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Test method for the microbial barrier properties of wrapping material; new approach.

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This study has been performed in order and for the account of the "Hoofdafdeling Geneesmiddelenvoorziening/WVC".

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PREFACE

As a result of deliberation within CEN TC102 wg4 the study described in this report was performed in three independent laboratories according to a mutual agreed protocol.
This report gives the details and the results of the tests done by the Wagner GmbH and the Laboratory for Medicines and Medical Devices (LGM) of the National Institute of Public Health and Environmental Protection (RIVM). The test results from the Laboratoire National d'Essais (LNE) were not received in time to be processed into this document.

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ABSTRACT

The study shows that the new approach, as proposed by CEN TC102 wg4, for the development of a test method for the determination the microbial barrier properties of packaging materials for medical devices does not give the expected advances over the in 1990 presented LGM test method (RIVM-report 919000001, June 1990).

The comments made by CEN TC102 wg4 on the LGM test method seem to be as much applicable on the final pack arrangement described in this report. The method is faster but needs more attention of personnel.

Without the use of two particle counters and active flow controllers the reproducibility proves to be poor. The use of additional equipment is necessary.
The results from the study let to the revision of the study protocol, which can be found in annex 1.

The material qualification obtained by means of a materials test does not provide any relevant information about the expected performance of the material when formed into a final pack. The authors recognize the desirability for a materials test in the production of medical devices but the results show that the design of every type of packaging concept must be validated by means of a final pack test. This does however not exclude the use of a materials test for the purpose of in process control in the field of production. Once both the concept and the forming of the pack is validated a materials test will be very usefull to check whether the quality of the wrapping material is identical to the quality of the materials used in the validated packaging concept.

SAMENVATTING

Dit onderzoek toont aan dat de door de CEN Technische Commissie 102, werkgroep 4 voorgestelde nieuwe aanpak om tot een testmethode voor de bacterie barrière eigenschappen van verpakkingsmaterialen voor medische hulpmiddelen te komen niet de verwachte voordelen over de methode welke door het LGM in 1990 is gepresenteerd (rapport. 919000001).

Het commentaar dat CEN TC102 wg4 heeft op de LGM test methode blijkt ook op de hier beschreven test op de eindverpakking onverminderd van toepassing te zijn. De nieuwe methode is iets sneller, maar verlangt meer personele inzet.

Zonder gebruik te maken van twee particle counters en actieve flowcontrollers blijkt de reproduceerbaarheid matig te zijn. Het gebruik van extra apparatuur is noodzakelijk.
De onderzoeksresultaten zijn verwerkt in een revisie van het onderzoeksprotocol, zie annex 1.

De kwalificatie van verpakkingsmaterialen door middel van een materiaaltest geeft geen relevante informatie over de te verwachten prestatie van het materiaal wanneer het in de praktijk wordt gebruikt. De auteurs erkennen dat een materiaaltest wenselijk is, maar de resultaten van het onderzoek geven aan dat het ontwerp van ieder type verpakkingsconcept middels een 'eindverpakkingstest' gevalideerd moet worden. Dit wil echter niet zeggen dat een materiaal test niet als parameter voor de procesbeheersing gebruikt kan worden. Indien zowel de eindverpakking als de produktie van de eindverpakking gevalideerd zijn kan de materiaaltest zeer wel zinvol zijn om vast te stellen of de kwaliteit van het verpakkingsmateriaal identiek is aan de kwaliteit van de materialen in het gevalideerde concept.

1. INTRODUCTION

The micro biological barrier properties of warpping materials for maedical devices are in general tested by complicated testing methods, using bacterial spores. The major disadvantage of these methods is that they are not general applicable because they are usually based on materials testing. Consequently packaging concepts such as containers or the variety of different wrapping methods cannot be tested.

To overcome these disadvantages the Laboratory for Medicines and Medical Devices developed a physical test method in which final packs can be tested. This method is described in RIVM-report 919000 001, June 1990.
The principle of this test method is that it determines the efficiency in which airborne particles are filtered out of the air passing the barrier formed by the pack. This test has the major advantage over the conventional test methods that it is capabel of testing all air permeable final packs including wrapped instruments trays, containers and pouches after sterilization.

1.1. Comments.

After publication of the report the work of the LGM was evaluated by CEN TC102 wg4 (packaging materials) and a number of comments were given. A summary of the major criticisms is given below.

1.1.1. Hardware influences on the test.

The test results were found to depend on:

- The material of the tubes used for sampling the air.
- The length of the tubes.
- The crookedness (curvature) of the tubes.
- The counters:
  - It was found to be necessary to have both counters sampling the same amount of contaminated air, because the method to sample 100% contaminated air with the outside measuring counter and only 10% mixed with 90% clean air with the inside measuring counter and then "correct" the values manual by multiplying the value of the inner counter by 10 (to obtain comparable figures relating to the same sampled volume again) was found to produce non repeatable results, especially with low contamination levels.
  - Concerning the exactness of the measurement: the used counters comply to the US Federal Standard 209 C. Appendix B of this Standard "Operation of optical particle counters" refers to calibration, system Limitations, inter instrument correlations and the allowed tare: a tare of 10% is allowed for the result when a calibrated counter measures a certain, known contamination.
    This means that two counters measuring one and the same air quality may show the result ±10% of the real value. With the LGM test method this will result in a fault of ±2% on a calculated efficiency of 90%.

1.1.2. Reproducability:

- It was found that a contamination peak outside needs typically 20-30 minutes until

it was fully registrated inside (depending on the material). This means that the calculation of the efficiency is based on wrong figures, as the high outside count is compared to a still low inside count or, later, when the outside count went down again, he is compared to a still high inside count. Therefore the efficiency diagrams are unsteady. For the above reason, a statistical/mathematical interpretation of the results is not possible: it must be judged on a subjective basis, so that it is definitely necessary to define the criterias for interpretation of the figures (as otherwise no reproducable results will be found).

- As a general requirement it is desirable to create and maintain a high level of contamination without rapid changes over several hours, otherwise the results may not be judged correctly.
- When repeated with one and the same final pack (untouched between the different tests), the results were found to be reproduceable within ±4% efficiency (at a total level of appr. 86% for 0,5 um particles).
- In some cases, the judgement of the efficiency was hard to do, as the efficiency showed a breakdown from 90 to 80% for several hours (without external count increase), but went up again to 90 for the remaining hours

1.1.3. Ability of the test to distinguish between good and bad:

- Tests with nonwoven packs, a valve container as well as with a filter container, all first without, later with intentional perforation lead to the same result: no significant decrease in efficiency detectable.

1.1.4. Test duration

- In any case it was necessary to run the test on a pack for several hours. Shorter sampling time led to too much varying results.

1.1.5. Test conditions:

- As a reduction of the sampling flow was found to produce a higher efficiency on the other hand, the question of a realistic airflow should be discussed, as it is a very important parameter, which certainly shall represent a worst-case situation on the one side but should not be too far away from reality on the other side.
- The tubing with an internal diameter of 6 mm already caused a hindrance for particles in sense of keeping them on surfaces due to too low flow speed and therewith avoid registration by the counter. It is likely that the same effect will also occur in a testpack with 1 sqm inner surface; a number of particles will penetrate inside the pack but will not be registrated.

