

**Steridoc Ltd.  
Martin-Luther-Universität Halle-Wittenberg  
Director Prof. Dr. M. Borneff-Lipp  
Johann-Andreas-Segner-Str. 12  
D-06097 Halle  
Germany**

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**Microbiological Testing of the Automated  
Reprocessing of Flexible Endoscopes in the  
Washer-Disinfector ETD 3 PAA-Version,  
Olympus Europe Ltd.,  
Hamburg, Germany**

# 1 Aim

With respect to the order dated 19<sup>th</sup> of March 2003 we tested the automated reprocessing of flexible endoscopes in the washer-disinfector ETD 3 PAA-version, Olympus Europe Ltd., Hamburg, using conditions close to the practical situation. The tests were carried out according to the recommendations of the Working Group Endoscopy, Germany (1995)<sup>1</sup>.

## 2 Methodology

### 2.1 Test objects

Two identical endoscopes, model CF-140i (Olympus Europe Ltd., Hamburg), length of biopsy channel 1330 mm, internal diameter 3.7 mm), were used as test objects.

### 2.2 Test organisms

Two different test organisms were used:

- *Enterococcus (E.) faecium* ATCC 6057 with an average initial count of  $2.2 \times 10^{10}$  colony forming units/ml. This test organism was used to determine the disinfection's efficacy.
- Spores of *Bacillus (B.) subtilis* ATCC 6051 with an average initial count of  $1.9 \times 10^9$  colony forming units/ml. This test organism was used to determine the cleaning's efficacy.

#### 2.2.1 Determination of the initial colony count of the original suspension (*E. faecium* and *B. subtilis*)

During the test period the initial colony count of the original suspension was checked once a week.

Beneath a sterile work bench 9990  $\mu$ l of 0.9 % sterile NaCl-solution was pipetted in each of 3 test tubes. Then 10  $\mu$ l of original suspension were added to each test tube. Following this, a decimal dilution sequence was prepared. For the suspension containing *E. faecium*, the dilution from 1:10<sup>8</sup> to 1:10<sup>9</sup>, and the suspension containing spores of *B. subtilis* the dilution from 1:10<sup>7</sup> to 1:10<sup>9</sup> were mixed with 20 ml of tryptic-soy-agar USP in triplicate using the pour plate method according to Robert Koch.

#### 2.2.2 Determination of heat resistance for the organism *E. faecium*

To determine the heat resistance, 20 test tubes were required. Due to safety reasons the temperature of disinfection in the test device cannot be altered by the user. Therefore the heat resistance test was modified by doing the test in a water bath instead of in the test device.

10  $\mu$ l of the original suspension were added to 10 ml tryptic-soy-broth USP in each of the test tubes. The test tubes were then heated in a water bath for  $70 \pm 1^\circ\text{C}$  for 10 min, then cooled in an icebath at  $0 \pm 1^\circ\text{C}$  for 5 min and incubated at  $36 \pm 1^\circ\text{C}$  for 24 h. Following this, a

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<sup>1</sup> Working Group Endoscopy: Testing and Evaluating the Cleaning and Disinfection Efficacy of Endoscope Washer/Disinfectors and Disinfection Automats as of 1<sup>st</sup> November 1994. Hygiene und Medizin 1995 (20): 40-47

subculture was done on a selective solid medium (Canamycin-esculin-acid-agar) and incubated at  $36 \pm 1^\circ\text{C}$  for 24 h. The heat resistance is supposed to be sufficient when 18 out of 20 test tubes (90 %) show growth of the test organisms.

## **2.3 Mode of inoculation**

The test organisms were suspended in defibrinated sheep blood, then protamin was added.

10 ml of the respective suspension containing the test organism and blood were inoculated into the biopsy channel through the closure at the hand piece of the endoscope with the help of a single-use syringe. Then the endoscope was placed horizontally and turned around by  $360^\circ$ . Following this 20 ml of air was pushed through the biopsy channel, then a sterile biopsy forceps was guided through the channel to guarantee patency. This was followed by a drying phase of 60 min.

