Microbiological Validation according to EN-ISO 11137-2:2012, Method VD_{MAX}^{25} Initial Validation

PharmaHelpBag

Sample code: 201306000256
Quotation: 2013313L

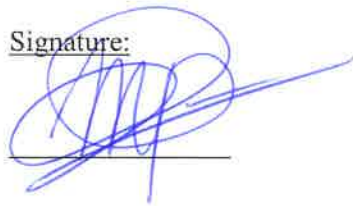
Applicant : Inpakomed B.V.

Performed by: Synergy Health Pharmaceutical Laboratories (SHPL)
Microbiological Laboratory
EDE - The Netherlands

APPROVAL PAGE

SYNERGY HEALTH UTRECHT BV

Responsible for the work performed at the microbiology laboratory of Synergy Health Utrecht BV, being executed according to internal procedures, and for the writing of this report.

Report made by:	Signature:	Date:
Manon Puister Supervisor Microbiology		26 June 2013

Approved by:		
Dick Wullink QA Officer Laboratory		27 June 2013

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SUMMARY

Test	:	**Microbiological Validation according to ISO 11137-2:2012 VD_{MAX}^{25} Initial Validation**			
Applicant	:	Inpakomed B.V.			
Product description	:	PharmaHelpBag			
Batch number	:	220413	230413	240413	250413

Test description		*Batch number*	*Test results*	
Bioburden validation		220413	Correction factor	1.2
Bioburden assay (cfu/unit)		230413	Average bioburden	34
		240413	Average bioburden	58
		250413	Average bioburden	28
			Bioburden for Verification	40
Verification Dose (kGy)	8.7	250413	Applied Dose	Min. 8.6 kGy; Max. 9.1 kGy
Sterility test		250413	All 10 tests negative, thus no growth	
Conclusion	The microbiological validation was successful and the Sterilization Dose of 25 kGy for S.A.L. 10^{-6} has been validated.			

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1 REFERENCES

EN-ISO 11137-2:2012	Sterilization of health care products – Radiation – Part 2: Establishing the sterilization dose
EN-ISO 11737-1:2006	Sterilization of medical devices – Microbiological methods – Part 1: Determination of a population of micro-organisms on products
EN-ISO 11737-2:2009	Sterilization of medical devices – Microbiological methods – Part 2: Tests of sterility performed in the definition, validation and maintenance of a sterilization process

2 INTRODUCTION

Sterility is an absolute term and the assurance that any given item is sterile is a probability function. The Sterility Assurance Level (SAL) is defined as the probability of any given unit being non-sterile after exposure to a validated sterilization process.

The ISO 11137-2:2012 method VD_{MAX}^{25} is used to substantiate the use of 25 kGy as a routine dose for sterilisation. This method substantiates using a Verification Dose of a SAL of 10^{-1} to attain a SAL of 10^{-6} and may only be applied to products whose Bioburden is on average below 1,000 cfu/unit.

The VD_{MAX}^{25} method is an alternative approach to substantiation of 25 kGy as an appropriate sterilization dose to attain a SAL of 10^{-6}. The application of this method is not limited by batch size or production frequency and the number of product units irradiated in the Verification Dose Experiment remains constant. The method employs as its basis components of the Standard Distribution of Resistances (SDR) on which Method 1 is also founded and embodies the following 3 principles:

1. Existence of a direct link between the outcome of the Verification Dose Experiment and the attainment of a SAL of 10^{-6} at a sterilization dose of 25 kGy.

2. Possession of a level of conservativeness at least equal to that of the SDR.

3. For a given Bioburden, use a maximum Verification Dose (VD_{MAX}^{25}) commensurate with substantiation of 25 kGy.

3 OBJECTIVE

To substantiate a sterilisation dose of 25 kGy to attain a S.A.L. of 10^{-6} using the VD_{MAX}^{25} Initial Validation method for the PharmaHelpBag of Inpakomed B.V. The procedures carried out for the substantiation of 25 kGy are the determination of the average Bioburden and the performance of a Verification Dose Experiment. The outcome of this Experiment ultimately allows the substantiation of 25 kGy.