1.2. New approach.

1.2.1. Goal:

To overcome all these problems one of the members of CEN TC102 wg4, Mr. Wagner, suggested a new approch for a physical testing method. This method is developed by an adhoc working group of CEN TC102 wg4, further called the working group.
Independent from the method of registration (microbial or particulate) the question was raised, how a test arrangement for a final pack could look in principle to:

- Allow the pack's exposure to the simulated critical environmental conditions as defined in the standard without influence on the registration unit
- Allow the pack's exposure to the actual environmental conditions (on site conditions for transport, storage) for validation purposes, also without influence on the registration unit
- Avoid all risks of wrong results caused by post-sterilization or postexposure handling of the pack.
- Reduce the costs for the registration and evaluation of the results as far as possible, i.c. avoid special instruments or special, non routine calibration of instruments, reduce procedure time and use only one particle counter.

1.2.2. Principle of the new approach: (Final pack test arrangement)

- A testpack is needed, modified in a way, that there are two (sealed) tube connectors availabe (may possibly be reduced to one combined connector)
- After sterilization, this testpack may be exposed to whatsoever environmental conditions (cool down, particle challange, varying humidity, long term shelf storage, light exposure, simulated or real transport and handling)
- After the exposure phase is finished, the testpack will be invesigated for particle penetration by means of a particle counter. The "air exit" of this counter is connected to the test pack in order to "re-feed" the pack with exactly the same amount of HEPA filtered air which was taken from it (see figure 1, page 5). This allows the use of any model and of any sampling speed without influence on the results. The testpack stays free from differences of pressure, only its enclosed airvolume will be exchanged rapidly and examinated for particles. The sampling may be stoped when no more particles are counted.
- From the outside contamination value and the counted partcles inside the testpack the efficiency of pack can be calculated.

1.2.3. Advantages of the new method.

- The testpack (any size) may be handled freely and can easily be exposed to whatever test arrangements or environmental conditions - without any influence to the registration units.
- Testpacks can be used for on site validation without having the need of an expensive registration unit to be with the pack all the time (shelf life survey: transport and handling)
- For registration, both microbial and particulate methods are possible and acceptable. The concept allows to use nearly any standard equipment, as it is independent of suction speeds (one of the major problems until now), and needs no special calibration routines.
- With standardized "connection units", available in different sizes in order to fit into all possible shapes and volumes of packs, it is easy and unexpensive to test a lot of packs and have the results evaluated later at a central place, which again makes the use of the registration unit cost effective.

2. MATERIALS AND STUDY PROTOCOL

2.1 Materials.

2.1.1. Test chamber big enough to receive 1 STU volume, sealable against internal overpressure of 17,5 KPa, with pressure recording device.
2.1.2. Optical particle counter, capable to count particles > 3um. (Met-One 217A)
2.1.3. Absolute HEPA-filter. (Pall DFA 3001 V002PV 0.2 um)
2.1.4. PE tubing with an internal diameter of 6 mm to connect the counter with the testpack inside the test chamber and a gastight connector on the test chamber.
2.1.5. Suitable sized pressure pump and reduction valve to produce a constant pressure gradient in the range 1,4 - 7 kPa/min from normal atmospheric pressure up to +17,5 kPa.
2.1.6. Suitable apparatus to create and maintain the desired contamination.
2.1.7. Standard instrument tray 480x250x60 mm. (580x245x110mm: volume 0.55 ft^3)
Optional a material holder capable of holding two discs of the sampled material with a diameter of 80 mm.
2.1.8. Suitable connectors to fit the tubing to the test packs.
2.1.9. Dust powder to make the particle challange. (Quarz powder Mikro Dorsilit 110).

2.2. Study protocol

2.2.1 Validate the dust distribution inside the test chamber so that the desired contamination level (10^8/m3) of contaminating particles may be reached and maintained for the duration of the test.
2.2.2 Validate the pressure increase to meet the following requirements:
7 kPa/min x 2.5 min = 17.5 kPa (in case of wrapped packs or material sample testing),
1.4 kPa / min x 12 min = 16.8 kPa (in case of containers),
over normal pressure. The gradient must be constant.
Note: With other package sizes than those 1/2 STU, the corresponding gradient for cool down (and subsequently pressure) needs to be established before.
2.2.3 Install and seal the tube connectors to the test material before forming the final pack. Take care that two connectors are not opposite to each other but have the maximum distance from each other which the package size allows.
For testpacks which are formed of sheets, use the bottom surface of the pack for installation of the connectors.
For rigid containers, install the connectors in half the height, diagonal to each other on the long sides.
2.2.4 When using sheet materials form the testpack by wrapping the instrument tray according to DIN 58953/10 "A" mode.
If material tests are performed, seal a 80 mm diameter sample disc to the standardized holder
2.2.5 Close the tube connectors and sterilize the final pack with the sterilization method for which it is intended to be used.
After sterilization, allow equilibration to normal room air conditions.
2.2.6 Insert the test pack into the test chamber and connect the tubing to the particle

counter outside of the chamber in sense of a "closed circuit" according to figure 1. (The closed circuit contains an absolute filter to avoid multiple registration of particles)

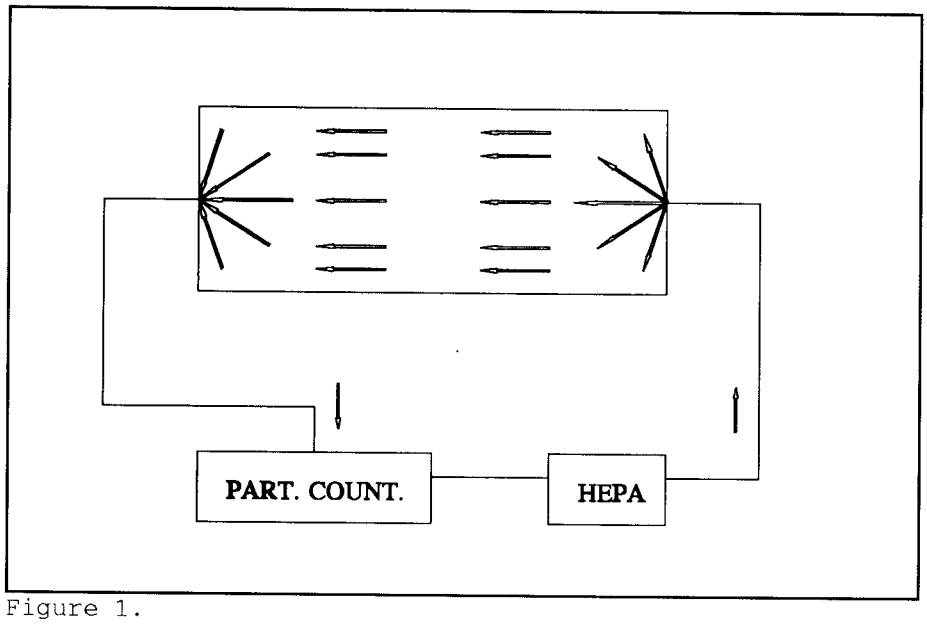

Figure 1.

2.2.7 Start the particle counter in the "absolute particle count mode" >3um in order to remove the initial particle soiling from the test pack.

2.2.8 Create the desired contamination as described by the validation in 2.2.1 without increasing the pressure inside the testchamber.

