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EVALUATION OF HELIX TEST VERSUS BOWIE DICK TEST AS A CHEMICAL INDICATOR FOR ASSESSMENT OF STERILIZATION OF DENTAL HANDPIECE

Fatma A. Mohamed[1*] BDS, Amel G. ElSheredy[2] PhD,
Eglal A. El sherbini[3] PhD

ABSTRACT

INTRODUCTION: Dental handpiece is an important device popularly used in dental procedures for drilling, filling, cleaning, and extraction of teeth. Dental HP is considered "Narrow lumen double ended open space device." not a simple hollow device. After every patient, the HPs should be cleaned, and sterilized. Assessment of sterilization and steam penetration of HP should be evaluated. The nature of the steam penetration test may be using Bowie Dick test or test helix.

OBJECTIVES: Evaluation of helix test versus bowie dick test for assessment of sterilization of dental handpiece.

MATERIALS AND METHODS: Fifty handpieces were included in the study, of them water irrigation samples were collected from 25 dental handpieces after its use and before sterilization. Water irrigation samples were collected from all the 50 handpieces after sterilization. Water samples were cultured for bacteriological examination on Blood Agar, Mannitol Salt Agar, MacConkey Agar, and Sabouraud Dextrose Agar.

RESULTS: Bowie dick test was successful to assess the sterilization and steam penetration of dental handpiece as confirmed by the absence of bacterial growth from all the 50 water irrigation samples taken from HPs after sterilization, while helix test showed discrepancy in its result as only 48% of the PCD tested showed sufficient air removal and 52% showed partially sufficient air removal and steam penetration in-spite of the negative results of bacterial culture after sterilization.

CONCLUSIONS: BD test is successful to assess the sterilization and steam penetration of dental handpiece. Using Helix test for assessment of sterilization of dental handpiece is not conclusive.

KEYWORDS: Dental handpiece, Sterilization, Helix test, Bowie dick indicator, Contamination with oral fluid.

RUNNING TITLE: Evaluation of PCD versus bowiedick for handpiece sterilization.

1-BDS 2014, Faculty of Dentistry, Faculty of Dentistry, Alexandria University, Alexandria, Egypt
2-Assistant Professor in Microbiology, Microbiology Department, Medical Research Institute, Alexandria University, Alexandria, Egypt
3-Professor of Microbiology, Microbiology Department, Medical Research Institute, Alexandria University, Alexandria, Egypt

Corresponding author:
tahafatma339@yahoo.com

INTRODUCTION

Dental handpieces (HP) are medical instruments popularly used in dental procedures. Once they are used in a patient's mouth, they are definitely soiled and contaminated exterior surface by biological fluids such as blood, saliva, pus, or dentinal smear layer (1).

Due to the small size and length of lumens [0.9-2.3 mm diameter], sophisticated working parts (which require lubrication), and inability to be easily disassembled, dental handpieces are extremely difficult to clean, examine, and sterilize. A compressed air supply tube and water-cooling tubes extend through the neck, body, and coupler, providing water and compressed air to the head. Furthermore, many studies report backflow when the HP is turned off (2,3), as a result, the inner surfaces of these devices (narrow air/water manifolds, drive shafts, and gears) are soiled and contaminated (4), potentially allowing cross-infections and a hazardous microbial quality of the water that runs through the dental unit (5,6).

Dental HP is considered a double ended open space device as according to its design, the first open end (couplar part) which attached to DUWL and the second open end carrying the bur (head) which in contact with the oral cavity (7). As shown in (Figure 1)

According to ISO11140-6, dental HP is considered "Narrow lumen double ended open space device." not a simple hollow device (8) because the ratio of the length to diameter of the dental HP is beyond the range mentioned for simple hollow double ended open space item (L/D=115/0.9)

The Spaulding classification assort dental HPs as semi-critical; investigations have shown that during use, their internal parts can get contaminated with patient materials and next patient may be potentially exposed to infectious

materials if these devices are not adequately cleaned, and heat sterilized (9). So, dental HP should be sterilized between patients (10). Sterilization process monitoring should involve a variety of process parameters, such as mechanical, chemical, and biological (11).

