative Mean Cell viability (%) ± SD	Interpretation
1. Swivel Elbow (Disposable) (n=20)	100%	1	82.28 ± 1.79	Non-cytotoxic
	75%	1	82.27 ± 2.21	Non-cytotoxic
	50%	1	81.38 ± 5.02	Non-cytotoxic
	25%	1	82.41 ± 3.75	Non-cytotoxic
	Control	0	100	Non-cytotoxic
	Negative Control	0	95.09 ± 2.46	Non-cytotoxic
	Positive Control	4	1.07 ± 1.0	cytotoxic
2. Flexible Tube (Reusable) (n=20)	100%	1	75.03 ± 1.18	Non-cytotoxic
	75%	1	79.15 ± 4.67	Non-cytotoxic
	50%	1	83.85 ± 5.71	Non-cytotoxic
	25%	1	88.41 ± 4.87	Non-cytotoxic
	Control	0	100	Non-cytotoxic
	Negative Control	0	97.24 ± 0.96	Non-cytotoxic
	Positive Control	4	0.43 ± 0.38	cytotoxic

Data are presented as mean ± SD

Clinical reprocessing sterilization validations and functionality studies

Reprocessed medical devices were evaluated for functionality with repeated procedures for cleaning and sterilization. From the results of the leakage and physical tests conducted on the disposable ventilator circuits and disposable HFNC circuits from each three brands (one set/brand available in the hospital. Test results of all circuit sets remained within the acceptable limit (≤3 mbar/min) throughout the ten reprocessing cycles (Table 4). Although minor fluctuations were observed among brands, no progressive increase beyond the acceptance threshold was detected. Because only one set per brand was tested, statistical comparison was not performed; however, the leakage patterns (Figure 9) showed stable performance with no indication of material degradation and remaining in usable condition.

Table 4 Comparison of leakage of three brands disposable ventilator and HFNC circuits when reusing 10 cycles at 50 mbar for 1 min of circuit pressure. (n = 1 per brand)

Cycle	Disposable ventilator circuits (mbar/min)			Disposable HFNC circuits (mbar/min)			Visual Inspection
	Brand A	Brand B	Brand C	Brand D	Brand E	Brand F	
0	1.1± 0.25	1.19 ± 0.25	0.47 ± 0.33	0.19 ± 0.12	0.31 ± 0.21	0.35 ± 0.11	Passed
1	0.39 ± 0.22	0.88 ± 0.22	0.54 ± 0.22	0.35 ± 0.09	0.33 ± 0.17	0.46 ± 0.08	Passed
2	0.37 ± 0.1	0.51 ± 0.1	0.33 ± 0.07	0.35 ± 0.05	0.59 ± 0.19	0.51 ± 0.18	Passed
3	0.52 ± 0.27	1.26 ± 0.27	0.53 ± 0.2	0.40 ± 0.10	0.67 ± 0.08	0.54 ± 0.20	Passed
4	0.82 ± 0.04	0.78 ± 0.04	1.08 ± 0.18	0.65 ± 0.16	0.69 ± 0.17	0.66 ± 0.03	Passed
5	0.87 ± 0.07	0.92 ± 0.07	1.25 ± 0.12	0.71 ± 0.06	0.73 ± 0.12	0.75 ± 0.03	Passed
6	0.86 ± 0.07	0.93 ± 0.07	1.14 ± 0.17	0.79 ± 0.05	0.75 ± 0.11	0.77 ± 0.07	Passed
7	0.85 ± 0.1	1.04 ± 0.10	1.04 ± 0.05	0.81 ± 0.07	0.77 ± 0.08	0.84 ± 0.09	Passed
8	0.96 ± 0.09	1.1 ± 0.09	1 ± 0.12	0.96 ± 0.06	0.84 ± 0.08	1.03 ± 0.07	Passed
9	1.06 ± 0.1	1.03 ± 0.10	1.04 ± 0.06	1.14 ± 0.09	1.10 ± 0.07	1.11 ± 0.08	Passed
10	1.02 ± 0.06	1.05 ± 0.06	1.15 ± 0.06	1.19 ± 0.09	1.25 ± 0.12	1.29 ± 0.08	Passed

Data are mean values ± SD

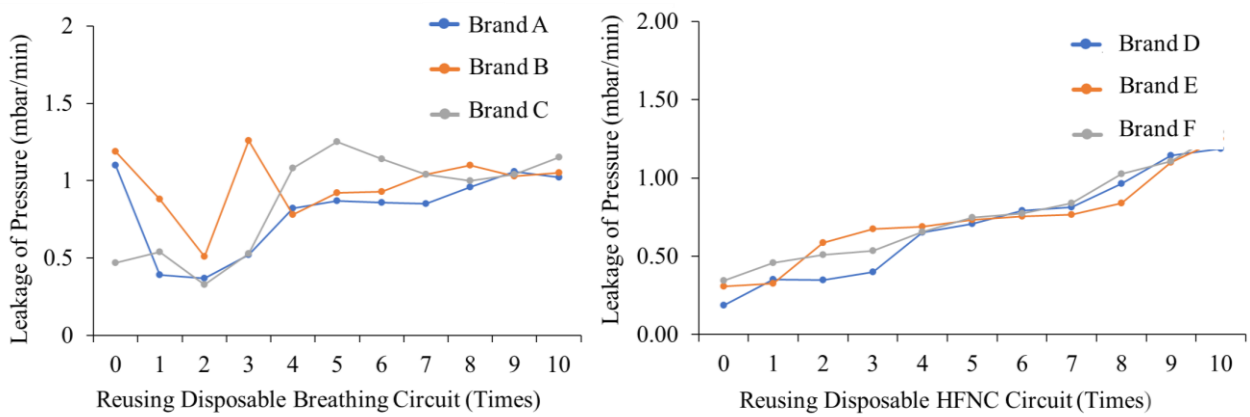


Figure 9 Leak testing of reused disposable ventilator circuits and HFNC were conducted, comparing three different brands over ten reuse cycles