2.2.9 If the test chamber is not already sealed, seal the test chamber now pressure tight.

2.2.10 Wait for the steady state of the particle counter. The particle counter has reached the steady state when the abosolute increase of the number of counted particles is zero.

Measure the outside contamination and record the value in particles/m^3.

2.2.11 Reset the particle counter in order to start over at zero.

2.2.12 Apply the pressure increase which is required for the inserted type of test pack and record the total amount of particles > 3um which are registered until the registration unit reaches the steady state again.

2.2.13 Calculate the concentration of penetrated particles in part./m^3 by dividing the total no. of registrated particles by the volume (in m^3) of the test pack.

2.2.14 Express the results as an efficiency in percentage ($[conc._{outside} - conc._{inside}]/conc._{outside}$) and as a reduction factor ($conc._{outside}/conc._{inside}$).

2.2.15 If desired, and provided that the chamber contamination still meets the requirements, repeat the measurement by starting again at 2.2.10.

3. RESULTS

At the RIVM the tests were performed in two stages. The results of the tests done in the first stages were discussed in de working group on 15 March 1993 which led to some modifications of the test protocol; stage 2 of the study. At Wagner the the test were performed in one stage. The results from Wagner are presented in tables 4-9.

3.1. Making of the dust challance and validation. (step 7 in the procedure). (RIVM).

3.1.1. Stage 1 (RIVM)

In the first stage of the study the test dust was distributed throughout the test chamber by means of an electrical powered fan. A small open container (6x6x0.5 cm) was attached to the fan with a piece of tape. Approximately two grams of the testpowder was put into the container before every test. The rotation of the fan resulted in distribution of the particles.

To validate the dustdistribution method it was performed seventeen times. The number of particles was counted at intervals; 1, 3, 5, 10, 15, 20 minutes after the fan was switched on. The figures 2 and 3 give a graphical presentation of a typical number of particles/m^3 >3 um in time.

These data show that:

a. When using the same method of distribution, the establishment of the dust in the chamber is more or less reproducible, but not very good.
b. The concentration of the dust is not stable in time but drops rather rapidly; after 10-15 minutes the concentration is less than $10^8/m^3$. The figures show that the halflife of the dustconcentration is not reproducible.
c. The dust is not evenly distibuted throughout the chamber. The concentration at the top of the chamber is 5-10% less than in the middle of the chamber.

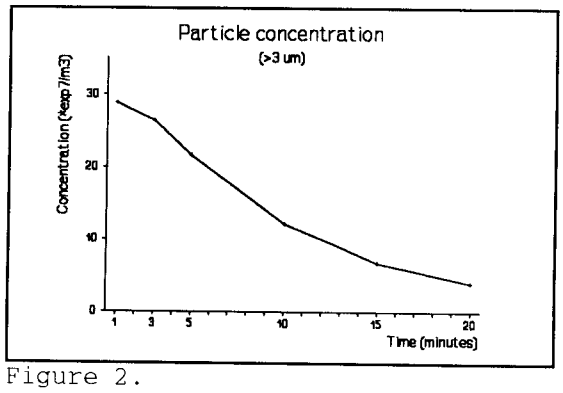

Figure 2.

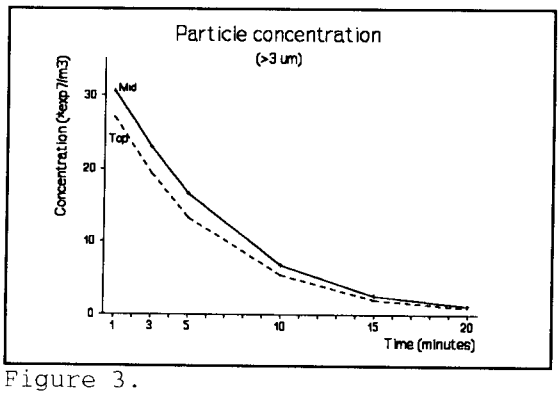

Figure 3.

3.1.2. Stage 2. (RIVM)

In stage two of the study very high levels of contamination were created by blowing pressurised air into a glass measuring cilinder containing a given amount of the dust powder. During earlier attemps to make very high contaminations, the used particle counter could never detect more than $11x10^6$ particles per ft^3.
Since sensor overload is allready indicated at $3x10^6$ particles/ft^3 the sensor is probably saturated at very high contamination levels and is simply not able to measure without a large coincidence error. When the sensor overload is indicated the coincidence error is already more than 5%.

This problem was avoided by mixing one tenth of the flow from the testchamber with nine tenths HEPA-filtered air. In general this gave good results, but could not in all cases prevent sensor overload.

The distribution of the dust with pressurised air gave contamination levels up to $35x10^8/m^3$. The minimum requirement level of at least $10^8/m^3$ was maintained for over two hours.
Figure 4 shows typical concentration levels in time. In comparison with the contamination levels measured during stage 1 the level is much higher and the relative concentration drop in time is lower.

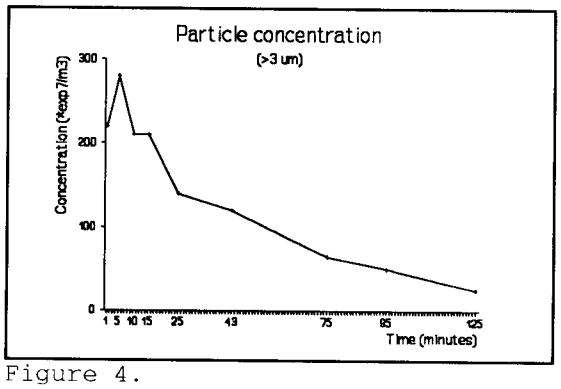

Figure 4.

3.2. Waiting for the steady state. (Step 2.2.2.10 of the study protocol) (RIVM).

When the air is circulated through the counter, HEPA-filter and the test pack the number of particles counted per time should drop to zero. The initial soling inside the testpack is then removed. All particles counted in the next steps of the procedure have penetrated through the barrier.

3.2.1. Stage 1.

After removal of the initial partical soling from the inside of the testpack the fan was started, thus creating the nescessary contamination. At this moment the particle counter was still running.

Although no pressure increase was applied, a certain penetration of particles was detected, starting from the moment the contamination was created.
The amount of penetration was depended on material from which the testpack was assembled. Penetration was high when the instrument tray was wrapped in a porous material such as a normal cotton textile sheet or some of the better quality non wovens. The penetration was minimum when the testpack was a container whereas creped paper with a small porosity showed hardly any penetration.
Figure 5 shows the typical particle count values for textile. From the moment the count reaches its maximum value the graph shows a similar decrease as the concentration of the contamination.

