Original Article

Journal of Infection Prevention

Journal of Infection Prevention
1–6
DOI: 10.1177/1757177416679879

jip.sagepub.com
SAGE

Is a chlorine dioxide wiping procedure suitable for the high-level disinfection of nasendoscopes?

Noureddine Henoun Loukili, Nadine Lemaitre, Benoit Guery, Olivier Gaillot, Dominique Chevalier and Geoffrey Mortuaire

Abstract

Background: Nasendoscopes are widely used in the outpatient ENT setting. Their reprocessing requires high-level disinfection (HLD). Recently, a wiping procedure using chlorine dioxide (ClO_2) has been proposed as an alternative to HLD traditional procedures.

Objective: To assess the effectiveness of the HLD wiping procedure versus soaking procedure on a contaminated nasendoscope.

Method: A nasendoscope was contaminated with four strains of bacteria and *Bacillus subtilis* spores. After HLD either with the wiping procedure or with the soaking procedure (PA), the reduction of the initial contamination was determined.

Findings: The wiping procedure with ClO_2 displayed more than 5 log reduction for vegetative bacteria after 30 s contact time (CT) and 4 log reduction on *B. subtilis* spores after 2 min CT. The soaking procedure with PA displayed similar results on planktonic bacteria after 10 min CT but the log reduction of *B. subtilis* remained below 4.

Conclusion: The ClO_2 wiping procedure showed bactericidal and sporicidal efficacy on a contaminated nasendoscope in a shorter time compared to the PA soaking procedure. Thus, ClO_2 wiping procedure might be considered as an alternative to the traditional HLD procedure for nasendoscopes.

Keywords

Nasendoscopes, disinfection, chlorine dioxide, high-level disinfection (HLD)

Date received: 28 December 2015; accepted: 18 October 2016

Background

Ear/nose/throat (ENT) diagnostic endoscopes are widely used instruments in the outpatient ENT setting. They are non-lumened heat-sensitive items that come into contact with mucous membranes and are categorised as semi-critical medical devices according to the Spaulding classification. Therefore, as a minimum, they require a disinfection level that should kill or inactivate all microorganisms (vegetative bacteria, mycobacteria, fungi, enveloped and non-enveloped viruses) except large numbers of bacterial spores (Rutala et al., 2004). In general, such level of disinfection is defined as high-level disinfection (HLD). By entering ENT cavities, nasendoscopes may become soiled and contaminated with blood, body fluids, organic debris and potential pathogenic microorganisms. International guidelines report varying methods for HLD. These guidelines state recommendations on use of manual or automated washer disinfector process (AER), the chemical actives that are capable of achieving HLD, the contact times (CTs) required and outline advantages and disadvantages of each methods (ASGE Ensuring

Centre Hospitalier Regional Universitaire de Lille, Lille, France

Corresponding author:
Noureddine Henoun Loukili, Centre Hospitalier Regional Universitaire de Lille, 1 av Oscar Lambret, Lille, 59037, France.
Email: Nourredine.loukili@chru-lille.fr

Safety in the Gastrointestinal Endoscopy Unit Task Force et al., 2014; ASGE Quality Assurance in Endoscopy Committee et al., 2011; Bellenhoff et al., 2008; Department of Health, 2016; Systchenko et al., 2016). In the United Kingdom, the guidelines recommend the use of AER as the 'Standard' method for endoscope decontamination whereas in the United State of America, Germany, France or some Asian countries, both manual and automated methods are accepted to complete the decontamination of endoscopes. Nevertheless, all the guidelines advice against the use of aldehydes because they are tissue fixative and have maximum exposure limits. Irrespective of the method used, all procedures include pre-cleaning, high level disinfection and rinsing steps.