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4 GENERAL PROCEDURES INITIAL VALIDATION METHOD VD_{MAX}^{25}

In general the Microbiological Validation of a sterilization process with the use of Gamma radiation consists of the following steps:

- Bioburden validation, and from the results the calculation of the correction factor
- Bioburden estimation on a defined number of non radiated samples
- Establishing of the Verification Dose, based on the Bioburden Estimation
- Delivery of the Verification Dose to a defined number of samples
- Test of sterility on the samples that received the Verification Dose
- Bacteriostasis / Fungistasis testing

4.1 Bioburden validation according to EN-ISO 11737-1

The Bioburden Validation is a test to determine the efficacy of the method that is used to estimate the Bioburden on the product. With the results of the Bioburden validation a correction factor is calculated which is used in the estimation of the product's Bioburden.
EN-ISO 11737-1 describes two general methods for the Bioburden validation:

1. Repetitive Method; the product is repeatedly subjected to a treatment. The Bioburden is determined after each treatment. The decrease in the Bioburden recovery after each treatment gives information about the efficacy of the method.

2. Inoculation Method; the (sterile) product is inoculated with a known number of micro-organisms (Max. 100 CFU *S. aureus*), after which the product will be allowed to dry for a defined period of time. Subsequently, the recovery of the inoculated micro-organism is determined. The correction factor can be calculated using the difference between the number of micro-organisms inoculated and the number of micro-organisms that is recovered. The inoculation method is the preferred method used by SHPL.

4.2 Bioburden estimation according to EN-ISO 11737-1

In this stage the average Bioburden per product unit of 3 batches is determined. At random 10 product units are selected, from each of a minimum of 3 production batches, immediately prior to the sterilization phase of production. The number of product units that are sampled shall be sufficient to validly represent the Bioburden on the product to be sterilized. An entire product should be used for testing; however, in practice this is not always possible. In these situations, a selected portion of a product unit that is convenient to handle during testing may be substituted. This Sample Item Portion (SIP) should be a portion of the product, that is as large as possible, to manipulate readily in the laboratory. The SIP may be calculated on the basis of length, weight, volume, surface area or the Bioburden distribution of the product unit to be tested.

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EN-ISO 11737-1 describes a number of methods that can be combined in order to obtain the best result in recovering the Bioburden from the product. There are methods to release the Bioburden from the product into a rinsing fluid, such as stomaching, ultrasonication, shaking, vortex mixing, flushing, blending or swabbing. Also there are methods as contact plating or agar overlaying to determine the Bioburden without initially removing it from the product.
For the transfer into culture medium, there are descriptions for membrane filtration, pour plating, spread plating and spiral plating. The best method to be used for a specified product is determined in the first stage of the validation process (Bioburden validation).

4.3 Establishing the Verification Dose according to EN-ISO 11137-2

According to EN-ISO 11137-2 one of the following should be used to determine the Verification Dose:

- Overall Bioburden average: if each of the batch averages is less than 2 times the overall Bioburden average.
- Highest batch Bioburden average: if 1 or more of the batch averages is equal to or larger than 2 times the overall Bioburden average.

Use the Verification Dose table in EN-ISO 11137-2 method VD_{MAX}^{25}, to determine the Verification Dose as follows:

- For a SIP = 1, find the closest Bioburden value larger than or equal to the average Bioburden estimate determined above. Select the corresponding Verification Dose from the table.

4.4 Perform the Verification Dose Experiment according to EN-ISO 11137-2

At random 10 product units from a single production batch are selected. The 10 product units may be selected from any one of the batches for which a Bioburden was obtained in the Bioburden estimation mentioned before, or from a new batch manufactured under conditions that are representative for normal production.

Irradiate the 10 product units or portions thereof with the Verification Dose determined in section 4.3.

The actual dose may vary from the selected dose by no more than +/-10%. If the delivered dose is less than 90% of the Verification Dose, the Verification Experiment may be repeated.

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4.5 Test of Sterility according to 11737-2

Each of the product units or portions thereof subjected to the Verification Dose is tested on sterility according to EN-ISO 11737-2. This international standard describes two methods to perform a test of sterility on medical devices.

1. Direct immersion in growth medium.
   The product is directly transferred into growth medium and incubated. Preparatory activities, as for example disassembly of the product, can be necessary, based on the nature of the product. For a specific product this is determined in the first stage of the validation process.

2. Extraction from the product and transfer of the micro-organisms to growth medium.
   The product is rinsed with a specific diluent, which is subsequently tested. This method is only used when direct immersion is not possible, for example because of Bacteriostatic / Fungistatic activity of the product or when there is a claim of sterility on a part of the device only and disassembly is not an option (for example sterile fluid path).

4.6 Interpretation of Results

(A) If no more than 1 positive test of sterility is obtained in the 10 tests, a sterilization dose of 25 kGy is substantiated.