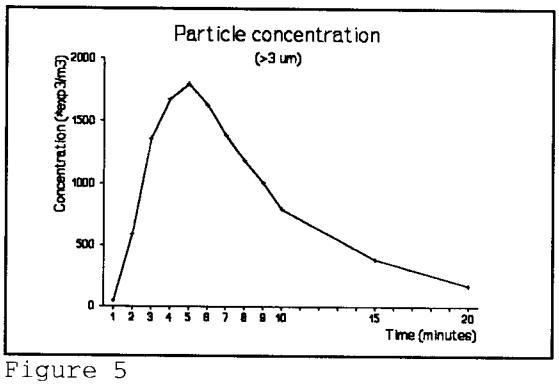

Figure 5

The results in table 1 show that for a number of wrapping materials the 'steady state' as meant in point 2.2.2.10 of the testprotocol is only, if at all, reached after a relative long period. As a consequense of having to wait for a period of more than 10-15 minutes the concentration of particles in the size range of >3um drops under the required number of at least 10^8 per m^3. In those cases that the time waiting for the steady state takes more than 10 minutes it has no use to apply the necessary pressure increase for the actual test of the wrapping concept. The required testing condition is no longer available.

The working group concluded that the use of a fan is not the best way to distribute the particles in the testchamber.

- The maximum concentration of dust particles is too low. Because of the short half live value the required concentration of at least $10^8/m^3$ is only available for a small period of time.
- The pressure-less penetration effect might be caused by the fan. The turbulence in the air could cause some small pressure differences which force an airflow into the pack. If the particles are distributed without using a fan, the noticed pre pressure increase penetration should be far less and the soling inside the testpack should be vanished within 20 minutes.

Material	Highest count per ft^3	Time to zero count	Remaining background count
Textile, single layer	275000	±50 minutes	0
Non wowen(1), single layer	49000	>65 minutes	100-220/ft^3
Non woven(2), single layer	267000	±40 minutes	0
Non woven(3), single layer	3700	>30 minutes	10-30/ft^3
Non woven(4), single layer	1900	±28 minutes	0
Non woven(5), single layer	48000	±30 minutes	0
Paper(1), single layer	20	±10 minutes	0
Paper(2), single layer	540	±30 minutes	0
Container(1)	0	0	0

Table 1. (RIVM)

Material	Highest count per ft^3	Time to zero count	Remaining background count
Textile, single layer	120000	>218 minutes	200-400/ft^3
Textile, double layer	730	>80 minutes	120-140/ft^3
Non wowen(1), single layer	25000	>86 minutes	350-500/ft^3
Non woven(1), double layer	0	0	0
Paper(1), single layer	780	<16 minutes	0
Paper(1), double layer	0	0	0
Container(1)	0	0	0
Container(2)	no data	no data	no data

Table 2. (RIVM)

3.2.2. Stage 2.

Table 2 show the results when the test dust is distributed through the test chamber with the use of pressurised air. The tables 1 and 2 show similary results on the aspect of time to zero count.
Although the dust was distributed without the use of a fan, the 'steady state' as meant in point 2.2.2.10 of the testprotocol is for porous wrapping materials again not reached, even after a long period. Exept for the paper wrap a background count remained.

In contrary to the situation in stage 1 it is not problematic to have to wait for the steady state. After the waiting period the concentration of particles in the size range >3um is still well within the required minimun.
However, the fact that for a number of materials the steady state cannot be reached because a backgrond count remains indicates that the method is not suitable to test all possible packaging concepts.

Note:
At the Wagner laboratory the pre-penetration was also noticable, however only as a small effect. The penetrated particles were removed within a few minutes.

3.3. Testing of the packaging concepts.

Despite the fact that for a number of wrapping materials the testconditions were not according to protocol (backgrond count; no steady state) packaging concepts were in stage 2 (RIVM) of the study actually tested conform point 2.2.2.12 of the test protocol.
Table 3 show the results from these tests.
Wagner found no problems in the performance of the tests.

The calculated efficiencies and reductionfactors have not been corrected for the back-ground count. The concentration of the dust challenge is assumed to be constant in the period between the concentration measurement and the application of the pressure increase, as well as during the period of the pressure increase. This assumption is of course not correct because of the on going drop of the concentration, especially at high concentrations.

Note: The results are not expressed as an absolute number of penetrated particles as is required by prEN 868-1.
In the opinion of the authors this method of expressing the results in an absolute number of particles/m^3/m^2 is not the best one. The use of the surface factor gives that for different shaped packs with identical volumes the number of penetrated particles and therefore the probability of contamination is allowed higher for the pack with the larger surface. The actual probability of contamination is of course higher when the surface is larger, thus this shall not be compensated by introducing a correction factor in the result.
In the here presented test results the number of penetrated particles is given as a concentration. The relation to the concentration on the outside of the pack is expressed as an efficiency in percentage ([$conc._{outside}$ - $conc._{inside}$]/$conc._{outside}$) and as a

reduction factor ($conc._{outside}/conc._{inside}$) for the total pack. When the results are expressed in this manner the packs with a relative large volume to a small surface are in favour, which is in line with the probability of contamination.

3.3.1. Explanation of the tables:

Outside contamination: The concentration (in particles/m^3) on teh outside of the test pack.

# of particles: The total amount of particles (>=3 um) which penetrated the pack as consequence of the pressure increase.

Concentration per m^3: The concentration of particles (>=3 um) inside the pack calculated by relating the # of particles to the volume of the testpack.

Eff %: The filtration effiency in % according to the fomula:
$E\% = 100 \times ([conc._{outside} - conc._{inside}]/conc._{outside})$.

Reduction: The factor between the concentration of penetrated particles and the outside contamination according to the formula:
$Rf = Conc_{outside}/Conc_{inside}$.

sgl: Single layer of wrapping material.

dbl: Double layer of wrapping material.

RIVM

Material	Outside contamination (/m^3)	# of particles	Concentration per m^3	Effectivity (%)	Reduction-factor
Textile, single layer	$7.2x10^8$			-	-
	$2.5x10^8$			-	-
	$1.5x10^8$	9796	$0.629x10^6$	99.58(1	239(1
Textile, double layer	$9.7x10^8$		$1.03x10^6$	99.83	939
Non woven(1), single layer	$5.8x10^8$	106760	$6.8x10^6$	98.8	85
Non woven(1), double layer	$22.3x10^8$	194208	$12.5x10^6$	99.44	179
	$8.3x10^8$	28026	$1.8x10^6$	99.78	461
Paper(1), single layer	$24.5x10^8$	1161295	$7.6x10^7$	96.90	32
Paper(1), double layer	$29x10^8$	1496342	$96.04x10^6$	96.69	30
	$9x10^8$	602844	$38.7x10^6$	95.7	23
	$1.7x10^8$	42358	$2.7x10^6$	98.4	63
Container 1	$35X10^8$	1974298	$1.14x10^8$	96.7	31
	$20x10^8$	486760	$2.8x10^7$	98.6	71
	$3x10^8$	39520	$2.3x10^6$	99.2	131
Container 2	$21x10^8$	12490	$7.2x10^5$	99.97	2905
	$10x10^8$	3620	$2.1x10^5$	99.98	4801
	$2.4x10^8$	1673	$0.96x10^5$	99.96	2490
Container 3	$14x10^8$	89120	$5.16x10^6$	99.6	271
	$6.1x10^8$	20878	$1.21x10^6$	99.8	505
	$1.8x10^8$	5272	$0.3x10^6$	99.8	590
Container 4	$13.7x10^8$	47670	$2.2x10^6$	99.84	623
	$4.7x10^8$	4172	$1.95x10^5$	99.96	2410
Container 5	$11.5x10^8$	1798390	$14.5x10^7$	87.4	8
	$1.8x10^8$	117638	$9.44X10^6$	94.8	19
Container 6	$17.6x10^8$	2838532	$1.54X10^8$	91	11

Table 3. Results from RIVM

1) The efficiency and reduction factor are given without correction for the background count. When the number of penetrated particles is corrected for the back ground count (400/ft^3) the efficiancy is 99.6% and the reductionfactor 250.