In the last few years, two alternatives to soaking disinfection have emerged: covering a nasendoscope with a disposable sheath and a HLD wiping procedure that utilises chlorine dioxide (ClO_2) chemistry (Alvarado et al., 2009; Tzanisakis et al., 2012). Sterile disposable sheaths physically cover nasendoscopes and protect them from patient contact and contamination. However, the use of sheaths does not rule out the requirement for reprocessing. Indeed, after removing the sheath, the operator has to check its integrity and perform low-level disinfection of the nasendoscope. If the sheath is perforated or torn, a HLD would be required. The other alternative involves a HLD wiping procedure. It is patented under the name Tristel Trio Wipe System® (TTWS) and is carried out in three steps: a cleaning step that uses a pre-cleaning wipe containing an enzymatic detergent, a disinfection step performed with a wipe that contains ClO_2 and a rinsing step which is achieved with a wet sterile wipe. The manufacturer claims that HLD can be achieved in 2–3 min (30 s for bactericidal activity and 2 min for sporicidal activity on *Bacillus subtilis*). Compared to the soaking procedures, this wiping technique would offer a swift and suitable alternative to disinfect non-lumened endoscopes and would allow a rapid turnaround of the instrument.

ClO_2 is a disinfectant with a wide spectrum of antimicrobial activity. Earlier studies have reported ClO_2 liquid solution as effective HLD for endoscopes (Gilling et al., 2013). Published data have also reported that wipes containing ClO_2 display mycobactericidal activity according to European Standard EN14563 in 30 s CT with mechanical action and 60 s without mechanical action (Hernandez et al., 2008). However, very few studies have highlighted the efficacy of TTWS on medical devices like nasendoscopes or ultrasound probes, while many public or scientific health authorities (Cavaliere et al., 2012; Government of Western Australia, 2012; Swift, 2010) worldwide have approved its use in HLD disinfection for non-lumened heat-sensitive medical devices. In this work, we assessed the bactericidal and sporicidal efficacy of a marketed wiping procedure, TTWS, using ClO_2 in the disinfection step, and compared its results to a manual soaking procedure involving PA.

Methods

Detergent and disinfectant products

Tristel Trio Wipes System®, a disinfecting procedure that is ready-to-use, was provided by (Tristel Solutions Limited, UK). It includes three types of wipes and an activator foam. PA (Anioxyde1000®) and a detergent disinfecting product (AniosymeDD1®) were purchased from the manufacturer (Anios, Hellemmes, France). These products were used according to the manufacturers' instructions for HLD on nasendoscopes.

Bacteria strains

According to European Standards for chemicals used for medical devices disinfection, *Escherichia coli* 54117, *Enterococcus hirae* CIP58.55, *Pseudomonas aeruginosa* CIP 103467, *Staphylococcus aureus* CIP4.83 and *Bacillus subtilis* spores CIP52.62 were used as the test organisms. Stock cultures were stored at −80°C prior to testing. To prepare the working culture, the stock cultures were sub-cultured by streaking onto plates and incubated at 37°C ± 1°C. After 20–24 h, a second run of sub-cultures from the first sub-cultures was prepared and incubated in the same way. A third run of sub-cultures (the working cultures) was produced in the same way as the second run.

Test suspensions N

Test suspensions were prepared by suspending the harvested bacteria in diluent (tryptone sodium chloride solution) and homogenising them with sterile glass beads for 5 min. Ten millilitres of distilled water were added, stirred and the suspension left to settle for 10 min. This supernatant fluid was adjusted by spectrophotometer absorbance to obtain a concentration range of 1.5×10^9 to 5.0×10^9 CFU/mL. The test suspension was enumerated by performing 10-fold dilutions in diluent up to 10^{-7} to 10^{-8}, and then cultured onto Trypton Soy Agar (TSA) plates which were incubated at 37 ± 1°C for 20–24 h. One millilitre of each suspension dilution was plated onto TSA plate in duplicate. Test suspension N was used for preparing the validation suspension and the contamination of the nasendoscope.

The disinfectant activity was tested with an organic load by preparing test suspensions in 3 g/L bovine serum albumin (BSA). Freshly prepared test suspensions with organic matter were used as initial inoculum for nasendoscope contamination in all tests and used in a 2-h time period.

