Interlaboratory Test

„Microbial barrier testing of packaging materials for medical devices which are to be sterilized“

according to DIN 58953-6:2010

Test report

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1. General Information on the Interlaboratory Test

1.1 Organization

Organizer of the Interlaboratory Test: Sterile Barrier Association (SBA)
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1.2 Occasion and Objective

In order to demonstrate compliance with the requirements of the ISO 11607-1:2006 „Packaging for terminally sterilized medical devices -- Part 1: Requirements for materials, sterile barrier systems and packaging systems“ validated test methods are to be preferably utilized.

For the confirmation of the microbial barrier properties of porous materials demanded in the ISO 11607-1, the DIN 58953-6:2010 „Sterilization – Sterile supply – Part 6: Microbial barrier testing of packaging materials for medical devices which are to be sterilized“ represents a conclusive method which can be performed without the need for extensive equipment.

However, since momentarily no validation data on DIN 58953-6 is at hand concerns emerged that the method may lose importance against validated methods in a revision of the ISO 11607-1 or may even not be considered at all.

Within the framework of this interlaboratory test, data on the reproducibility of the results obtained by means of the analysis according to DIN 58953-6 shall be gathered.

1.3 Time Schedule

September 2010: The Sterile Barrier Association queried ISEGA Forschungs- und Untersuchungsgesellschaft about the technical support for the interlaboratory test. For the realization, the Verein zur Förderung der Forschung und Ausbildung für Faserstoff- und Verpackungschemie e. V. (VFV) was won over.

November 2010: Preliminary announcement of the interlaboratory test / Seach for interested laboratories

January to December 2011:	Search for suitable sample material / Carrying out of numerous pre-trials on various materials
January 2012:	Renewed contact or search for additional interested laboratories, respectively
February 2012:	Sending out of registration forms / preparation of sample material
March 2012:	Registration deadline / sample despatch
May / June 2012:	Results come in / statistical evaluation
July 2012:	Despatch of samples for the re-examination
September 2012:	Results of the re-examination come in / statistical evaluation
November 2012:	Results are sent to the participants
December 2012/ January 2013:	Compilation of the test report

1.4 Participants

Five different German laboratories participated in the interlaboratory test. In one laboratory, the analyses were performed by two testers working independently so that six valid results overall were received which can be taken into consideration in the evaluation.

To ensure an anonymous evaluation of the results, each participant was assigned a laboratory number (laboratory 1 to laboratory 6) in random order, which was disclosed only to the laboratory in question. The complete laboratory number breakdown was known solely by the ISEGA staff supporting the proficiency test.

2. Sample Material

2.1 Sample Description and Execution of the Test

Utmost care in the selection of suitable sample material was taken to include different materials used in the manufacture of packaging for terminally sterilized medical devices.

With the help of numerous pre-trials the materials were chosen covering a wide range of results from mostly germ-proof samples to germ permeable materials.

2.1.1 Materials for the Analysis of Germ Proofness under Humidity according to DIN 58953-6, section 3:

Sample designation	Sample material	Comment
Sample F1	Sterilization paper, smooth	
Sample F2	Sterilization paper, one-sided smooth	Paper sheet of a sterilization reel
Sample F3	Sterilization paper, creped	

The participants were advised to perform the analysis on the samples according to DIN 58953-6, section 3, and to protocol their findings on the provided result sheets.

The only deviation from the norm was that in case of the growth of 1 -5 colony-forming units (in the following abbreviated as CFU) per sample, no re-examination 20 test pieces was performed.

2.1.2 Materials for the Analysis of Germ Proofness with Air Permeance according to DIN 58953-6, section 4:

Sample designation	Sample material	Comment
Sample L1	Sterilization paper, one-sided smooth	Paper sheet of a sterilization reel
Sample L2	Nonwoven sterilization wrap	Each test piece was perforated by an acupuncture needle several times
Sample L3	Sterilization paper, one-sided smooth	Sampling from a paper pouch sterilization packaging
Sample L4	Nonwoven sterilization wrap	Additional sample, tested in the re-examination (July / August 2012)

The participants were advised to perform the analysis on the samples according to DIN 58953-6, section 4, and to protocol their findings on the provided result sheets.