Wagner

Material	Test #	outside contamin.	# of particles	concentration per m^3	eff.%	Reduc-tion
Textile sgl.	1	$2,3\ 10^9$	2270	$3,14\ 10^5$	99,986	7324
	1b	$1,7\ 10^8$	644	$8,8\ 10^4$	99,95	1902
Textile sgl.	2	$7,5\ 10^8$	2597	$3,6\ 10^5$	99,951	2049
Textile sgl.	13a	$3,57\ 10^8$	1396	$1,94\ 10^5$	99,946	1841
	13b	$1,57\ 10^8$	320	$4,45\ 10^4$	99,97	3517
Textile dbl.	14a	$8,9\ 10^8$	325	$4,5\ 10^4$	99,995	19724
	14b	$4,04\ 10^8$	113	$1,57\ 10^4$	99,996	25737
	14c	$1,57\ 10^8$	34	$4,7\ 10^3$	99,997	33333

Table 4.

Wagner

Material	test #	outside contamin.	# of particles	concentration per m^3	eff.%	Reduction
Non woven single layer	4a	$2,8\ 10^8$	1500	$2,1\ 10^5$	99,93	1339
Non woven single layer	4b	$6,4\ 10^7$	371	$5,16\ 10^4$	99,92	1238
Non woven single layer	5a	$1,57\ 10^8$	2100	$2,9\ 10^5$	99,81	538
Non woven single layer	5b	$3,5\ 10^7$	565	$7,85\ 10^4$	99,78	446
Non woven single layer	12a	$8,03\ 10^8$	22280	$3,1\ 10^6$	99,61	257
Non woven single layer	12b	$2,42\ 10^8$	7597	$1,06\ 10^6$	99,56	229
Non woven single layer	17a	$8,39\ 10^8$	423	$5,88\ 10^4$	99,993	14250
Non woven single layer	17b	$3,57\ 10^8$	220	$3,05\ 10^4$	99,991	11676
Non woven double layer (with hole)	17e	$3,03\ 10^8$	14500	$2\ 10^6$	99,34	153
Non woven single layer inner of 17	18a	$4,3\ 10^8$	4200	$5,8\ 10^5$	99,86	734
Non woven single layer inner of 17	18b	$9,6\ 10^7$	352	$4,9\ 10^4$	99,95	1957

Table 5.

Wagner

Material	test #	outside contamin.	# of particles	concentration per m^3	eff.%	Reduction
Paper, single layer	7b	$1{,}86\ 10^7$	395	$5{,}5\ 10^4$	99,7	336
Paper, single layer	7d	$2{,}14\ 10^8$	1856	$2{,}58\ 10^5$	99,88	825
Paper single layer	7e	$7{,}85\ 10^7$	968	$1{,}34\ 10^5$	99,83	584
Paper single layer	7h	$4{,}11\ 10^8$	5456	$7{,}6\ 10^5$	99,81	541
Paper single layer	7i	$1{,}43\ 10^8$	1883	$2{,}6\ 10^5$	99,82	547
Paper single layer	15a	$3{,}78\ 10^8$	16380	$2{,}27\ 10^6$	99,40	165
Paper single layer	15b	$1{,}18\ 10^8$	4080	$5{,}66\ 10^5$	99,52	208
Paper single layer (bottom up)	9a	$1{,}36\ 10^8$	8200	$1{,}14\ 10^6$	99,16	119
Paper double layer (bottom up)	9b	$4{,}28\ 10^7$	1780	$2{,}47\ 10^5$	99,4	171

Table 6.

Wagner

Material	test #	outside contamin.	# of particles	concentra-tion per m^3	eff.%	Reduction
Container 2	3a	$6,5\ 10^8$	1723	$8,7\ 10^4$	99,99	7453
Container 2	3b	$4,3\ 10^8$	229	$1,14\ 10^4$	99,997	37570
Container 2	6a	$2,14\ 10^8$	332	$1,9\ 10^4$	99,992	12844
Container 3	29c	$7\ 10^8$	4020	$2,2\ 10^5$	99,97	3468
Container 3	30a	$8\ 10^8$	8500	$4,25\ 10^5$	99,95	1880
Container 4	25a	$5,35\ 10^8$	7270	$3,65\ 10^5$	99,93	1468
Container 4	25c	$1,17\ 10^9$	10966	$5,45\ 10^5$	99,95	2147

Table 7.

Wagner

MATERIAL TESTS : 3 um values

Material	test #	outside contamin.	# of particles	concentra-tion per m^3	eff.%	Reduction
Textile sgl.	3a	$>10^9$	74400	$1{,}14\ 10^8$	88,6	10
Textile sgl.	3b	$8\ 10^8$	45700	$7\ 10^7$	87,9	10
Textile sgl.	4a	$1\ 10^9$	22160	$3{,}4\ 10^7$	96,6	30
Paper sgl.	1a	$5\ 10^8$	99	$1{,}52\ 10^5$	99,97	3290
Paper sgl.	1/2a	$> 10^9$	336	$5{,}16\ 10^5$	99,95	1930
Paper sgl.	1/2b	$5\ 10^8$	83	$1{,}27\ 10^5$	99,97	3918
Pouch paper single	5b	$8\ 10^8$	3890	$6\ 10^6$	99,25	133
Pouch paper single	5/2c	$8\ 10^8$	4120	$6{,}3\ 10^6$	99,20	127
Non woven single	2b	$8\ 10^8$	43420	$6{,}7\ 10^7$	90,0	12
Non woven single	2/2a	$8\ 10^8$	49900	$7{,}67\ 10^7$	90,4	11
Non woven double	6a	$1\ 10^9$	5634	$8{,}66\ 10^6$	99,1	115
Non woven double	6/2a	$8\ 10^8$	4196	$6{,}45\ 10^6$	99,18	124

Table 8.

Wagner

MATERIAL TESTS : 1 um values

Material	test #	outside contamin.	# of particles	concentra-tion perm^3	eff.%	Reduction factor
Textile sgl.	3c	8 10^8	164200	2,5 10^8	68,4	3
Textile dbl.	4c	1,2 10^9	120500	1,85 10^8	84,1	7
Paper sgl.	1b	4,8 10^8	1305	2 106	99,58	240
Paper sgl.	1/1c	5,6 10^8	1430	2,2 10^6	99,61	254
Pouch paper sgl.	5a	1,6 10^9	48500	7,45 10^7	95,3	22
Pouch paper sgl.	5d	6,5 10^8	17730	2,7 10^7	95,8	24
Pouch paper sgl.	5/2b	1,8 10^9	56300	8,65 10^7	95,15	21
Non woven sgl.	2c	8 10^8	199700	3,07 10^8	61,6	2,6
Non woven sgl.	2/2c	5,6 10^8	129200	2,10^8	63,6	2,7
Non woven double	6b	1,2 10^9	38560	5,9 10^7	94,9	20
Non woven double	6/2b	8 10^8	25450	3,9 10^7	95,1	20

Table 9.