### **2.3.1 Determination of colony count of the controls**

The initial colony count was determined using endoscopes that were not disinfected. Following the drying phase, the endoscope was rinsed with 50 ml of a solution containing a neutralising agent (sterile distilled water + 3 % tween 80 + 0.1 % histidine + 0.3 % lecithine + 0.5 % sodium thiosulfate according to DGHM-guidelines (1981)<sup>2</sup>), followed by 2 x 50 ml of air. The distal end of the endoscope was placed in a conical flask to which a vacuum pump was attached.

Beneath a sterile work bench the recovered fluid was divided equally into two sterile test tubes filled with 5 glass beads each. The test tubes were closed with screw caps, inverted twice and shaken on a vortex for 2 min.

Then the recovered fluid was filled back into the conical flask and sonicated for 5 min. The conical flask was shaken for 30 s, then a decimal dilution sequence was done using sterile 0.9 % NaCl-solution. 1 ml of the solution was added to 20 ml of tryptic-soy-agar USP and mixed. When the agar was solidified, the plates were incubated at  $36 \pm 1^\circ\text{C}$  for 48 h. The visible colonies were counted with the help of a colony counter as colony forming units (cfu) per endoscope. Only those dilution factors that contained a colony count between 30 and 300 cfu were evaluated.

### **2.3.2 Recovery of the test organisms after automated cleaning and disinfection**

Immediately after the endoscope was taken out of the washer-disinfector ETD 3, the endoscopes were rinsed just like the control endoscope. The recovered fluid in the conical flask was shaken and sonicated. Then 1 ml of the recovered fluid was taken and a decimal dilution sequence prepared. 1 ml of each dilution factor was added to 20 ml of tryptic-soy-agar USP and mixed.

From the remaining 49 ml of the recovered fluid 1 ml was pipetted each to three plates and additionally 20 ml of tryptic-soy-agar USP, as well. 11.5 ml were added each to 4 large plates

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<sup>2</sup> Richtlinie für die Prüfung und Bewertung chemischer Desinfektionsverfahren. Erster Teilabschnitt. Stand 1.1.1981. Zbl. Bakt. Hyg. 1. Abt. Orig. B 172 (1981): 534-562 ff.

(145 mm x 21 mm) and 30 ml of tryptic-soy-agar USP. Following the solidification of the media, all plates were incubated at  $36 \pm 1^\circ\text{C}$  for 48 h. The visible colonies were counted using a plate counter and documented as cfu per endoscope.

## 2.4 Test device

A washer-disinfector ETD 3 PAA-version (Olympus Europe Ltd., Hamburg) was used as test device. The program tested was STD ENDODIS DRY. This program consists mainly of 7 phases (see also the instruction manual):

**Table 1: Temperature phases and holding time**

**Program: STD ENDODIS DRY**

**Cycle: process with EndoDis<sup>TM</sup>, EndoAct<sup>TM</sup> and EndoDet<sup>TM</sup>**

Phase	Temperature (°C)	Holding time with T = constant (min)	Time duration Process phase (*) (min)
Leak test	n. d.	1.5	1.5 min
Pre cleaning	no heating	0.5	3.0 min
Cleaning	35	3.0	7.0 min
Disinfection	35	5.0	9.0 min
1. Rinsing	no heating	1.0	3.5 min
Final rinse	no heating	1.0	3.5 min
Drying / Optional	57	15.0	15.0 min
<b>Complete cycle time without drying</b>			<b>27.5 min</b>
<b>Complete cycle time with drying</b>			<b>42.5 min</b>

n. d. = no data

(\*) = these times were determined with a water temperature at the inlet at approximately  $25^\circ\text{C}$ . The time duration of the process phase is depending on the temperature of the water at the inlet. This may run up to  $37^\circ\text{C}$ .

### 2.4.1 Disinfecting and cleaning agent

As a disinfecting agent 1.2 % EndoDis<sup>TM</sup> (Olympus Europe Ltd., Hamburg) using peracetic acid and hydrogen peroxide was used. 1.2 % EndoAct<sup>TM</sup> (Olympus Europe Ltd., Hamburg), an activator containing potassium hydroxide was added. Both agents were added together simultaneously. 0.6% of EndoDet<sup>TM</sup> (Olympus Europe Ltd., Hamburg), which contains surfactants, was used as a cleaning agent.

## 2.5 Set-up in the test device

According to the recommendations of the Working Group Endoscopy (1995), the endoscopes were taken out of the washer-disinfector following the disinfection and prior to drying. One endoscope was disinfected in one cycle. Five cycles were run for each test organism.