Chemical indicators: The chemical monitoring system is made up of six types of indicators that are based on unique needs such as equipment monitoring, pack monitoring and exposure monitoring. the nature of the steam penetration test is based on a tubular device, such as a test helix, or a test based on a textile pack, such as the Bowie and Dick test (11).

In the early 1960s Bowie and dick developed the first steam penetration test (12). The Bowie-Dick test sheet is class 2 indicator, it operates at 134°c for 3.5 minutes / 132°c for 4 minutes to detect air leaks, inadequate steam and vacuum pump failures. The test sheet is designed to meet the ISO 11140-1/3, test method for evaluating the sterilizer's effectiveness of steam penetration and air removal (13).

The current European standards suggested that a «Process challenge device for hollow instrument loads » can be used as steam penetration test for small sterilizers with Type B and specific Type S. often these PCD are helix shaped and are in practice referred to as helix (14). As shown in (Figure 2)

Depending on the application in sterilization process monitoring, the PCD may contain (11) a) a biological indicator, b) a biological indicator and a Type 5 integrating chemical indicator, c) a Type 5 integrating chemical indicator, or d) a Type 6 emulating indicator.

Numerous studies of the penetration of steam into the lumens of medical devices in a laboratory setting have provided technical evidence for the necessity of air removal from lumens (15). Due to the complex construction and internal lumens that may lead to trapped air comprising steam penetration, international and regional standards require hollow instruments, such as dental handpieces, to be sterilized using a vacuum steam sterilization type B process with fractionated pre-vacuum and post-vacuum phases. This is also advised by sterilizer and dental handpiece makers (16).

The aim of the present study was to evaluate of helix test versus bowie dick test for assessment of sterilization of dental handpiece.

The null hypothesis was that there will be no difference in air removal and steam penetration assessment of tubular devices as dental handpiece using bowie dick test and helix test chemical indicator.

MATERIALS AND METHOD

In This cross-sectional study, Sterilization, and efficiency of air removal assessment in 50 dental HP were carried out. Five steam sterilizers with the same sterilization parameters were used to sterilize 50 dental handpieces. In Each sterilization cycle, evaluation of air removal and steam penetration was done using both Bowie Dick test and Helix test chemical indicator.

Sample size estimation

The sample size was estimated based on results obtained from previous studies of similar nature (2,17,18). The total sample size is 50 dental handpieces.

I-A-The 50 dental handpieces were divided into 2 groups:

1st group: included 25 dental handpieces subjected to internal irrigation before sterilization to detect internal contamination of narrow tubing system inside the handpieces and water samples were collected for bacteriological examination.

2nd group: 25 dental handpieces not subjected to internal irrigation.

B-All the 50 dental handpieces went to sterilization cycles with helix test and Bowie dick test to evaluate the efficiency of air removal and steam penetration of narrow hollow tubes of handpieces.

Water samples were collected from the 50 handpieces (25 water samples before sterilization and 50 water samples after sterilization), then cultured on Blood Agar, MacConkey Agar, Sabouraud Dextrose Agar and Mannitol Salt Agar after incubation for 24:72 hours at 37 C and results were recorded.

II-Handpiece sampling

Ten milliliter of sterile water was injected by sterile disposable syringe in the water-cooling narrow tube of handpiece from coupler part, sterile water was allowed to flow inside narrow tube from coupler to the head, then received in a sterile falcon tube, sample was collected and immediately processed in centrifugation device. Twenty-five micro liter was cultured from precipitate on each blood agar, MacConkey agar, Mannitol salt agar and Sabourad dextrose agar plates and incubated for 24:72 hours at 37°c.

III-Identification of microorganisms

Isolates were identified microscopically and by using standard biochemical identification tests {(Gram stain, catalase test, oxidase test, triple sugar iron agar, urease test, phenylpyruvic acid test, Indole test, methyl red test, voges Proskauer test, citrate test and motility test)}. Some gram-negative bacteria were subjected to further identification using automated system (VITEK® 2).

IV-Steps of Preparation of Dental handpieces for sampling and sterilization:

Handpiece was flushed about 20:30 seconds before removing from DUWL to expel any excess of patients' infectious materials after dental treatment.