4. DISCUSSION.

4.1. Validation of the dustdistribution in the test chamber.

4.1.1. The distribution method with the use of a fan is no longer considered a suitable method. The distribution by means of chattering the dust with pressurised air gave very high concentrations of air borne particles. Validation of this method is however not possible. In practice only part of the dustpowder is actually brought into an air borne state. There is no way in telling which fraction of the powder this will be.

4.1.2. The concentration of air borne particles is not steady in time. Therefore it is not correct to assume that the concentration will be constant in the period between the measurement of the contamination and the start of pressure increase. This argument is also valid for the period in which the pressure is increased, especially during the 12 minutes period when measuring containers. The influenze on the result is not calculated. At high concentrations the fault is also larger due to the fact that the absolute drop in concentration per time unit is larger than at low concentrations.

Both 4.1.1. and 4.1.2. point out that it is nescessary to monitor the concentration of airborne particles with a second counter at the start of and during the pressure increase.

4.1.3. When measuring the concentration sensor overload of the counter must be prevented. In the overload situation the accuracy of the counter is smaller than 5%, which is unacceptable. Mixing 10% of the contaminated air with clean air is not in all cases sufficient to prevent sensor overload.
To mimize the measuring fault the mixing of the airflows should must be done with the use of accurate flowcontrollers. For each percentage error in the flowrate a fault of one percent is introduced in the particle concentration of contaminated air. Both the total flow as the flow of clean air must be controled.

4.2. Suitability of the test for various package concepts.

Packs which are made of porous materials show particle penetration without the application of a positive pressure gradient. The effect is both dependent on the porosity as well off the barrier properties. The results in table 1 and 2 show that through the non woven material nr.1 in comparison to paper nr.1 considerable more particles penetrate. The efficiency of the non woven is however much better than the efficiency of the paper wrap (table 3).

The explanation that the fan, as used in stage 1 of the study, is the cause of the particle penetration before the application of the positive pressure gradient is not valid. Also without the use of a fan the particle penetration is considerable. Therefore the properties of the different materials must be taken into consideration when explaning the phenomon.

Although the amount of air which is taken from the testpack is exactly replaced by HEPA-filtered air this does not guarantee that there is absolutely no interaction between the inside of the pack and the environment. It is very likely that depending on the porosity of

the material, part of the air which is sucked from the testpack is coming through the wrapping material. The wrapping is removing a large part of the contamination but since no wrapping material is an absolute filter "some" particles will penetrate. Due to the high contamination "some" particles may in fact be a lot of particles!

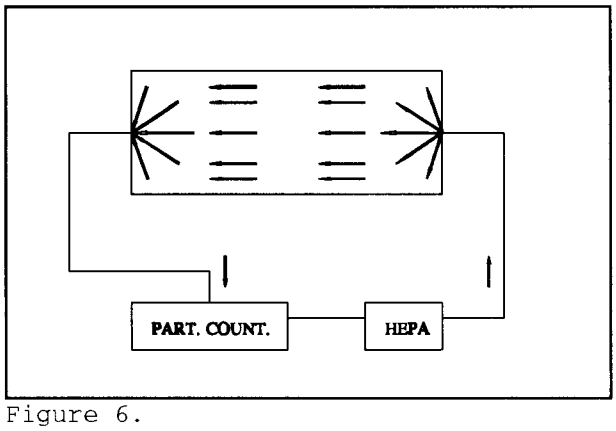

Figure 6.

Ideal situation.
The pack is non permeable, all the HEPA filtered air which is blown into the pack will reach the particle counter. There is no influence from the environment.
Instrument containers come close to this ideal situation.

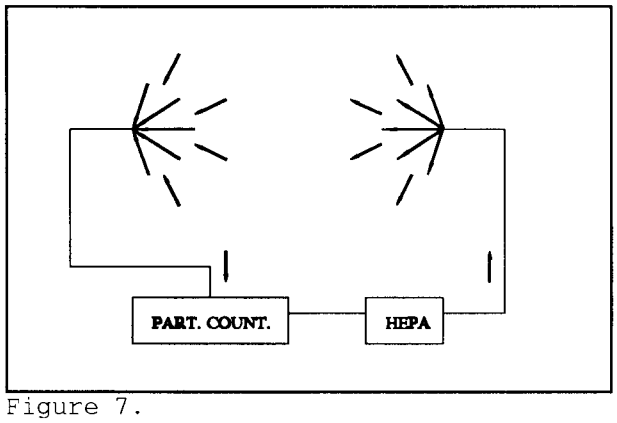

Figure 7.

No pack situation.
The air that is pumped into the particle counter has no relation at all with the HEPA filtered air which is blown from the opposite direction. The contamination of the airflow with particles from the environment is maximum.

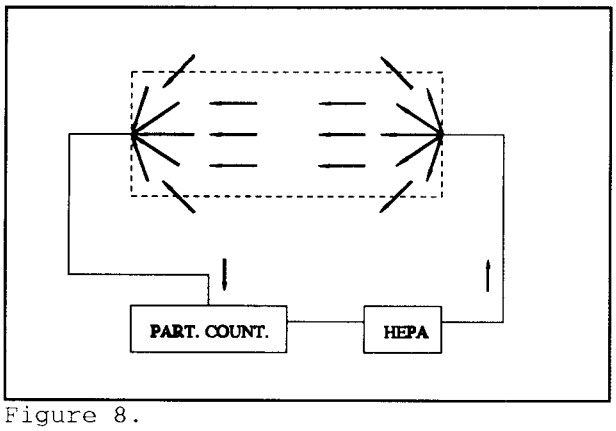

Figure 8.

Porous pack.
Since the wrapping material is porous, part of the HEPA filtered air flow will leak out of the pack. Consequently part of the air flow to the particle counter will come from outside the pack. Although this air flow is very small, it will be contaminated with particles.
The influence by the environment will therefore be dependent on both the porosity of the material which determines the amount of "leakage" from the pack as well as on the barrier properties which determine the fraction of the contamination to move through the material.

For a number of packaging concepts this penetration effect leads to a back ground count (see table 2). This means that it is not possible to make the total amount of air in the pack free from particles. The total particle count after the application of the positive pressure gradient might be corrected for this background count under the condition that it is a constant value in time. Since the particle penetration due to "air leakage" is dependent on the outside concentration of the particle contamination, which is not a constant value, this is not to be expected. An uncertain factor remains; did the counted particles come into the pack as a result of the pressure gradient or as an effect of the leakage phenoman?

The authors suspect that this effect is strongly influenced by the load of particles which are sedimented on the pack's surfaces. This suspicion is given by the fact that measure of the effect depends on the position of the testpack in the testchamber (See figure 9). When one of the larger surfaces of the pack is facing upwards (RIVM) the effect is by far worse than in the situation when one of the smaller surfaces is facing up (Wagner). Also during the pressure increase the effect of the position in the testchamber is noticable.
In case of less porous packs (paper, double layers, containers), this effect is not given when the pack is positioned in a way that one of the smaller surfaces is facing upwards. The test may be performed within appr. 30 minutes. The revised study protocol in annex 1 specifies the position of the testpack.