Initial contamination of the endoscope (Bioburden)

For each microorganism, the following test was performed in duplicate. Prior to each assay, the nasendoscope underwent a HLD procedure with PA as described in (Immersion

disinfection procedure HLD B). In order to determine the bioburden on the nasendoscope (negative control), the insertion tube (24 cm length) of this instrument was introduced into a bacterial suspension of ≃ 2 × 10^9 CFU/mL freshly prepared (respectively ≃ 2 × 10^7 CFU/mL spores of *Bacillus subtilis*). After 10 min CT, the nasendoscope was removed from the bacterial suspension and left to dry for 10 min at room temperature. Thereafter, the insertion tube was introduced into a sampling tube containing 40 mL of sterile solution (Trypton Soy + 5 g/L sodium thiosulfate) for 10 min CT. Volumes of 1 mL and 0.1 mL were then sampled in duplicate from the insertion tube and transferred into a separate membrane filtration apparatus with 50 mL of distilled water. The membranes were rinsed with 100 mL of sterile water and placed onto TSA plates for incubation at 37 ± 1°C for 24 h. The number of CFU per assay was counted and their average determined.

Wiping disinfection procedure (HLD-A)

The wiping disinfection procedure was performed with TTWS. First, the nasendoscope was wiped with the pre-cleaning wipe to perform the cleaning step. Afterwards, the disinfecting wipe was prepared by applying a double dose of the activator foam onto the wipe. This procedure takes 15 s and mixes the components of the wipe (organic acids, preservatives, buffers and corrosion inhibitor) and the activator (sodium chlorite) to generate 200 ppm of ClO_2. Immediately, the activated disinfecting wipe was mechanically applied to the surface of the nasendoscope for 30 s and timed with stopwatch. Finally, the nasendoscope was wiped with a sterile wipe in order to remove the remaining ClO_2. For *B. subtilis* spores, an additional run using a 2 min contact time between ClO2 and the nasendoscope was performed in order to assess the sporicidal action claimed by the manufacturer.

Soaking disinfection procedure (HLD-B)

The nasendoscope was manually cleaned by immersion in the detergent disinfectant (AniosymeDD1®) for 10 min then rinsed with sterile water. Afterwards, the endoscope was soaked in the PA solution (Anioxyde1000®) for 10 min and finally was rinsed with sterile water to remove the remaining PA.