The external surface of handpiece was manually cleaned using a damp disposable cloth with mild detergent to make the handpiece surface clean of all dirt, dust, and bio-matter before sterilizing. If some dirt left on the handpiece, cleaning under running water using a soft to medium, non-metallic brush was done.

The handpieces were transported to the decontamination room using a lockable **transport box according to** Standard instrument transportation protocols**.**

After cleaning by hand, the external surfaces have been disinfected with Unisepta (which is Rapidly acting intermediate level disinfectant for pre-cleaned surfaces of medical devices, each 100g Unisepta® Plus contains: Ethanol (55g), Oxyethylammonium didecylmethyl propionate (0,11g), fragrance. (Quaternary ammonium compounds) plus solution used undiluted on pre-cleaned surfaces of medical devices. Spray on area to be treated until completely dampened. Contact time: 30 seconds minimum.

Drying the external surface of handpieces (waiting until evaporation of ethanol).

Water samples were collected from the handpieces twice from the 1st group (irrigation and after sterilization), and once from the 2nd group (only after sterilization)

Before sterilization application of lubricant into the drive air hole of the couplar part of handpieces and until only lubricant comes out of the head to eliminates excess oil.

Each handpiece was wrapped in sterilization pouch and inserted in the autoclave for sterilization.

ALL The 50 handpieces were sterilized using large steam sterilizer class B which parameter was 134°c for 3.5 minutes, 2.1 BAR with total cycle time 47 minutes and samples were collected from handpieces after sterilization.

In Each sterilization cycle, the chamber of autoclave had contained Bowie dick test pack, Helix test with class 6 chemical indicator and 2 dental handpieces each one was wrapped in separate sterilization pouch to simulate ordinary sterilization condition.

In the sterilizer, the BD test sheet is placed within a towel pack at the most difficult to sterilize region. Every day the sterilizer is utilized, a Bowie-Dick test is performed, either before the first processed load or at the same time each day. The HELIX TEST complies with standards: EN 867-5 /ISO 11140-1. PCD (Helix test) is made from material specified in the standard described in EN867-5. PCD is constructed from polytetrafluoroethylene (PTFE) which has high thermal mass and low thermal conductivity with a melting point of around 327°C. the helix tube length 1500 mm and internal diameter of 2 mm (15).

The parameters necessary for sterilization to obtain an ink color change indicator are: 134 C saturated steam for 3.5 minutes (19).

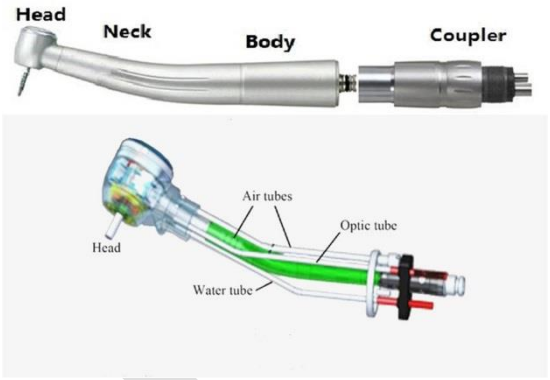

Figure 1: Showing Designing high-speed dental air-turbine handpiece.

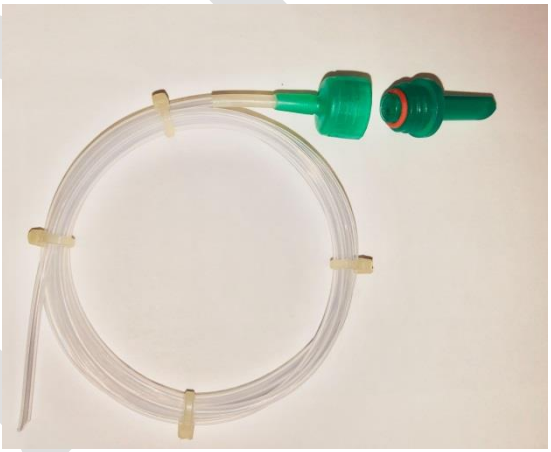

Figure 2: Showing design of PCD (Helix test).