2.2 Sample Preparation and Despatch

For the analysis of the germ proofness under humidity, 10 test pieces in the size of 50 x 50 mm were cut out of each sample and heat-sealed into a sterilization pouch with the side to be tested up.

Out of the 10 test pieces, 5 were intended for the testing and one each for the two controls according to DIN 58953-6, sections 3.6.2 and 3.6.3. The rest should remain as replacements (e.g. in case of the dropping of a test piece on the floor etc.).

For the analysis of the germ proofness with air permeance, 15 circular test pieces with a diameter of 40 mm were punched out of each sample and heat-sealed into a sterilization pouch with the side to be tested up.

Out of the 15 test pieces, 10 were intended for the testing and one each for the two controls according to DIN 58953-6, section 4.9. The rest should remain as replacements (e.g. in case of the dropping of a test piece on the floor etc.).

The sterilization pouches with the test pieces were steam-sterilized in an autoclave for 15 minutes at 121 °C and stored in an climatic room at 23 °C and 50 % relative humidity until despatch.

2.3 Additional Sample and Re-examination

For the analysis of the germ proofness under humidity another test round was performed in July / August 2012. For this, an additional sample (sample L4) was sent to the laboratories and analysed (see 2.1.2). The results were considered in the evaluation.

For validation or confirmation of non-plausible results, occasional samples for re-examination were sent out to the laboratories. The results of these re-examinations (July / August 2012) were not taken into consideration in the evaluation.

3. Results

3.1 Preliminary Remark

Since the analysis of germ proofness is designed to be a pass / fail – test, the statistical values and precision data were meant only to serve informative purposes.

The evaluation of the materials according to DIN 58953-6, sections 3.7 and 4.7.6 by the laboratories should be the most decisive criterion for the evaluation of reproducibility of the interlaboratory test results. Based on this, the classification of a sample as “sufficiently germ-proof” or “not sufficiently germ-proof” is carried out.

3.2 Note on the Record of Test Results:

The exact counting of individual CFUs is not possible with the required precision if the values turn out to be very high. Thus, an upper limit of 100 CFU per agar plate or per test pieces, respectively, was defined. Individual values above this limit and values which were stated with “> 100” by the laboratories, are listed as 100 CFU per agar plate or per test piece, respectively, in the evaluation.

3.3 Comment on the Statistical Evaluation

The statistical evaluation was done based on the series of standards DIN ISO 5725-1ff.

The arithmetic laboratory mean X_i and the laboratory standard deviation s_i were calculated from the individual measurement values obtained by the laboratories.

The overall mean X of the laboratory means as well as the precision data of the method (reproducibility and repeatability) were determined for each sample

3.4 Outlier tests

The Mandel's h-statistics test was utilised as outlier test for differences between the laboratory means of the participants.

A laboratory was identified as a “statistical outlier” as soon as an exceedance of Mandel's h test statistic at the 1 % significance level was detected.

The respective results of the laboratories identified as outliers were not considered in the statistical evaluation.

3.5 Record of Test Results

On the following pages, the records of the test results for each interlaboratory test sample with the statistical evaluation and the evaluation according to DIN 58953-6 are compiled.