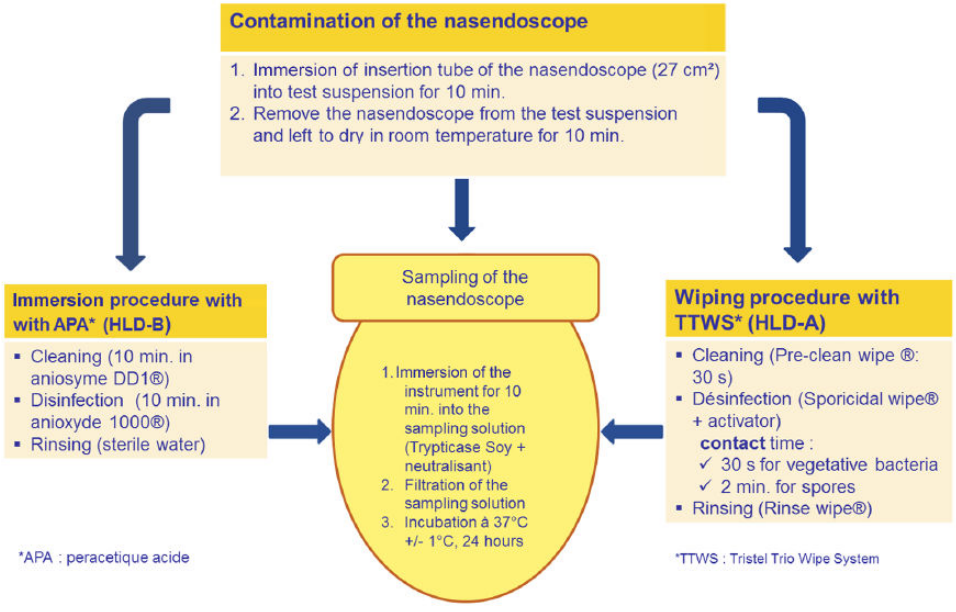
Assays

After the initial contamination, the insertion tube of the scope underwent either HLD-A or HLD-B. At the end of the rinsing step, the insertion tube was sampled and cultured as described above. The results were expressed as a $\log_{10}$ reduction of the initial contamination of the nasendoscope (Figure 1). Each assay has been performed in triplicate.

Interpretation of the results

The results were interpreted according to the European Standards for chemical disinfectants and antiseptics for the medical area where it is stated that bactericidal activity can

Figure 1. Evaluation of the effectiveness of wiping procedure involving chlorine dioxide compared to HLD soaking involving peracetic acid.



be claimed when reduction by a factor of at least 10^5 in the initial inoculum has been demonstrated. The sporicidal activity is achieved when the reduction factor recovered is at least greater than 10^4.

Results

The bacterial concentration of the test suspension for the planktonic bacteria was of 2×10^9 to 3×10^9 CFU/mL. After the initial contamination, the bacterial burden recovered from the nasendoscope ranged from 1.6×10^6 to 4.0×10^6 CFU/mL for planktonic strains and from 2.5×10^4 to 1.6×10^5 CFU/mL for *B. subtilis* spores.

Following the implementation of either the HLD-A (30 s CT) or HLD-B (10 min CT), the bacterial concentration recovered from the nasendoscope was below 15 CFU/mL ($\log_{10}$ reduction >5) for the vegetative bacteria while the burden of *B. subtilis* spores counted 15 CFU/mL on average ($\log_{10}$ reduction <4). Nevertheless, when the CT with the ClO_2 has reached 2 min, the concentration of *B. subtilis* spores was reduced to <1 CFU/mL ($\log_{10}$ reduction >5). The results recovered for each bacterial strain and for each HLD procedure are summarised in Tables 1 and 2.

Discussion

The reprocessing of semi-critical devices requires HLD procedure. The effectiveness of PA in HLD soaking procedures is well recognised. As expected, the procedure involving PA (10 min CT) has resulted in an effective bactericidal action on all planktonic strains tested ($\log_{10}$ reduction >5) whereas the sporicidal activity has failed ($\log_{10}$ reduction <4). The wiping procedure involving ClO2 has displayed the same results on both of vegetative bacteria and spores after 30 s CT. Notably, when the CT with ClO_2 was extended to 2 min, the wiping procedure has led to a significant decrease of the burden of *B. subtilis* spores ($\log_{10}$ reduction >5); thus the sporicidal activity was highlighted. There are few published studies that evaluate the in vitro bactericidal activity of a wiping procedure involving ClO_2. Hence, comparison has been difficult to ascertain. In our knowledge, this is the first reported study that used a nasendoscope as an in vitro test carrier in order to assess the sporicidal activity of a HLD wiping procedure. Nevertheless, a valuable study performed according to EN14561 standard (European Committee for standardization (CEN) 2007), where a frosted glass contaminated with *Mycobacterium avium* has undergone a disinfection with ClO_2 wipe, has already displayed a mycobactericidal activity ($\log_{10}$ reduction >4) in 30 s CT with mechanical action (Tzanisakis et al., 2012). These results are consistent with those recorded in the present study despite the difference of the test carriers and the microorganisms.

European Standards for chemicals disinfectants used in medical devices state that a disinfectant is deemed bactericidal (except for mycobacteria) if it passes the following

Table 1. Bactericidal and sporicidal activity of HLD wiping procedure (TTWS) on a contaminated nasendoscope.

