

ASSESSMENT OF A CAR'S FILTER EFFICIENCY TOWARDS MICRO-ORGANISMS

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RECORDS

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1.OBJECT AND FIELD OF APPLICATION

The purpose of this method is to describe a test for measuring the efficiency of filters towards micro-organisms. This method describes the quantitative analysis of micro-organisms on a surface.

A list of selected micro-organisms, based on vehicle contamination is defined.

The device must control the ambient temperature (volume, T°, HR).

The efficiency of the system is evaluated by the quantity of micro-organisms collected on the surface.

2.DOCUMENTARY REFERENTIAL

2.1.NORMS

2.1.1.PSA INTERNAL NORMS

[A10 0156](#) TEST OR MEASUREMENT REPORTS - WRITING

2.1.2.EXTERNAL NORMS

NF-EN 1276 CHEMICAL DISINFECTANTS AND ANTISEPTICS - QUANTITATIVE SUSPENSION TEST FOR THE EVALUATION OF BACTERICIDAL ACTIVITY OF CHEMICAL DISINFECTANTS AND ANTISEPTICS USED IN FOOD, INDUSTRIAL, DOMESTIC AND INSTITUTIONAL AREAS - TEST METHOD AND REQUIREMENTS

2.2.REGULATIONS

Not applicable.

2.3.OTHER DOCUMENTS

Not applicable.

3.TERMINOLOGY

A dictionary (glossary) of the main terms and their definitions used within the activities of the R&D can be consulted in-house via the [DESP](#) or by clicking on the following link: [SCPO_MGPJ07_0116](#).

3.1.DEFINITIONS

BSC	Security device: guaranty the non contamination of the media used and of the persons.
Filter	Device to be tested, micro-organisms trapping.
Media MA or MEA	Culture medium on agar for mould growth.
Media PCA	Culture medium on agar for bacteria growth.
Test Bed	Device in Plexiglas with defined volume with controlled introduction of micro-organisms.

3.2.ACRONYMS

BSC	B iological S afety C abinet
CFU	C olony F orming U nit
HE	H igh E fficiency
MA or MEA	M alt A gar or M alt E xtract A gar : culture media for mould
PCA	P late C ount A gar : culture media for bacteria

4.TEST DESCRIPTION

The principle is to quantify the amount of micro-organisms trapped by a filter in a hermetic and specific test bed. The filter to be tested is introduced in a Plexiglas specific device. A specific microbiologic solution is introduced into

the device during a limited time. After the diffusion time, the filter is collected and crushed in order to collect the micro-organisms trapped into the filter.

The device is equipped with a collecting high efficiency (HE) filter (PSA reference: 1616959180) to collect the micro-organisms not stopped by the tested filter. After the test, the amount of micro-organisms trapped by the HE filter is measured following the same procedure than for the tested filter.

The efficiency of the filter is calculated seeing the concentrations found on the test filter and the collecting filter.

5.EQUIPEMENT

5.1.TEST BED

The testbed is a Plexiglas Cabinet. It is separated in 3 compartments.

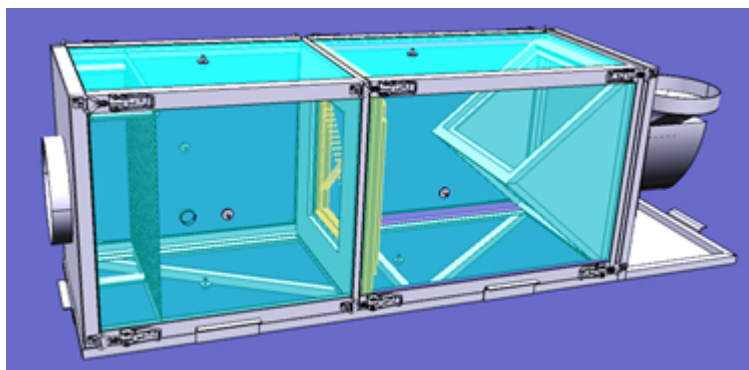
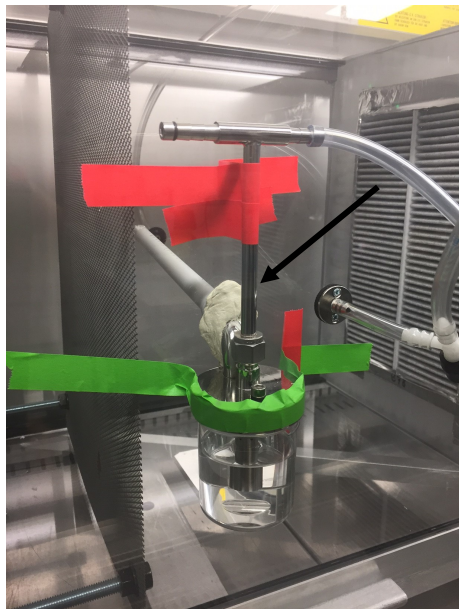