3.5.1 Record of Test Results Sample F1

Individual Measurement values:

Lab-oratory no.	Sum CFU	Result in CFU / agar plate						
		Individual Values					Laboratory Mean X_i	Lab. Standard Deviation s_i
Lab. 1	500	100	100	100	100	100	100.0	0.00
Lab. 2	500	100	100	100	100	100	100.0	0.00
Lab. 3	500	100	100	100	100	100	100.0	0.00
Lab. 4	10	2	2	3	1	2	2.0	0.71
Lab. 5	381	100	74	37	86	84	76.2	23.8
Lab. 6	393	28	100	100	65	100	78.6	32.1

Statistical Evaluation:

Comment: Laboratory 4, as an outlier, has not been taken into consideration in the statistical Evaluation.
Outlier criterion: Mandel's h-statistics (1 % level of significance)

Overall mean X:	91.0	CFU / agar plate
Repeatability standard deviation s_r:	17.9	CFU / agar plate
Reproducibility standard deviation s_R:	19.8	CFU / agar plate
Repeatability r:	50.0	CFU / agar plate
Repeatability coefficient of variation:	19.6	%
Reproducibility R:	55.5	CFU / agar plate
Reproducibility coefficient of variation:	21.8	%

Evaluation according to DIN 58953-6, Section 3.7:

Lab. 1 - 6: Number of CFU > 5, i.e. the material is classified as not sufficiently germ-proof.

Conclusion:

All of the participants, even the Laboratory 4 which was identified as an outlier, came to the same results and would classify the sample material as “not sufficiently germ-proof”

3.5.2 Record of Test Results Sample F2

Individual Measurement values:

Lab-oratory no.	Sum CFU	Result in CFU / agar plate						
		Individual Values					Laboratory Mean X_i	Lab. Standard Deviation s_i
Lab. 1	0	0	0	0	0	0	0.0	0.00
Lab. 2	0	0	0	0	0	0	0.0	0.00
Lab. 3	0	0	0	0	0	0	0.0	0.00
Lab. 4	5	3	2	0	0	0	1.0	1.41
Lab. 5	0	0	0	0	0	0	0.0	0.00
Lab. 6	0	0	0	0	0	0	0.0	0.00

Statistical Evaluation:

Comment: Laboratory 4, as an outlier, has not been taken into consideration in the statistical Evaluation.
Outlier criterion: Mandel's h-statistics (1 % level of significance)

Overall mean X:	0	CFU / agar plate
Repeatability standard deviation s_r:	0	CFU / agar plate
Reproducibility standard deviation s_R:	0	CFU / agar plate
Repeatability r:	0	CFU / agar plate
Repeatability coefficient of variation:	0	%
Reproducibility R:	0	CFU / agar plate
Reproducibility coefficient of variation:	0	%

Evaluation according to DIN 58953-6, Section 3.7:

Lab. 1 – 3:	Number of CFU = 0, i.e. the material is classified as sufficiently germ-proof
Lab. 4:	Number of CFU ≤ 5, i.e. a re-examination on 20 test pieces would have to be done
Lab. 5 – 6:	Number of CFU = 0, i.e. the material is classified as sufficiently germ-proof

Conclusion:

All of the participants, except for the Laboratory 4 which was identified as an outlier, came to the same results and would classify the sample material as “sufficiently germ-proof”.

3.5.3 Record of Test Results Sample F3

Individual Measurement values:

Lab-oratory no.	Sum CFU	Result in CFU / agar plate						
		Individual Values					Laboratory Mean X_i	Lab. Standard Deviation s_i
Lab. 1	167	59	58	42	5	3	33.4	27.7
Lab. 2	212	49	54	52	0	57	42.4	23.9
Lab. 3	9	0	2	5	1	1	1.8	1.92
Lab. 4	361	81	93	54	76	57	72.2	16.8
Lab. 5	0	0	0	0	0	0	0.0	0.00
Lab. 6	154	20	40	14	40	40	30.8	12.8

Statistical Evaluation:

Overall mean X:	30.1	CFU / agar plate
Repeatability standard deviation s_r:	17.2	CFU / agar plate
Reproducibility standard deviation s_R:	30.9	CFU / agar plate
Repeatability r:	48.2	CFU / agar plate
Repeatability coefficient of variation:	57.1	%
Reproducibility R:	86.5	CFU / agar plate
Reproducibility coefficient of variation:	103	%

Evaluation according to DIN 58953-6, Section 3.7:

Lab. 1 - 4:	Number of CFU > 5, i.e. the material is classified as not sufficiently germ-proof.
Lab. 5:	Number of CFU = 0, i.e. the material is classified as sufficiently germ-proof.
Lab. 6:	Number of CFU > 5, i.e. the material is classified as not sufficiently germ-proof.