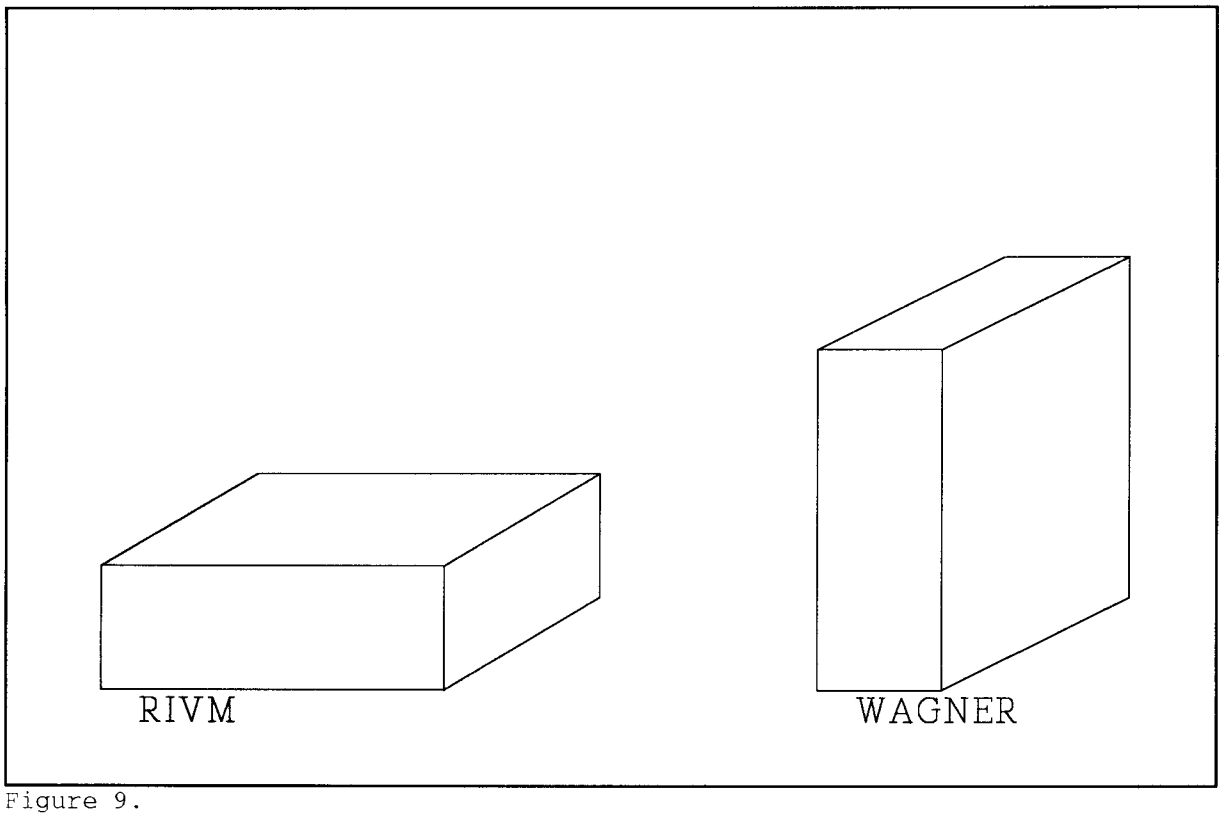

Figure 9.

4.3. Results versus requirements.

The desired reduction factor of 10^5 as laid down in EN 868-1 was with the present test circumstances (see clause 2) not reached by any of the tested packs when tested with particles >= 3 um of the Mikrodosilit 1-10 um range distribution.

The material test (tables 8 and 9) show that for smaller particle sizes (1 um) there is again appr. 1 decade less reduction factor.

The authors expect that the requirements can be met by changing the test parameter "duration of pressure increase" to a smaller value e.g. 1 minute, which corrolates to the first minute of cool down after opening of the sterilizer. This is in line with the first RIVM study.

4.4. Reproducibilty.

4.4.1. Inter laboratory reproducibility

When a single pack is tested several times after another the results are not reproducibel. This is noted in both laboratories although the results from Wagner are considerable better.
The following factors may lead to inaccuracies in the final results. Most of these factors were not recognized at the time the study protocol was described.

a. To measure the concentration of the particle challenge accurately the airflow from the testchamber must be diluted. Accurate flow controllers must be used.
b. The accuaracy of the used particle counter is dependent on the concentration. At high particle concentration levels as used in the test chamber the is not accurate even with dilution of the flow.
c. It is not easy to make a constant positive pressure gradient without the help of a flow controller.
d. The flow through the particle counter and the testpack must be controlled to a value which is the same for all kinds of test packs. This to assure the accuracy of the particle counter.

4.4.2. Intra laboratory reproducibility

The results obtained by the RIVM and Wagner differ a lot from each other. The authors suspect a number of reasons for these differences.

a. Calculation of the outside contamination. Since the measurement of the outside contamination made it necessary to dilute the airflow from the testchamber. The dilution introduces a uncertainty in the contamination calculation because of the mistake in the flowrates. The resistance in the flowmeter will decrease the flow through the particle counter whereby the calibration of the counter is no longer valid.
   The RIVM used the same particle counter for both outside and inside contamination. Wagner was able to use seperate counters.
b. The position of the test pack in the test chamber. This detail was not mentioned in the testprotocol.

c. The connectors used on the test pack. The RIVM used simple "see through" connectors; the flow rate nor the direction of the flow was altered by the connector. Wagner used "snap" connectors. In this kind of connectors the flow is forced into a curved path was might filter out part of the particles.
d. The way the pressure was increased. The RIVM did not have means to make a constant pressure gradient, especially during the 12 minutes period, wheras Wagner used a needle valve.
e. The forming of the testpack. Since both RIVM and Wagner found out that the DIN A method was not usefull an alternative test method was used. RIVM used a non standardized method, while Wagner used the DIN B method.
f. Sizes of the test packs. The size of the RIVM test pack was twice the size of the Wagner test pack.

4.5. Total pack test versus materials test

4.5.1. The results of the final pack test and the material sample test show:
a. That for materials of poor drapebility (like paper) the final pack test approach tests the success of pack forming rather then the material. For example single layer paper is performing better in the materials test than in the final pack test.
b. That vice versa, materials with good drapebility perform better in the final pack test than in the matrial sample test.

4.5.2. The final pack test is capable of detecting package problems which are independent of the material's barrier properties. This is confirmed by the following facts:
a. When a pack is tested more then once without being handeled inbetween, the test results are more or less reproducable.
When a new pack is formed (of the same material batch), the results of this one are not directly comparable.
b. Both the RIVM and Wagner found that it was not possible to form a good single pack when using the DIN 58953/10 Type A diagonal method. Reduction factors larger than 10 could not be reached.
c. When properly formed packs are intentionally perforrated (needle hole), the reduction factor breaks down nearly two decades (see table 5 test 17 b to e)

4.6. Particle counter used.

The Met-One 217 particle counter is not designed to measure high levels of particle levels. At levels higher than $3x10^6/ft^3$ sensor overflow is indicated and the error is larger then 5%. The accuracy of the particle counter depends also on the flow of air through the sensor. The sensitivity of the sensor is calibrated at a flow level of 0.1 ft^3/minute. When the test pack and HEPA filter are connected the flow is reduced. Under those conditions the calibration of the counter is no longer valid.
The flow should be kept to a constant value in an active way by means of a mass flow controller and the particle counter should be calibrated at this flow.