Figure 1: CAD Computer aided design



Microorganisms diffusion
with an air compressor

Figure 2: Microorganism diffusion system – compartment 1



Figure 3: Middle compartment with tested filter.

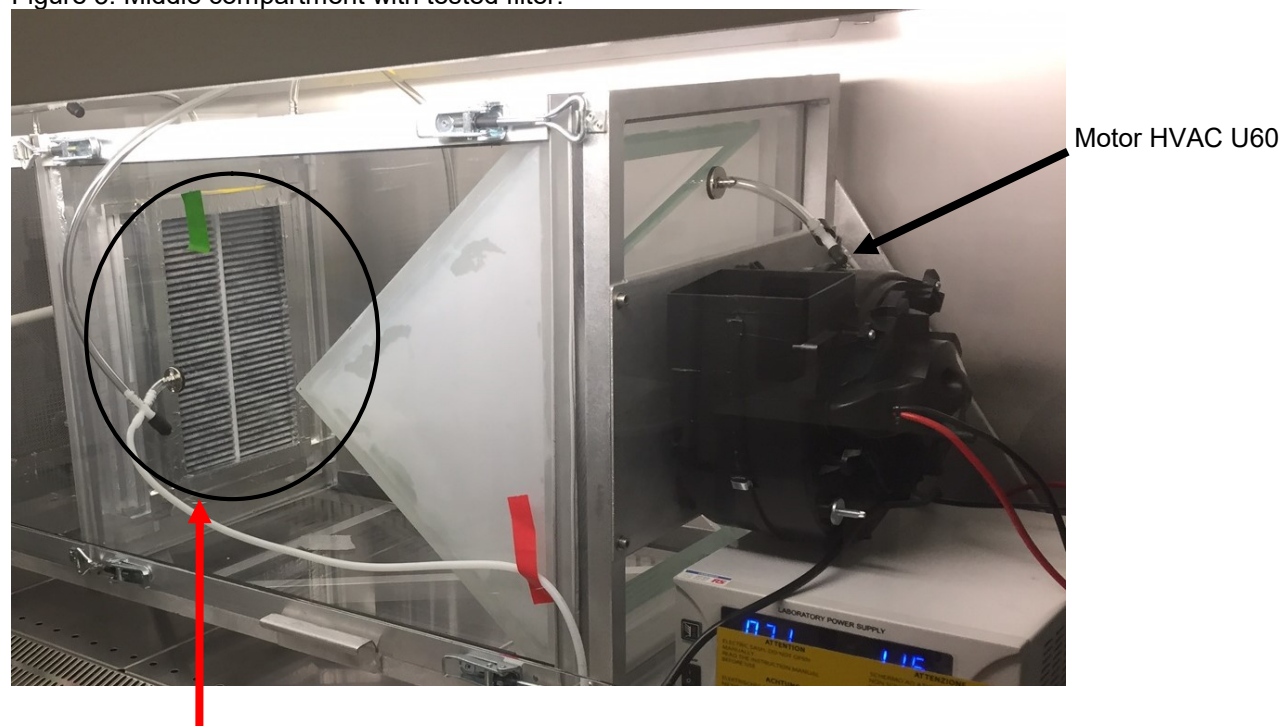
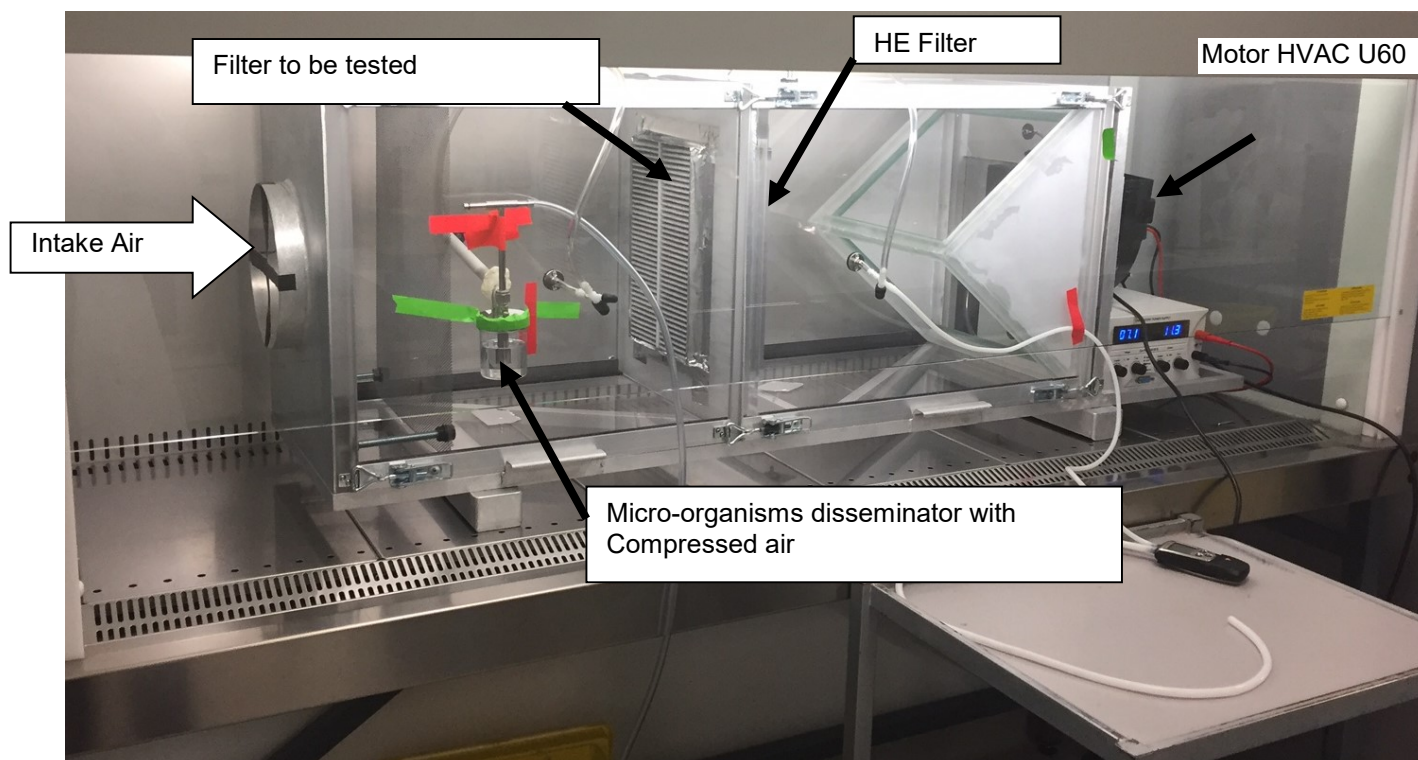


Figure 4: 3rd compartment – collecting HE filter

The testbed is placed in a Biological Safety Cabinet (type 2).



This specific testbed, developed by PSA, is available in Conidia laboratory.

Contact : Sébastien Vacher (sebastien.vacher@conidia.fr).

The technical nomenclature for the testbed are available at PSA : **DQI/DRIA/ACBI/THQA (Denis Dumur)**

5.2 SPECIFIC EQUIPEMENT FOR MICROBIOLOGIC TESTS (SEE CHAPTER 4)

- Biological Savety Cabinet.
- Thermoregulated incubator.
- Autoclave.
- Agitator (Vortex).
- Mixer.
- Precision balance 0,001 g.
- Microscope.
- Spectrophotometer (or colorimeter).
- Propette (1000 µl, 500 µl, 100 µl).
- HE filter (PSA reference 1616959180).