RESULTS

Microbiologic culture Results of water sample from internal irrigation of dental handpiece before sterilization (n=25 dental handpieces) (Table 1)

Streptococci species were isolated from 6 internal water irrigation samples either as single isolate or in mixed growth, Staphylococcus aureus were isolated from 6 internal irrigation samples either as single isolate or in mixed growth, Klebsiella species were isolated from 2 internal irrigation samples, Acinetobacter lwoffii was isolated from 2 samples, Pseudomonas species were isolated from 8 internal irrigation samples, Cupriavidus pauculus was isolated from 2 samples, and Bacillus species were isolated from 2 samples as shown in table 1.

All organisms were identified as a single isolate except for sample (3) was mixed growth of streptococci and Bacillus species, and Sample (1 &6) were mixed growth of staphylococcus and streptococcus species.

Microbiologic culture Results of water sampling from dental handpieces after sterilization (n=50 dental handpieces)

The results of cultured water Samples after steam sterilization of the 50 dental HP showed no bacterial growth on Blood Agar, MacConkey Agar, Sabouraud Dextrose Agar and Mannitol salt Agar after incubation from 24:72 hours at 37 C.

Results of assessment of Air removal and steam penetration (Table 2)

In the present study, the results of assessment of air removal in the sterilization cycles using the 2 types of chemical indicator; bowie dick test and Helix test were compared. Using Bowie dick test, All the 25 sterilization cycles (2 HPs/ cycle) showed successful results regarding air removal and steam penetration as shown in (Figure 3) While Using Helix test, only 12 out of 25 sterilization cycles (24 HPs out of 50 HPs) showed sufficient steam penetration and air removal as shown in (Figure 4-a) while other 13 sterilization cycles (26 HPs out of 50 HPs) showed partially sufficient

Steam penetration and air removal as shown in (Figure 4-b).

Comparing bacterial culture results of water samples collected after sterilization with the results of the 2 chemical indicators used (Table 3) Regarding the results of bowie dick test, no bacterial growth was observed among the 25-sterilization cycle that shows successful air removal using bowie dick test. On the other hand, the results of helix test show no bacterial growth among the 25 sterilization cycles using helix test, although 12 sterilization cycles were successful and showed sufficient steam penetration results and the other 13 sterilization cycles showed partial steam penetration results, as shown in table 3.

Figure 3: Show color change of Bowie dick test in successful sterilization cycle Color change to black. (A) Before sterilization (B) After sterilization.

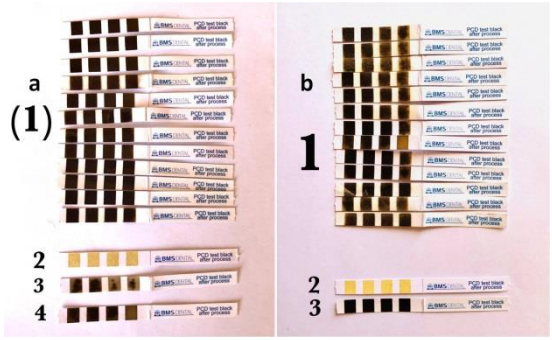

Figure 4: a-shows (1a) sufficient steam penetration of chemical indicator of Helix test, (2a) unprocessed chemical indicator, (3a) in sufficient steam penetration, (4a) partially sufficient steam penetration. b- shows (1b)- color of partially sufficient steam penetration of chemical indicator of Helix test, (2b) unprocessed chemical indicator, (3b) sufficient steam penetration.

Table 1: The microorganisms isolated from water irrigation sample of 25 dental handpiece before sterilization.

Type of Microorganism	Number of Positive Samples
Streptococcus species	6 samples (2 HG, 1 IG, 3 MG)
Staphylococcus aureus	6 samples (4 HG, 2 IG)
Klebsiella species	2 samples MG
Acinetobacter lwoffii	2 samples MG
Pseudomonas species	8 samples (1 IG & 7 MG)
Bacillus species	2 samples HG
Cupriavidus pauculus	2 samples IG

Heavy Growth (HG)≥100 CFU, Intermediate Growth (IG) (10<IG<100), Mild Growth (MG) <10 CFU/plate

Table 2: Comparison between the results of air removal and steam penetration using bowie dick test and helix test.