(B) If 2 positive tests of sterility are obtained in the 10 tests, a Confirmatory Verification Dose Experiment as outlined below shall be performed:
At random 10 product units from a single batch are selected. The 10 product units may be from 1 of the batches sampled previously or from a new batch manufactured under conditions that are representative of normal production. The 10 product units are irradiated with the same Verification Dose as detailed before. The same tolerances apply. Each of the product units is then subjected to a test of sterility as detailed before.
If, on re-test, no positive tests of sterility are obtained in the 10 tests, a sterilization dose of 25 kGy is substantiated. This finding represents a total of 2 positive tests of sterility from the Initial and Confirmatory Verification Dose Experiment.

If, on re-test, 1 or more positive tests of sterility are obtained in the 10 tests, a sterilization dose of 25 kGy has not been substantiated. This finding represents a total of 3 or more positive tests of sterility from the Initial and Confirmatory Verification Dose Experiments. An alternative dose setting method shall be used.

The dose substantiated shall not be repeated unless the results can be ascribed to incorrect performances of the estimation of Bioburden, the sterility testing or the delivery of the Verification Dose, e.g. the delivered dose was less than 90% of the Verification Dose.

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(C) If 3 or more positive tests of sterility are obtained in the 10 tests, a sterilization dose of 25 kGy has not been substantiated. An alternative dose setting method shall be used.

This Verification Dose Experiment shall not be repeated unless the results can be ascribed to incorrect performances of the estimation of Bioburden, the sterility testing, or the delivery of the Verification Dose, e.g. the delivered dose was less than 90% of the Verification Dose.

4.7 Bacteriostasis and Fungistasis test according to EN-ISO 11737-2

Products under test can possibly have antimicrobial activity. Antimicrobial activity can prevent the growth of micro-organisms leading to false negative results in the sterility test. To exclude the possibility of false negative results a bacteriostasis and fungistasis test is performed. Under the circumstances of the sterility test a maximum of 100 CFU of specific micro-organisms are added to the test. After a defined period of incubation, the samples are visually checked for growth. When positive results (and thus growth) are found, it can be concluded that antimicrobial activity is either not present or otherwise sufficiently neutralised.

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5 MATERIALS & METHODS

5.1 Product

PharmaHelpBag. In all of the tests the total product was tested.

Bioburden validation:

- Batch no. 220413

Bioburden Estimation:

- Batch no. 230413
- Batch no. 240413
- Batch no. 250413

Verification dose experiment and subsequent sterility test:

- Batch no. 250413

Bacteriostasis/Fungistasis test.

- Batch no. 250413

5.2 Micro-organisms

• *Staphylococcus aureus*	ATCC 6538
• *Bacillus subtilis*	ATCC 6633
• *Pseudomonas aeruginosa*	ATCC 9027
• *Candida albicans*	ATCC 10231
• *Aspergillus brasiliensis*	ATCC 16404

5.3 Media

• Tryptone Soya Agar	TSA
• Sabouraud Dextrose Agar	SDA
• Tryptone Soya Broth	TSB
• Pepton Water	PW

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5.4 Test Procedures

5.4.1 Bioburden Validation

The Bioburden validation was carried out using the inoculation method.

- 5 products were irradiated with a dose of 25 kGy minimum.
- Each of the 5 samples was inoculated with 0.1 ml of a *Staphylococcus aureus* ATCC 6538 inoculum suspension with 500 cfu/ml. For items with a low Bioburden, a micro-organism concentration of less than 100 cfu on the product should be appropriate.
- The inoculated product was allowed to dry for 2-5 hours (under LAF, class 5).
- The inoculated product was put in PW aseptically (under LAF, class 5).
- The product and PW were shaken for 30 min at 240rpm.
- The eluent was filtrated by membrane filtration and the filter was put on TSA.
- 0.1 ml of the Staphylococcus aureus inoculum suspension was put on TSA directly (in duplicate).
- All plates were incubated for 2-3 days at 32.5°C ± 2.5°C.
- The number of colonies on the plates was counted.
- The number of micro-organisms removed from the inoculated products was expressed as a fraction of the number of micro-organisms inoculated on to the product.
- The mean recovery efficiency and correction factor were calculated.

5.4.2 Bioburden Estimation

The average Bioburden per product unit of the three batches was determined, according to the internal procedures.

- Each product was put in 400 ml PW aseptically (under LAF, class 5).
- The jar containing product and the PW was shaken for 30 min at 240 rpm.
- The eluent was filtrated by membrane filtration in two equal parts:
    - 1 X 50% of the eluent on TSA for aerobic micro-organisms
    - 1 X 50% of the eluent on SDA for yeasts and moulds.

  This will result in a dilution factor of 2.