Discussion

The present study aimed to validate the safety and feasibility of reprocessing breathing circuits used with ventilators and high-flow nasal cannula (HFNC) systems. The results confirmed that the cleaning and sterilization processes were highly effective, as no protein residues, blood residues, and microbial contamination were detected after reprocessing. These findings align with previous studies showing that enzymatic detergents combined with mechanical brushing can effectively reduce biofilm formation and remove organic contaminants from reusable medical devices.[14] A recent study reported that cleaning endpoints for reusable medical devices should be scientifically justified, noting that residual protein levels≥6.4 µg/cm² may pose potential toxicological or immunological risks. The authors suggested a safer acceptable range of 3-6.4 µg/cm² for post-cleaning protein residues.[15] Moreover, validated protein-detection methods have been shown to reliably assess residual protein on cleaned surfaces, supporting the use of colorimetric assays such as those employed in this study.[16] In the present study, no detectable protein was found in any samples, indicating levels well below this safety threshold and supporting the effectiveness of the hospital's cleaning process.

Cytotoxicity testing using L-929 fibroblast cells demonstrated cell viability exceeding 70% for all samples, consistent with the acceptance criteria specified in ISO 10993-5, indicating the absence of cytotoxic chemical residues. These results are comparable to previous reported,[9] who emphasized that cell viability ≥70% is the established threshold for declaring a device non-cytotoxic. The use of validated positive and negative controls in this study further ensured the reliability of the biological safety assessment. Together, these findings confirm the biological safety of the reprocessed breathing circuits.

Regarding functional performance, leakage testing across ten reprocessing cycles revealed a slight increase in leakage values; however, all remained within the ISO 5367 acceptance limit (≤3 mbar/min). Similar trends were described by Huang et al.,[17] who reported minor mechanical variation in medical devices after repeated cleaning and sterilization, without loss of functional performance when remaining within standard tolerances. Kwon et al. also emphasized the necessity of conducting routine integrity checks, including flow testing and careful physical inspection, to prevent ventilation failure caused by circuit obstruction or material degradation.[18] Notably, a review of the available literature indicates that no previous studies have evaluated the leakage performance of complete ventilator or HFNC breathing circuit systems across as many as ten reprocessing cycles. Most previous studies have focused on short-term extended use, single decontamination events, or isolated component-level assessments. Thus, the present findings contribute novel cycle-

indexed performance data, demonstrating acceptable functional integrity through ten reprocessing cycles under validated hospital procedures. The present results therefore suggest that, under validated hospital procedures and appropriate inspection, breathing circuits can be safely reused for up to ten cycles without compromising functional performance.

International guidance from the WHO[7] and the U.S. FDA[8] emphasizes that reuse decisions should rely on validated process function and performance rather than a fixed number of cycles, since material properties and sterilization methods vary among manufacturers. Our study contributes cycle-indexed performance data under an actual hospital reprocessing workflow and demonstrates consistent compliance through ten cycles, aligning with this guidance. Nonetheless, repeated low-temperature sterilization may eventually affect polymer materials and seals, underscoring the importance of local acceptance criteria, regular visual inspection, and prompt removal of devices from service when deviations are detected.

Potential sources of bias were minimized by assigning test interpretation to independent technical personnel and by validating all biological assays with standardized positive and negative controls to enhance objectivity and reproducibility. All instruments were calibrated before use, and data recording was independently reviewed to prevent operator influence. This study was a preliminary evaluation using one circuit set per brand for performance testing; therefore, while the results support functional safety for up to ten reprocessing cycles under current conditions, they should not be interpreted as a universal limit. Future studies involving larger sample sizes, multiple circuit sets per brand, and extended reprocessing cycles are recommended to confirm long-term durability and to develop standardized reprocessing protocols applicable to a broader clinical setting.

Conclusion

Overall, the findings of this study support the safe, effective, and environmentally sustainable reuse of ventilator and high-flow nasal cannula (HFNC) breathing circuits when validated cleaning and sterilization procedures are strictly applied. Implementing such reprocessing systems can substantially reduce hospital costs and medical waste while maintaining patient safety. The leakage assessment, performed as a preliminary engineering validation using one circuit set per brand, demonstrated consistent performance trends within ISO limits but did not allow for statistical comparison across brands. Therefore, these findings should be interpreted as preliminary evidence of functional safety. Future studies with larger sample sizes and multiple sets per brand are recommended to enable statistical validation and confirm the generalizability of these finding.

Suggestion

Reprocessing and reusing single-use medical devices in hospitals can be safely achieved through established policies, validated procedures, and continuous quality assurance. Each step of cleaning, disinfection, and sterilization should follow standardized protocols with documented verification and certification. Ongoing staff training, competency assessment, and process monitoring are essential to maintain consistency and prevent contamination. Hospitals should also define the maximum allowable number of reuse cycles based on performance and safety validation. Furthermore, outcomes of reuse should be systematically monitored, including device-related infection rates and adverse events, to ensure continuous improvement and uphold patient safety standards.

Acknowledgement

The authors gratefully acknowledge the Office of National Higher Education Science Research and Innovation Policy Council (NXPO) and the National Science, Research and Innovation Fund (NSRF) via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation (PMU-B), Thailand (grant number B01F640054) under MOU No. MOU-CO-2564-16159-TH between the National Science and Technology Development Agency (NSTDA) and the National Institute of Metrology Thailand (NIMT) for financial support. We also thank the Phrapokklao Hospital team for their assistance with data collection, supporting, and providing the facility and equipment.

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