Number of sterilization cycle	Bowie Dick test		Helix test		
	Pass	Failed	Sufficient	Partially sufficient	Insufficient
25	25	0	12	13	0

Table 3: Comparing bacterial culture results of water samples collected after sterilization with the results of chemical indicators used.

Indicator	Bowie Dick		Helix test		
	Successful (n=25)	Faile (n=0)	Sufficient steam penetration (n=12)	Partially Sufficient steam penetration (n=13)	Failed (n=0)
Culture results	NG	---	NG	NG	---

*NG= no growth

DISCUSSION

Reliable steam sterilization necessitates direct contact of moisture, often provided as saturated steam, onto the surfaces of the load, which must then be kept at a specific temperature for a defined period of time. The presence of residual air impedes saturated steam penetration, particularly into devices with lumen, and prevents the establishment of the moist conditions essential for microbial inactivation. Modern medical devices with intricate components with closed or open lumens (e.g., dental HP) enhance the likelihood of sterilization failure by trapping air e (the prevalent species in the human mouth cavity) parts (14).

The results of the current study showed contamination of dental HPs (n=25 dental handpieces) after its use with different types of bacteria. Level of bacterial contamination in 44% of the tested water irrigation samples exceed the maximum level guided by ADA and CDC (<500 CFU/ml) (20).

The source of bacteria isolated from the irrigation water samples taken from HPs was either from the oral cavity as (Streptococci species and Staphylococcus aureus which species predominate in the human oral cavity) due to back flow effect of HP in spite of the presence of anti-retraction valve or from biofilm related microorganisms of DUWL as (Klebsiella species, Acinetobacter lwoffii, Pseudomonas stutzeri and Cupriavidus pauculus).

Some other studies have been reported similar result of high level of contamination of dental HPs and also high bacterial count in water samples taken from several parts of dental units after patients use. The isolates were gram positive as streptococcus spp., staphylococcus aureus and gram negative as pseudomonas stutzeri. (Herd et al., 2007; Smith & Smith, 2014; Błaszczyk et al., 2022) (21-23).

Oral germs can enter the blood stream not just after invasive treatments like dental extractions and oral surgery, but also following routine daily activities like chewing, brushing, and flossing. However, these organisms only sometimes able to evade the immune system and harm immunocompromised patients (24).

The isolation of the different types of bacteria from internal irrigation water samples of dental HPs and its presence in a high load (>500 CFU/ml) as shown in the result of the current study and previously mentioned studies, confirm the internal contamination of dental HP and highlight the importance of the sterilization process and its assessment to prevent cross infection between patients.

In 2000, The Dutch Health Care Inspectorate highlighted her concern about the hospitals' lack of attention to the need of the daily steam penetration test. The Bowie & Dick test is no longer realistic for the sterilization loads since hospital autoclaves are sterilizing an increasing number of hollow lumen devices and very few textile packs. For the sterilisation of hollow devices, the sterilization procedures should be developed and optimized (25).

In 2001, Gömann et al reported that the increased usage of hollow devices such minimally invasive surgical tools, catheters, etc. in sterilization raised the question as to whether these complex instruments can be safely sterilized. Very little information is available on the penetration of steam in hollow devices. The Standard EN 867-4 describes how alternative tests must be tested in order to be deemed comparable with the standard Bowie-Dick test pack (26).

In 2005, Kaiser reported that standard bowie dick test is not able to detect NCG quantities below 50 ml. These NCG quantities are too high by a factor of 100 as far as lumened devices are concerned. European standard EN 867-5 describes a hollow device system "Hollow" that is capable of detecting NCG quantities below 1 ml (27).

According to AAMI and ISO, the steam sterilization equipment was qualified as part of a routine performance test or after substantial repair. Both using same commercially available monitoring devices such as BD type tests, PCD, and Cis, also specifies the time, pressure, and thermometric criteria for the exposure phase as well as other criteria to see if the equipment is working within the permitted parameter range (28,29).

In the current study, large steam autoclaves were used to sterilize dental HPs and to assess the effectiveness of steam penetration and air removal by using BD test and PCD (Helix test) as there is no difference between the operating cycles of large steam sterilizer and small (bench top) autoclaves (14). As a result, it is rational to utilize the same test instrument as a reference item for designing and monitoring the sterilization

process in large steam autoclaves as reported by *Bruijn et al,2005* and *Kirk et al,2016* (14,25).

