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DIN EN ISO/IEC 17025



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## Test Report 110721-1

### Microbial Barrier Testing of Type “S” Mouthpieces for the Breathalyzers Alco True P, Alco True C, Alco True AL, and Alco True M in the Case of Rebreathing

Order date: July 21, 2011

### 1. Test purpose

The function of the non-return valve in the Type "S" mouthpiece of the breathalyzer Alco True is to be tested with respect to possible contamination in the case of rebreathing. The mouthpiece is made from plastic and consists of an upper part that is connected to a lower part by means of a pressure lock. The non-return valve is located between these two parts and consists of a movable flap made from slightly flexible plastic. In the case of an abrupt intake of air from the upper part, the flap seals the narrowed opening between the upper and lower parts.

The bacteria in the exhaled air of the tested person, who blows forcefully into the device, reach the mouthpiece sleeve, which is not replaced after use. The purpose of the tests is to determine whether, in the case of rebreathing, any bacteria that have reached the mouthpiece sleeve would re-contaminate a newly inserted Type "S" mouthpiece past the non-return valve.

### 2. Principle of the test

Twenty-five Type "S" mouthpieces (LOT:2011-04-29) and two mouthpiece sleeves detached from the Alco True test device were made available for the testing.

### 3. Test methodology

- (1) Test organism: *Enterococcus faecium* ATCC 6057
- (2) Contamination of the inner surface of the mouthpiece sleeve with *E. faecium* bacteria (concentration approx.  $1 \times 10^8$  in 100 µl).
- (3) Preparation of the Type "S" mouthpiece: filled with sterile cotton above the non-return valve.
- (4) The lower part of the mouthpiece was connected to the infected mouthpiece sleeve, and the upper part was connected to a sterile syringe (20ml).
- (5) Simulation of rebreathing through an abrupt suction of the air through the mouthpiece using the sterile syringe until the safety flap of the valve closes.
- (6) Separation of the mouthpiece into upper part, cotton, non-return valve, and lower part for the re-dissolution of bacteria from each of the individual parts in 1 ml of physiological saline solution (NaCl).
- (7) Ten minutes of shaking to ensure the complete wetting of the individual parts and the removal of any existing bacteria by the saline solution.
- (8) Transfer of the inoculated saline solution onto blood agar.
- (9) Incubation of the plates for 24 hours at 37°C.
- (10) Analysis: Quantitative determination of *E. faecium* for each part.
- (11) Coating of individual parts with nutrient solution to be incubated for 24 hours.
- (12) For any nutrient solutions showing turbidity: loop smear onto blood agar and Enterococcus agar.
- (13) Analysis: Qualitative determination of *E. faecium*.

The test was performed 15 times.

In order to check the test set-up, the same procedure was performed six times with 300 µl of contamination solution parallel to the actual testing in order to bring about a contamination by means of bacterial overload. With 300 µl of bacterial suspension, the maximum capacity of the mouthpiece sleeve was reached.

*Note:* In the execution of the test, a risk of bacterial spread was associated with Step (6) (see above) owing to the force required for separating the mouthpieces into flap, upper part, and lower part.

### 4. Results

For table, see Appendix 1.

In the test with 100 µl of contamination solution, no test organisms *E. faecium* could be quantitatively detected on any of the parts.

Test organisms were only detected qualitatively on the cotton. A systematic error was suspected in the execution of the test. Therefore, the test was repeated with five Type "S" mouthpieces. In the test run performed on August 4, 2011, no test organisms could be detected on the mouthpiece parts or the cotton.

Owing to bacterial overgrowth, only one sample in the test with 300 µl of contamination solution could be quantified.

In the qualitative determination, five of the six lower parts tested positive in both test runs with 300 µl of contamination solution. Three of the six flaps placed in saline solution also showed positive results. On each test day, a positive detection are performed on the mouthpieces respectively. The cotton tested negative in all test runs with 300 µl of bacterial suspension.

### **Assessment**

The test results show that a large quantity of fluid is required in the mouthpiece sleeve in order to transfer a contamination into the mouthpiece through air intake. Even in the test runs with 300 µl of test bacterial suspension in the mouthpiece sleeve, the flap functioned effectively as a barrier for the test bacterial suspension in four out of six cases.

The single-use mouthpieces offer sufficient protection for blocking any existing bacteria in the mouthpiece sleeve in the case of rebreathing.

Professor Werner Solbach, M.D.

Nina Krause, graduate engineer

# Appendix 1 - Results -

Test Organism Initial Solution Batch July 25, 2011 Bacterial Count $1.17 \times 10^9$ /ml								
Use of 100 $\mu$ l Test Bacterial Solution								
Date/ No.	Lower Part		Flap		Upper Part		Cotton	
	CFU/ Part	Qual. Detection Test Bact.	CFU / Part	Qual. Detection Test Bact.	CFU / Part	Qual. Detection Test Bact.	CFU / Part	Qual. Detection Test Bact.
07/25/11								
1	0	neg.	0	neg.	0	neg.	0	pos.
2	0	neg.	0	neg.	0	neg.	0	pos.
3	0	neg.	0	neg.	0	neg.	0	neg.
4	0	neg.	0	neg.	0	neg.	0	pos.
5	0	neg.	0	neg.	0	neg.	0	pos.
6	0	neg.	0	neg.	0	neg.	0	neg.
7	0	neg.	0	neg.	0	neg.	0	pos.
8	0	neg.	0	neg.	0	neg.	0	neg.
9	0	neg.	0	neg.	0	neg.	0	pos.
Pre-test	0	----	0	---	0	---	0	---
08/04/11								
1	0	neg.	0	neg.	0	neg.	0	neg.
2	0	neg.	0	neg.	0	neg.	0	neg.
3	0	neg.	0	neg.	0	neg.	0	neg.
4	0	neg.	0	neg.	0	neg.	0	neg.
5	0	neg.	0	neg.	0	neg.	0	neg.
Use of 300 $\mu$ l Test Bacterial Solution								
Date/ No.	Lower Part		Flap		Upper Part		Cotton	
	CFU/ Part	Qual. Detection Test Bact.	CFU / Part	Qual. Detection Test Bact.	CFU / Part	Qual. Detection Test Bact.	CFU / Part	Qual. Detection Test Bact.
07/25/11								
1	10	pos.	0	pos.	0	neg.	0	neg.
2	>300	pos.	>300	pos.	>300	pos.	0	neg.
3	0	pos.	0	pos.	0	neg.	0	neg.
07/28/11								
1	>300	pos.	0	neg.	0	neg.	0	neg.
2	0	neg.	0	neg.	0	neg.	0	neg.
3	>300	pos.	0	neg.	>300	pos.	0	neg.
Negative control	0	neg.	0	neg.	0	neg.	0	neg.

Key:

neg.: negative

pos.: positive