Microorganisms	Bacterial burden on the endoscope before decontamination ($\log_{10}$ initial count)	Contact time of ClO_2 with nasendoscope	$\log_{10}$ reduction factor
E. coli	6.6	30 s	>5.5
E. hirae	6.4	30 s	6.4
P. aeruginosa	6.6	30 s	5.3
S. aureus	6.5	30 s	>5.4
B. subtilis spores	4.4	30 s	>3.4
	5.2	2 min	5.2

Table 2. Bactericidal and sporicidal activity of soaking procedure on a contaminated nasendoscope.

Microorganisms	Bacterial burden on the nasendoscope before decontamination ($\log_{10}$ initial count)	Contact time of PA with nasendoscope	$\log_{10}$ reduction factor
E. coli	6.4	10	>5.3
E. hirae	6.6	10	>5.5
P. aeruginosa	6.5	10	>5.6
S. aureus	6.2	10	>5.1
B. subtilis spores	4.4	10	>3.3

tests: EN13727 (European Committee for Standardization (CEN) 2013) (quantitative suspension test) and EN 14561 (quantitative carrier test). In the EN13727 Standard, the bactericidal activity is performed on liquid disinfectants such as glutaraldehyde or PA. Therefore, this standard was not suitable to assess a wiping disinfection procedure without a significant modification of the test protocol. In the EN14561 standard, the bactericidal effectiveness is assessed on a sterile frosted glass carrier which is inoculated, on a surface of 1 cm^2 with 50 µL of a bacterial suspension (10^8 CFU/mL), and left to dry before the test. Briefly, the contaminated glass carrier is immersed into a sample of the disinfectant. After the CT, the carrier is transferred into a neutralizer containing glass beads. The remained bacteria are recovered from the carrier surface by shaking. The numbers of surviving bacteria are determined and the reduction is calculated. If the $\log_{10}$ reduction is >5 for *P. aeruginosa* ATCC15442, *S. aureus* ATCC 6538 and *E. hirae* ATCC10541, the chemical product is regarded to be bactericidal for disinfectants of medical devices. Compared to the European Standards where the assays are performed on the chemical disinfectant only, our assays have been carried out on the full HLD procedures including cleaning, disinfecting and rinsing steps, as it is performed in the clinical setting. Furthermore, working on a nasendoscope as a carrier test, has allowed to work on a real material and over a large surface (27 cm^2), which is close to real-life conditions. As the HLD-B method is recognised as effective to disinfect semi-critical devices like endoscopes or ultrasound probes, it has been used as positive control in this experiment.

Before each assay and with each strain, the bioburden on the nasendoscope was determined according to the EN14561 standard. However, the instrument was not shaken with glass beads in order to avoid its damage. The concentration of vegetative bacteria recovered from the nasendoscope exceeded 10^6 CFU/mL (respective recovery of 10^4 CFU/mL for *B. subtilis* spores). These two contamination levels have allowed us to interpret the trial results according to European Standard specification for both bactericidal and sporicidal activity.

The results of the present study suggest that TTWS using ClO_2 as disinfectant achieves HLD in a shorter time than other soaking procedures. A prospective single-blind study has already reported the effectiveness of TTWS on nasendoscopes where the samples were free of bacteria, fungi and mycobacteria after the wiping procedure implemented (Tzanisakis et al., 2012). Nevertheless, further clinical studies are needed in order to confirm the effectiveness of such a wiping procedure in the decontamination of nasendoscopes in the clinical setting.

Acknowledgements

We thank the representative of Tristel Solutions Limited, UK. who has funded the cost of microbiological analysis.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Tristel Solutions Limited, Cambs., UK. has funded the microbiological analysis fees of this study.

Peer review statement

Not commissioned; blind peer-reviewed.

References

Alvarado CJ, Anderson AG and Maki DG. (2009) Microbiologic assessment of disposable sterile endoscopic sheaths to replace high-level disinfection in reprocessing: a prospective clinical trial with nasopharygoscopes. *American Journal of Infection Control* 37: 408–413.