Before starting the current work, the performance of the 5 tested autoclave was evaluated to assess the effectiveness of steam penetration and air removal, and it was found that one of it manifested failure of air removal in many sterilization cycles using both helix test and BD test, this autoclave was excluded from the study.

In the present study, all Bowie Dick tests (100%) showed successful air removal and steam penetration but only 48% of the PCD tested showed sufficient air removal and 52% showed partially sufficient air removal and steam penetration. When these findings compared to the microbiological results, we can observe the success of all sterilization cycles (25 cycles) as confirmed by the absence of bacterial growth from all the 50 water irrigation samples taken from dental HPs after sterilization and the success of all sterilization cycles (100%) using BD test compared to only 48% (12 out of 25 sterilization cycles) using helix test. These results denote failure of the helix test to confirm the validity (success) of the sterilization process.

Bruijn and Van Drongelen, 2005, carried out a research project at 20 hospitals to ascertain whether the sterilizers can pass the EN867-5-compliant standard helix test. The Bowie & Dick test was passed by all sterilizers, however 41% of the 476 tests using the helix were unsuccessful. The study demonstrates that the test conditions and the kind of air removal affect a sterilizer's capacity to pass the helix test. A sterilizing cycle's air removal stage that is appropriate for removing air from a textile pack may not always be appropriate for removing air from hollow devices (25).

In contrast to the results of the current study, helix test failed to detect failure of sterilization and efficiency of air removal were reported by *Gömann et al in 2001,* performed tests in two trans-atmospheric air removal cycles under otherwise similar conditions; tests revealed adequate steam penetration of the porous pack, but air was not sufficiently removed from 2 hollow devices (PCD) under these circumstances; in addition, after sterilisation, growth of biological indicator was found in all hollow PCDs (26).

Kirk et al in 2016, tested 14 PCDs, and as a reference device, a textile pack was employed. When subjected to a Pass cycle (134 °C for 5 minutes), all devices produced acceptable results. The authors reached the conclusion that just 27% of the flaws were obviously observable after being subjected to a range of fail situations, and that 40% failed to identify even a single problem. The authors also came to the conclusion that the EN 867-5 basic helix device could only identify the most severe of flaws (14).

In the current study, when comparing the parameter of PCD and the parameter of the dental HP (length= 115 mm and diameter 0.9 mm), we can observe the smaller diameter and shorter length of the dental HP compared to PCD which makes HP easier in sterilization also in air removal and steam penetration. This observation could explain the discrepancy of the results of the air removal and steam penetration using the 2 indicators (BD and PCD) which could be attributed to several factors related to PCD, as reported by Van *Doornmalen et al, 2013 and Kirk et al in 2016 (14,15).* The long length of PTFE tubing of PCD (250 to 1500 mm) is being most difficult to sterilize in addition to a wall thickness between 0.25 and 2 mm and an interior diameter between 1 and 2 mm, it seemed to have a complicated effect (14).

Van Doornmalen et al, in 2013 reported that the tube length of PCD is an important parameter during sterilization as with longer channels, steam penetration diminishes (15).

Although the lumens in helix shape devices may look like lumens as encountered in medical devices, yet its dimensional and physical properties differ. These differences will influence the penetration characteristics as confirmed in the previous studies. Further examples of known influential factors next to radii and length are wall thickness of the lumen, dimensions of the receptacle, coiling of the tubing, position of device in the sterilizer, and used materials (30).

So that, an International Standard that explains how a hollow load PCD may be created and evaluated against genuine medical equipment in a variety of sterilization procedures that represent the state of the art under full load circumstances is urgently needed.

CONCLUSION

Contamination of dental HP after its use in the oral cavity. The source of contaminant was either from oral cavity or DUWL biofilm. Assessment of air removal from autoclaves before starting the sterilization cycle is very important to exclude nonfunctional autoclave. BD test is sufficient for assessment of sterilization of dental HPs (narrow lumen device with short tube length). The use of PCD (Helix test) to assess sterilization and air removal from dental HP is not conclusive.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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