Conclusion:

Five of the six participants came to the same result and would classify the sample as “not sufficiently germ-proof”. Only laboratory 5 would classify the sample material as “sufficiently germ-proof”.

3.5.4 Record of Test Results Sample L1

Individual Measurement values:

Lab-oratory no.	Sum CFU	Result in CFU / test piece											
		Individual Values										Laboratory Mean X_i	Lab. Standard Deviation s_i
Lab. 1	3	2	1	0	0	0	0	0	0	0	0	0.30	0.67
Lab. 2	2	1	1	0	0	0	0	0	0	0	0	0.20	0.42
Lab. 3	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Lab. 4	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Lab. 5	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Lab. 6	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00

Statistical Evaluation:

Overall mean X:	0.09	CFU / test piece
Repeatability standard deviation s_r:	0.32	CFU / test piece
Reproducibility standard deviation s_R:	0.33	CFU / test piece
Repeatability r:	0.91	CFU / test piece
Repeatability coefficient of variation:	357	%
Reproducibility R:	0.93	CFU / test piece
Reproducibility coefficient of variation:	366	%

Evaluation according to DIN 58953-6, Section 4.7:

Lab. 1 - 6: Number of CFU < 15, i.e. the material is classified as sufficiently germ-proof.

Conclusion:

All participants came to the same result and would classify the sample as “sufficiently germ-proof”.

3.5.5 Record of Test Results Sample L2

Individual Measurement values:

Lab-oratory no.	Sum CFU	Result in CFU / test piece											
		Individual Values										Laboratory Mean X_i	Lab. Standard Deviation s_i
Lab. 1	16	4	4	4	2	1	1	0	0	0	0	1.60	1.78
Lab. 2	8	1	1	2	4	0	0	0	0	0	0	0.80	1.32
Lab. 3	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Lab. 4	2	1	0	0	0	0	0	0	0	1	0	0.20	0.42
Lab. 5	11	0	0	3	2	2	2	0	0	1	1	1.10	1.10
Lab. 6	6	0	0	0	3	0	1	1	0	0	1	0.60	0.97

Statistical Evaluation:

Overall mean X:	0.73	CFU / test piece
Repeatability standard deviation s_r:	1.10	CFU / test piece
Reproducibility standard deviation s_R:	1.18	CFU / test piece
Repeatability r:	3.07	CFU / test piece
Repeatability coefficient of variation:	151	%
Reproducibility R:	3.32	CFU / test piece
Reproducibility coefficient of variation:	163	%

Evaluation according to DIN 58953-6, Section 4.7:

Lab. 1:	Number of CFU > 15, i.e. the material is classified as not sufficiently germ-proof.
Lab. 2 - 6:	Number of CFU < 15, i.e. the material is classified as sufficiently germ-proof.

Conclusion:

Five of the six participants came to the same result and would classify the sample as “sufficiently germ-proof”. Only laboratory 1 exceeds the limit value slightly by 1 CFU, so that the sample would be classified as “not sufficiently germ-proof”.