4.7. Health risk.

The quartz powder used is merely siliciumdioxid. This material is known for the health risk it creates when distributed in air. It may lead to serious lung diseases (silicoses). The use of this test powder is therefore not recommandable. Other materials can be used, such as the powder used for surgical gloves.

5. CONCLUSIONS

5.1. Performance of the test.

The goal of the new approach was to make a test method which is both easy and fast to perform without the need for much sophisticated equipment. In the practical application of the proposed method a number of problems arose.

5.1. It is not possible to make a particle challenge with a constant concentration in time. Therefore it is necessary to constant monitor the concentration of particles. The use of a second particle counter is unavoidable.

5.2. To measure the concentration of the particle challenge accurately the airflow from the testchamber must be diluted. Accurate flow controllers must be used.

5.3. Even with dilution the concentration of particles is so high that soling of the sensor and internal tubing of the partcile counter is unavoidable. Regular maintenance of the system is necessary.

5.4 The used particle counter is not very accurate at high particle concentration levels. The use of this kind of particle counter is therefore not recommendable.

5.5. The testmethode cannot be used for packs which are made of materials with a high porosity value. In order to perform step 2.2.2.7 of the study protocol (waiting for the steady state) in a short time, the position of the testpack much be such that the surface of packs constructed out of materials with a high porosity value pointing upwards is as small as possible. This fact does however not implicate that these kind of materials are bad microbial barriers. Table 2 and 3 show that non woven(1) although being a more porous material than paper(1) has the better barrier properties of the two.

5.6. It is not easy to make a constant positive pressure gradient without the help of a flow controller.

5.7. The flow through the particle counter and the testpack must be controlled to a value which is the same for all kinds of test packs. This to assure the accuracy of the particle counter.

5.8. Table 3 shows that the reproducibility is poor. However, the results presented in this report come from tests which have been performed without the use of a second particle counter and the necessary flow controllers.

5.9. The method is faster than the original LGM test method. On the other hand it needs more attention of personnel, whereas the LGM methode can be performed completely automatic.

5.10 The test dust used presents a health hazard. The maximum allowable concentration is only 0.15 mg/m^3. The creation of the particle challenge by blowing pressurised air creates a dust cloud which partly escapes out of the test chamber into the environment. The MAC value is likely to be exceeded.

5.2. Results

5.2.1. The results from the material sample tests do not give any relevant information about the performance as a barrier when it is formed into a final pack. Depending on the parameters such as drapebility and wrapping method the barrier of the final pack may be better or worse than in the material sample test. The results from the

material sample test can therefore not be translated into usefull information about the final pack performance. The authors recognize the desirability for a materials test in the production of medical devices but the results show that the design of every type of packaging concept must be validated by means of a final pack test. This does however not exclude the use of a materials test for the purpose of in process control in the field of production. Once both the concept and the forming of the pack is validated a materials test will be very usefull to check whether the quality of the wrapping material is identical to the quality of the materials used in the validated packaging concept.

Annex 1.

Revised MATERIALS AND STUDY PROTOCOL

1. Materials.

1.1. Test chamber big enough to receive 1 STU volume, sealable against internal overpressure of 17,5 KPa, with pressure recording device.
1.2. Two optical particle counters, capable to count particles > 3um.
1.3. Absolute HEPA-filter.
1.4. PE tubing with an internal diameter of 6 mm to connect the counter with the testpack inside the test chamber and a gastight connector on the test chamber. Connectors shall not give any noticeable resistance to air or particle flow.
1.5. Suitable sized pressure pump and active flow controller to produce a constant pressure gradient in the range 1,4 - 7 kPa/min from normal atmospheric pressure up to +17,5 kPa.
1.6. Suitable apparatus to create and maintain the desired contamination.
1.7. Standard instrument tray 480x250x60 mm.
Optional a material holder capable of holding two discs of the sampled material with a diameter of 80 mm.
1.8. Suitable connectors to fit the tubing to the test packs. Connectors shall not give any noticeable resistance to air or particle flow.
1.9. Dust powder to make the particle challange.
1.10. Three active flow controllers working in the range of the flow produced by the air pumps in the particle counters.

2. Study protocol

2.1. Validate the dust distribution inside the test chamber so that the desired contamination level (10^8/m3) of contaminating particles may be reached and maintained for the duration of the test. To prevent overload of the partclecounter the flow from the test chamber shall be diluted at least 1 to 10 with HEPA filtered air. The dilution shall be performed using two active flow controllers, conrolling the amount of HEPA filtered air and the total flow through the counter. The total flow shall be identical to the flow at which the counter is calibrated.
2.2. Adjust the flow from the compressor to give a pressure increase of
7 kPa/min x 2.5 min = 17.5 kPa (in case of wrapped packs or material sample testing),
1.4 kPa / min x 12 min = 16.8 kPa (in case of containers),
over normal pressure. The flow must be constant.
Note: With other package sizes than those 1/2 STU, the corresponding gradient for cool down (and subsequently pressure) needs to be established before.
2.3. Install and seal the tube connectors to the test material before forming the final pack. Take care that two connectors are not opposite to each other but have the maximum distance from each other which the package size allows.
For testpacks which are formed of sheets, use the bottom surface of the pack for installation of the connectors.

For rigid containers, install the connectors in half the height, diagonal to each other on the long sides.

2.4. When using sheet materials form the testpack by wrapping the instrument tray according to DIN 58953/10 "B" mode.
If material tests are performed, seal a 80 mm diameter sample disc to the standardized holder

2.5. Close the tube connectors and sterilize the final pack with the sterilization method for which it is intended to be used.
After sterilization, allow equilibration to normal room air conditions.

2.6. Insert the test pack into the test chamber and connect the tubing to the particle counter outside of the chamber in sense of a "closed circuit". Make sure that the pack is put on one of the smallest surfaces. Containers are put in the normal position.
The closed circuit contains the particle counter, an absolute filter to avoid multiple registration of particles and an active flow controller to keep the flow through the particle counter at the value at which the counter is calibrated.

2.7. Start the particle counter in the "absolute particle count mode" >3um in order to remove the initial particle soiling from the test pack.

2.8. Create the desired contamination as described by the validation in 2.1 without increasing the pressure inside the testchamber.

2.9. If the test chamber is not already sealed, seal the test chamber now pressure tight.

2.10. Wait for the steady state of the particle counter. The particle counter has reached the steady state when the abosolute increase of the number of counted particles is zero.
Measure the outside contamination and record the value in particles/m^3.

2.11. Reset the particle counter in order to start over at zero.

2.12. Apply the pressure increase which is required for the inserted type of test pack and record the total amount of particles > 3um which are registered until the registration unit reaches the steady state again.

2.13. Calculate the concentration of penetrated particles in part./m^3 by dividing the total no. of registrated particles by the volume (in m^3) of the test pack.

2.14. Express the results as an efficiency in percentage ($[conc._{outside} - conc._{inside}]/conc._{outside}$) and as a reduction factor ($conc._{outside}/conc._{inside}$).

2.15. If desired, and provided that the chamber contamination still meets the requirements, repeat the measurement by starting again at 2.10.