6.MICRO-ORGANSIMS REPRESENTATIVENESS

The micro-organisms have to be characteristic of the media tested. The vehicle interior micro-organisms have been characterized by PSA.

The selection criteria must be mentioned into the test report [A10 0156](#).

In any doubt contact PSA [*DQI/DCHM/PMXP* or *DQI/DRIA/ACBI/ADTH*].

6.1. QUANTIFICATION

See preparation of the micro-organisms mixture (chapter 7.2.4)

6.2. PREPARATION DEVICE AND CULTURE MEDIA

- Graduated pipette (10 ml, 2 ml).
- Petri dish 90 mm diameter.
- Glass beads.
- Sterile vials (100 ml).
- Swabs+ recovery liquid.
- Agar media.
- Recovery liquid.
- Water.
- Crusher: Bagmixer 400 interscience.

The culture media can be:

- Agar PCA for bacteria.
- Agar MA for mould.

6.1. LABORATORY SAFETY EQUIPEMENT

Personal Protection Equipment :

- Safety glasses.
- Lab Coat.
- Chemistry gloves.

7. TEST PROCEDURE

7.1. CLEANING OF THE TESTBED

Before every test the surfaces of the test bed should be cleaned with an alcohol solution (70%) (single use tissue).
The diffusion system has to be cleaned with the same solution.

7.2.PREPARATION OF CULTURE MEDIA

7.2.1.RECOVERY LIQUID

Composition:

Tryptone, pancreatic enzyme digests of caseine	1,0 g
Sodium Chloride	8,5 g
Water	1L

Mix the different compound and introduce it into an autoclave (sterilization).

7.2.2.PCA MEDIA

Composition

Peptone	5,0 g
Yeast extract	2,5 g
Glucose.....	2,0 g
Agar	15,0 g
Water	qsp 1L

Mix the different compound and put into an autoclave. pH must be $7,2 \pm 0,2$.

Flow 15-20 ml into the Petri dish of 90 mm diameter or keep the solution bottle at 65 °C approximately.

7.2.3.MA MEDIA

Composition

Malt extract	30,0 g
Agar	15,0 g
Water	qsp 1L

Mix the different compound and put into an autoclave. Le pH must be $5,6 \pm 0,2$.

Flow 15-20 ml into the Petri dish of 90 mm diameter or keep the solution bottle at 65 °C approximately

7.2.4.PREPARATION OF THE MICROBIOLOGIC SOLUTIONS

Different micro-organisms have been selected based on standards and on field analysis of the vehicle interior (2014 vehicles micro-biologic tests).

- *Penicillium brevicompactum*.
- *Aspergillus niger*.
- *Staphylococcus aureus*.
- *Pseudomonas aeruginosa* or *Escherichia coli*.

Two mixtures (one for bacteria and one for moulds are prepared (concentration from 10^7 for moulds and 10^8 CFU/ml for bacteria. Before essay, the two solutions are mixed in the same volume proportion.

7.2.5.FILTER PREPARATION

No specific treatment of the filter.

7.2.6.TEST INTO THE TESTBED

The filters are introduced into the test bed: into the specific area. The filters are maintained with aluminium tape all around the support. To increase the link, a double-sided tape is applied between the same filters (set of test filter and set of recovery filter).

7.2.7.MICROORGANISMS QUANTIFICATION

Microbiologic sampling procedure:

- Each filter (set of 2) are cut into two samples.
- Cut the samples into small piece.
- Put the pieces into a sterile bag with 250 ml of recovery liquid (volume to be adapted if needed).
- Mix during 2 X 3 minutes.

The liquid is analysed and the germs quantified

- Dilution range with a factor 10 (adapted for the expected amount).
- Deposit 1 ml of each dilution (in double) on a empty Petri dish.
- Add 15-20 ml of under cooling media (at 40-45 °C approximately).
- Homogenize with a circle movement.
- Note: to increase the amount of moulds on the recovery tests, for each sample, prepare 10 Petri dishes.

7.2.8.INCUBATION AND COUNT

The temperature and the time depend on the micro-organisms:

- For bacteria: 37 °C during 2 days.
- For Mould: 25 °C during 5 days.

After the incubation phase a count of micro-organisms is performed.