## **2.6 Determination of surviving test organism in the test objects**

Following the end of the program the door was opened and the colony count of the surviving test organism determined as described in chapter 2.3.2.

## **2.7 Interpretation of the efficacy of disinfection and cleaning**

According to the recommendations of the Working Group Endoscopy (1995) the disinfection is effective when at the given temperature of disinfection and holding time for the test organism *Enterococcus faecium* the reduction factor (RF) is equal or larger than 5. The reduction factor is calculated as follows:

$RF = \log N_0 - \log N$  ( $\log N$  = the logarithm of initial colony count of the control endoscope prior to cleaning and disinfection;  $\log N$  = the logarithm of the number of surviving test organism after cleaning and disinfection).

The cleaning is effective, when the reduction factor (RF) of the test organism *Bacillus subtilis* is equal or larger than 3.5.

## **3 Results**

On the whole, the test included 16 endoscopes (disinfected endoscopes each and control-endoscopes, i. e. 1 control endoscope per test day and test organism).

### **3.1 *Enterococcus faecium***

For the test organism *Enterococcus faecium* the mean of the reduction factor was 8.2, the median 8.5 and the average standard deviation 0.9 (for detailed results see table 1, attachment).

### **3.2 *Bacillus subtilis***

For the test organism *Bacillus subtilis* the mean of the reduction factor was 5.3, the median 6.0 and the average standard deviation 1.2 (for detailed results see table 2, attachment).

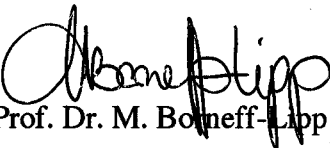
## 4 Expert interpretation

The testing of the efficacy of cleaning and disinfection in the ETD 3 PAA-version led to the following results:

1. The initial colony count of the artificially contaminated endoscopes fulfilled the recommendations of the Working Group Endoscopy (1995).
2. The disinfection of the endoscope is to be called effective since the disinfection process led to a reduction factor of 8.2 for the test organism *Enterococcus faecium*.
3. The cleaning of the endoscope is effective since the cleaning process led to a reduction factor of 5.3 for the test organism *Bacillus subtilis*.

In view of the microbiological testing the thermo-chemical disinfection of the washer-disinfector for endoscopes, ETD 3 PAA-version (Olympus Europe Ltd., Hamburg) at 35°C fulfilled the recommendations of the Working Group Endoscopy (1995) and thus can be recommended for practise.

Halle, Nov. 28<sup>th</sup>, 2003



Prof. Dr. M. Boneff-Lipp

Attachment

**Attachment**

**Table 1: Reduction of Enterococcus faecium ATCC 6057 (DSM 2146) in a flexible endoscope CF-140i after cleaning and disinfection phase**

Test day	Endoscope serial number	Average initial colony count (No)	log No	Average number of surviving organisms (N)	logN	RF
1	29 11 236	6.50E+08	8.8	1.09E+00	0	8.8
1	28 10 376	6.50E+08	8.8	1.36E+00	0.1	8.7
2	28 10 376	3.20E+08	8.5	7.60E+01	1.9	6.6
2	28 10 376	3.20E+08	8.5	1.00E+00	0	8.5
2	29 11 236	3.20E+08	8.5	1.00E+00*	0	8.5
Mean						8.2
Median						8.5
Standard deviation						0.9

Remark: \* This sample showed no growth of the test organisms, but Log of 0 cannot be calculated, so log of 1 is used.

**Table 2: Reduction of spores of Bacillus subtilis ATCC 6051 (DSM 10) in a flexible endoscope CF-140i after cleaning and disinfection phase**

Test day	Endoscope serial number	Average initial colony count (No)	log No	Average number of surviving organisms (N)	logN	RF
1	28 10 376	9.60E+07	8.0	3.60E+04	4.6	3.4
2	28 10 376	2.60E+08	8.4	4.40E+03	3.6	4.8
2	29 11 236	2.60E+08	8.4	1.50E+02	2.2	6.2
3	29 11 236	5.20E+07	7.7	5.70E+01	1.8	6.0
4	29 11 236	3.90E+07	7.6	3.70E+01	1.6	6.0
Mean						5.3
Median						6.0
Standard deviation						1.2