ASGE Ensuring Safety in the Gastrointestinal Endoscopy Unit Task Force, Calderwood AH, Chapman FJ, Cohen J, Cohen LB, James Collins J, Day LW and Early DS (2014) Guidelines for safety in the gastrointestinal endoscopy unit. *Gastrointestinal Endoscopy* 79: 363–372.

ASGE Quality Assurance in Endoscopy Committee, Petersen BT, Chennat J, Cohen J, Cotton PB, Greenwald DA, Kowalski TE, Krinsky ML, Park WG, Pike IM and Romagnulo J; Society for Healthcare Epidemiology of America, Rutala WA. (2011) Multisociety guideline on reprocessing flexible gastrointestinal endoscopes. *Gastrointestinal Endoscopy* 73: 1075–1084.

Bellenhoff U, Neumann CS, Rey FJ, Biering H, Blum R, Cimbro M, Kampf B, Rogers M and Schmidt V; ESGE Guidelines Committee; European Society of Gastrointestinal Endoscopy; European Society of Gastroenterology and Endoscopy Nurses and Associates. (2008) ESGE-ESGENA Guideline: Cleaning and disinfection in gastrointestinal endoscopy. Endoscopy 40: 939–957.

Cavaliere M and Iemma M. (2012) Guidelines for reprocessing non-lumened heat-sensitive ear/nose/throat endoscopes. *Laryngoscope* 122:1708–18.

Department of Health. (2016) *Technical Memorandum 01–06: Decontamination of flexible endoscopes. Part A: Policy and management*. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/530418/HTM0106_PartA.pdf.

EN14561. (2007) Chemical disinfectants and antiseptics - Quantitative carrier test for the evaluation of bactericidal activity for instruments used in medical area - Test method and requirements (phase 2, step 2) - Désinfectants chimiques et antiseptiques. *European Committee for Standardization (CEN)*.

EN13727 A+. (2013) Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity in the medical area - Test method and requirements (phase 2, step 1) - Antiseptiques et désinfectants chimiques *European Committee for Standardization (CEN)*.

Gilling PJ, Reuther RM, Addidle M, Lockhart MM, Frampton CM and Fraundorder MR. (2013) A randomised single-blind comparison of the effectiveness of Tristel Fuse (chlorine dioxide) as an office-based fluid soak, with Cidex OPA (ortho-phthaldehyde) using an automated endoscopic reprocessor (AER) as high-level disinfection for flexible cystoscopes. *BJU International* 112 (Suppl. 2): 69–73.

Government of Western Australia. Department of Health. *Prevention of cross infection in diagnostic ultrasound*. Available at: http://www.health.wa.gov.au/circularsnew/pdfs/12913.pdf.

Hernandez A, Carrasco M and Ausina V. (2008) Mycobactericidal activity of chlorine dioxide wipes in a modified prEN 14563 test. *Journal of Hospital Infection* 69: 384–388.

Rutala WA and Weber DJ. (2004) Reprocessing endoscopes: United States perspective. *Journal of Hospital Infection* 56 (Suppl. 2): 27–39.

Swift A. (2015) Guidance on the decontamination and sterilization of rigid and flexible endoscopes. Available at: https://entuk.org/sites/default/files/…/endoscopes.pdf.

Systchenko R, Sauterau D and Canard JM. (2013) Recommendations from the French Society of Digestive Endoscopy (SFED) for a technical support center dedicated to digestive endoscopy. Available at: http://www.sfed.org/files/documents_sfed/files/recommandations/Plateautechnique_orgafnnt.pdf.

Tzanisakis K, Choudhury N, Bhat S, Weerasinghe A and Marais J. (2012) Evaluation of disinfection of flexible nasendoscopes using Tristel wipes: a prospective single blind study. *Annals of the Royal College of Surgeons of England* 94: 185–188.