- Incubation conditions:
    - TSA: 3 – 5 days at 32.5°C ± 2.5°C
    - SDA: 5 – 7 days at 22.5°C ± 2.5°C

The result was calculated by multiplying the counted colonies on the plate with the dilution factor and the correction factor. When 0 colonies are counted on the plate, the result is reported as "< 1 x (dilution factor) x (correction factor)".
The Total Bioburden is the sum of Aerobic micro-organisms and Yeast /Moulds.

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5.4.3 Application of the verification dose

The 10 product were sent to the Synergy Health AST facility in Ede (NL) where they were irradiated with the calculated verification dose. After receiving back the samples from Synergy Health AST, sterility testing was executed on the samples.

5.4.4 Test of Sterility

The sterility test was performed according to the internal procedures.

- The sterility test was carried out in the cleanroom.
- The 10 samples were tested on sterility with the direct immersion method using TSB as the growth medium.
- Incubation conditions: 28°C – 32°C for 14 days.
- A negative control was tested under the same test conditions.
- During the preparation of the sterility test the environment of the cleanroom was monitored for the presence of micro-organisms.
- The number of positive results was observed by determining visual growth.

5.4.5 Bacteriostasis and Fungistasis test.

After the test of sterility 5 negative samples were each inoculated with 0.1 ml (approximately 100 cfu) of either *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans* or *Aspergillus brasiliensis*. The samples were incubated at 28°C - 32°C. Inoculated samples were checked for visual growth after 2-3 days.

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6 RESULTS

6.1 Bioburden Validation

The product was inoculated after which the recovery of the micro-organism was determined. Recovery was found to be 87.9%. This has resulted in a correction factor of 1.2. This value will be used in all future Bioburden estimations.

Detailed results are listed in the table below.

Table 1. Detailed results of the Bioburden validation.

Recovery:				
	Product No.	***Batch No.***	***Result***	***Unit***
	PharmaHelpBag	220413	68	cfu/unit
	PharmaHelpBag	220413	79	cfu/unit
	PharmaHelpBag	220413	72	cfu/unit
	PharmaHelpBag	220413	78	cfu/unit
	PharmaHelpBag	220413	70	cfu/unit
Inoculation:				
	Staphylococcus aureus ATCC 6538, sample 1		81	cfu/unit Added
	Staphylococcus aureus ATCC 6538, sample 2		86	cfu/unit Added
			84	cfu/unit added (mean value)
	Mean recovery		87.9	%
	Standard deviation recovery		5.8	%
	Recovery factor		1.14	
	Correction factor*		1.20	

** Correction factor = recovery factor + (standard deviation recovery x recovery factor)*

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6.2 Bioburden Estimation

The average overall Bioburden is: 40 cfu/unit.
The average Bioburden of the individual batches is:

- Batch 230413: 34 cfu/unit
- Batch 240413: 58 cfu/unit
- Batch 250413: 28 cfu/unit

These are the results of the number of micro-organisms removed from the product multiplied with the dilution factor (= 2) and the correction factor (= 1.20).

The detailed results of the Bioburden estimation are shown in table 2.

Table 2. Detailed results of the Bioburden estimation.

No.	*Identification sample* *Product No.*	*Batch No.*	*Aerobic bacteria cfu/unit*	*Yeast cfu/unit*	*Moulds cfu/unit*	*Total [1] bioburden cfu/unit*	*Mean value per batch cfu/unit*
No. 1	Pharma help bag	230413	7	< 2	14	22	
No. 2	Pharma help bag	230413	34	< 2	17	50	
No. 3	Pharma help bag	230413	14	< 2	2	17	
No. 4	Pharma help bag	230413	12	< 2	< 2	12	
No. 5	Pharma help bag	230413	46	< 2	17	62	
No. 6	Pharma help bag	230413	24	< 2	7	31	
No. 7	Pharma help bag	230413	50	< 2	26	77	
No. 8	Pharma help bag	230413	17	< 2	17	34	
No. 9	Pharma help bag	230413	12	< 2	7	19	
No. 10	Pharma help bag	230413	14	< 2	2	17	34
No. 11	Pharma help bag	240413	122	< 2	31	154	
No. 12	Pharma help bag	240413	38	< 2	17	55	
No. 13	Pharma help bag	240413	72	< 2	26	98	
No. 14	Pharma help bag	240413	22	< 2	10	31	
No. 15	Pharma help bag	240413	70	< 2	19	89	
No. 16	Pharma help bag	240413	24	< 2	5	29	
No. 17	Pharma help bag	240413	14	< 2	< 2	14	
No. 18	Pharma help bag	240413	14	< 2	< 2	14	
No. 19	Pharma help bag	240413	55	< 2	10	65	
No. 20	Pharma help bag	240413	22	< 2	12	34	58
No. 21	Pharma help bag	250413	12	< 2	2	14	
No. 22	Pharma help bag	250413	14	< 2	2	17	
No. 23	Pharma help bag	250413	12	< 2	14	26	
No. 24	Pharma help bag	250413	7	< 2	17	24	
No. 25	Pharma help bag	250413	17	< 2	22	38	
No. 26	Pharma help bag	250413	14	< 2	2	17	
No. 27	Pharma help bag	250413	24	< 2	10	34	
No. 28	Pharma help bag	250413	29	< 2	17	46	
No. 29	Pharma help bag	250413	36	< 2	2	38	
No. 30	Pharma help bag	250413	17	< 2	12	29	28