8.RESULTS

Quantity of bacteria or moulds on tested filter and collecting filter

$$Q = \left[\frac{n_1 + n_2}{2 \times v \times D} \times V_T \right] + [...] + \dots$$

Q = Quantity on filter (CFU)

n = Number of colonies on Petri dish (dilution choice)

v = Deposited volume on dish (1 ml) volume

D = Dilution factor

V_T = Total Volume (250 ml)

Efficiency formula (log):

The tested filter efficiency is calculated with collected microorganisms in the tested filter and in the collecting filter which is placed downstream of the tested filter.

$$E = \log [(Q_{\text{collecting}} + Q_{\text{tested}})/Q_{\text{collecting}}]$$

E: efficiency - log

Q_{collecting}: Quantity of microorganisms on the collecting filter with the tested filter upstream

Q_{tested}: Quantity of microorganisms on the collecting filter with the tested filter upstream

Efficiency formula (percentage %):

The tested filter efficiency is calculated with collected microorganisms in the tested filter and in the collecting filter which is placed downstream of the tested filter.

$$E = [Q_{\text{tested}}/(Q_{\text{collecting}} + Q_{\text{tested}})] \times 100$$

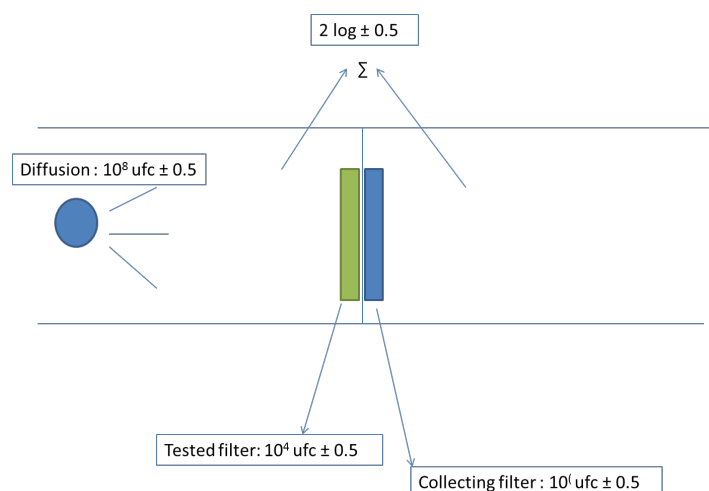
E: efficiency - %

Q_{collecting}: Quantity of microorganisms on the collecting filter with the tested filter upstream

Q_{tested}: Quantity of microorganisms on the collecting filter with the tested filter upstream

The results are expressed in Colony Forming Units (CFU) /filter

The ratio of the microorganisms on the tested filter HE defines the rate expressed in logarithmic unit.



In reference of the NF-EN 1276 standard, if a result is different from the others (for example: $15 \text{ UFC} < \text{exploitable result} < 300 \text{ UFC}$), the measure is considered to be useless.

We will define on another hand for our test method the following conditions:

- Tests for which the quantity of bacteria collected on the tested filter or on the collecting filter are different by more or less one power of 10 compared to the average of 2 closest test results are considered as useless.
- The acceptable difference between 2 tests results from a same filter reference is maximum 1 log for bacteria and fungus.

Application example:

Test number	Quantity on tested filter (UFC)	Quantity on collecting filter (UFC)	Total bacteria on filters (UFC)	Efficiency LOG (bacteria)	Efficiency % (bacteria)
1	3,11E+06	4,36E+04	3,15E+06	1,86	98,62%
2	1,53E+07	1,37E+06	1,67E+07	1,09	91,78%
3	1,94E+07	1,06E+06	2,05E+07	1,29	94,82%

First line in red is different by more than a power of 10 compared to the average of the two other tests (1 and 2).

Moreover if the difference between the total bacteria on the 2 filters (collecting and tested) and the quantity of microorganisms diffused is more than 2 log, the test is also considered as useless.

9.TEST REPORT

See [A10 0156](#) standard.

The test report shall include:

- The concentration results,
- The standard used,
- The name and the address of the laboratory,
- The name of the technician,
- The device reference,
- The test date,
- The incidents or unforeseen events shall be reported.