3.5.6 Record of Test Results Sample L3

Individual Measurement values:

Lab-oratory no.	Sum CFU	Result in CFU / test piece											
		Individual Values										Laboratory Mean X_i	Lab. Standard Deviation s_i
Lab. 1	1	1	0	0	0	0	0	0	0	0	0	0.10	0.32
Lab. 2	2	1	1	0	0	0	0	0	0	0	0	0.20	0.42
Lab. 3	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Lab. 4	13	0	1	0	0	0	5	4	1	2	0	1.30	1.83
Lab. 5	1	0	0	0	0	0	0	0	0	1	0	0.10	0.32
Lab. 6	7	0	3	0	0	0	4	0	0	0	0	0.70	1.49

Statistical Evaluation:

Overall mean X:	0.36	CFU / test piece
Repeatability standard deviation s_r:	1.00	CFU / test piece
Reproducibility standard deviation s_R:	1.06	CFU / test piece
Repeatability r:	2.79	CFU / test piece
Repeatability coefficient of variation:	274	%
Reproducibility R:	2.98	CFU / test piece
Reproducibility coefficient of variation:	293	%

Evaluation according to DIN 58953-6, Section 4.7:

Lab. 1 - 6: Number of CFU < 15, i.e. the material is classified as sufficiently germ-proof.

Conclusion:

All participants came to the same result and would classify the sample as “sufficiently germ-proof”.

3.5.7 Record of Test Results Sample L4

Individual Measurement values:

Lab-oratory no.	Sum CFU	Result in CFU / test piece											
		Individual Values										Laboratory Mean X_i	Lab. Standard Deviation s_i
Lab. 1	1000	100	100	100	100	100	100	100	100	100	100	100.0	0.00
Lab. 2	570	20	20	30	50	50	50	50	100	100	100	57.0	32.0
Lab. 3	22	1	4	2	4	1	0	1	4	5	0	2.2	1.87
Lab. 4	10	5	2	0	0	1	0	0	0	1	1	1.0	1.56
Lab. 5	205	46	1	6	2	60	1	58	15	15	1	20.5	24.4
Lab. 6	156	7	1	7	70	37	7	0	22	5	0	15.6	22.3

Statistical Evaluation:

Overall mean X:	35.1	CFU / test piece
Repeatability standard deviation s_r:	18.8	CFU / test piece
Reproducibility standard deviation s_R:	42.6	CFU / test piece
Repeatability r:	52.7	CFU / test piece
Repeatability coefficient of variation:	53.7	%
Reproducibility R:	119	CFU / test piece
Reproducibility coefficient of variation:	122	%

Evaluation according to DIN 58953-6, Section 4.7:

Lab. 1 - 3:	Number of CFU > 15, i.e. the material is classified as not sufficiently germ-proof.
Lab. 4:	Number of CFU < 15, i.e. the material is classified as sufficiently germ-proof.
Lab. 5 - 6:	Number of CFU > 15, i.e. the material is classified as not sufficiently germ-proof.

Conclusion:

Five of the six participants came to the same result and would classify the sample as “not sufficiently germ-proof”.

4. Overview and Summary

Sample desig-nation	Type of germ proofness analysis	Overall mean X	No. of parti-cipants	Valid results	No. of evaluations as		Con-sensus
					“sufficiently germ-proof”	“not sufficiently germ-proof”	
Sample F1	Humidity	91.0	6	5	0	5	100 %
Sample F2	Humidity	0.00	6	5	5	0	100 %
Sample F3	Humidity	30.1	6	6	1	5	83 %
Sample L1	Air permeance	0.09	6	6	6	0	100 %
Sample L2	Air permeance	0.73	6	6	5	1	83 %
Sample L3	Air permeance	0.36	6	6	6	0	100 %
Sample L4	Air permeance	35.1	6	6	1	5	83 %

Summary:

In case of four of the overall seven tested materials, a 100 % consensus was reached regarding the evaluation as “sufficiently germ-proof” and “not sufficiently germ-proof” according to DIN 58 953-6.

As for the other three tested materials, there were always 5 concurrent participants out of 6 (83 %). In each case, only one laboratory would have evaluated the sample differently.

It is noteworthy that the materials about the evaluation of which a 100 % consensus was reached were the smooth sterilization papers. The differences with one deviating laboratory each occurred with the slightly less homogeneous materials, such as with the creped paper and the nonwoven materials.