[1] Total bioburden = Aerobic bacteria + Yeast + Moulds

Bioburden for verification 40

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6.3 Application of the verification dose

None of the batch averages is ≥2 times the overall Bioburden average. Therefore, the overall Bioburden average of 40 cfu/unit has been used for determining the verification dose.

Using the Verification Dose table in EN-ISO 11137-2, method VD_{MAX}^{25}, it was determined that with a Bioburden of 40 cfu/unit a Verification Dose of 8.7 kGy should be used.

The application of the verification dose was performed at the Synergy Health AST facility in Ede (NL). The actual dose delivered was min. 8.6 kGy and max. 9.1 kGy. This complies with the limit of 8.7 kGy +/- 10% as described in section 4.4. The Certificate of Gamma irradiation is shown in Appendix I.

6.4 Sterility tests

The samples were tested on sterility after application of the verification dose. No positive results have been observed in the test of sterility (thus all samples were sterile). Furthermore, the monitoring of the cleanroom during the preparation of the sterility test and the positive and negative controls complied with the limits.

6.5 Bacteriostasis and Fungistasis test

After the test of sterility, 5 negative samples were inoculated each with a different micro-organism, as described in section 5.4.5. The product did not demonstrate any inhibitory effect on the micro-organisms examined, because growth was confirmed visually for all of the 5 micro-organisms. It can therefore be concluded that the product either does not have antimicrobial activity or that this is sufficiently neutralized.

7 CONCLUSION

The microbiological validation has been successful and the Sterilization Dose of 25 kGy for S.A.L. 10^{-6} has been validated for the PharmaHelpBag of Inpakomed B.V

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APPENDIX I

Certificate of Gamma Irradiation

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Synergy Health Pharma Lab.
Microbiologie Laboratorium Ede
Morsestraat 3
6716 AH EDE

Certificate of Gamma Irradiation

Synergy Health Ede B.V., Morsestraat 3, 6716 AH Ede, the Netherlands, Telephone: +31 (0)318 637476, certifies the following irradiation treatment in accordance with :

* licence number KEW 2012/0265-05, issued by the Dutch Ministry of Economic Affairs, Agriculture and Innovation.
* the national and international Quality Assurance System Standards: ISO 9001:2008 and additionally for CE marked medical devices: EN ISO 13485:2003 and ISO 11137-1:2006 (if proper validated).

The irradiation dose applied is controlled by Synergy Health Ede B.V. The calibration of the dosimeters is carried out by the National Physical Laboratory in the United Kingdom.

Product description:	**Samples packaging**
Customer reference:	**Verification 1305256**
Irradiation facility:	**JS6500 Tote Box Irradiator**
Irradiation dose:	**7,9 kGy Min. 9,5 kGy Max.**
Irradiation date:	**31-5-2013**
Order number:	**20141487**

Synergy Health Ede QA

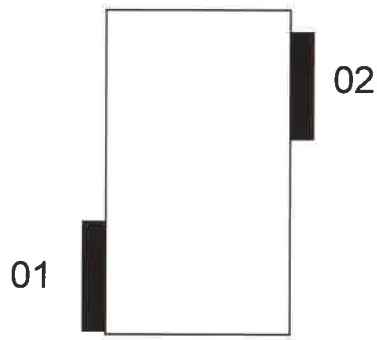
3-06-2013.

ISO11137 VERIFICATION VALIDATION

- Site: Ede
- Machine: Test tube
- Customer: Synergy Health Utrecht B.V.
- Item description: Pharma help bag
- Customer reference: verification 1305256
- Isotron orderno.: **20141487**
- Irradiation date: 31-05-2013

- Requested dose: 8.7 kGy ± 10%
- Dose range: 7.9 kGy – 9.5 kGy

- Dosimeterpositions:



Results:

Dosimeterposition		Measured dose (kGy)
2014148701	01	9.1
	02	8.7
2014148702	01	8.7
	02	8.6

Minimum Dose : 8.6 kGy
Maximum Dose: 9.1 kGy