

## Efficiency study of a surface disinfectant

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### **Sample identification and sampling conditions**

- Analysis N° : 21.03.847 (7 pages)
- Company or / and client No. : P-Divers
- Description of the tests : **Study of the effectiveness of a disinfectant on a work surface**
- Preparation of two bacterial suspensions and deposition on treated and untreated surfaces at different times after spraying the disinfectant.  
Total of 46 surface samplings
- Collection procedure : Sampling with a contact agar applicator (500g pressure)
- Culture medium : CT3P (count-tact with neutralizing agents) Biomérieux Lot : 1008520100  
Exp :02.08.2021
- Date of analysis : 31.03.2021
- Protocol : A melamine-coated wooden work surface covered with a decorative resin is separated into two parts, one area for the study of the disinfectant with the *Staphylococcus aureus* strain (area A), the other with the *Klebsiella pneumoniae* strain (area B).
- 1st step : Determination of areas A and B which will be used for testing with the two bacterial strains. These are subdivided into 100 cm<sup>2</sup> areas noted T (control areas without product), and E (test areas with spraying of disinfectant product according to the manufacturer's recommendations). These surfaces are duplicated 5 times on each of the area A and B in order to carry out the tests at times 0, 6 hours, 24 hours, 48 hours, 72 hours.
- 2nd step : Determination of the "natural" contamination before the tests on both surfaces A and B in viable microorganisms (bacteria, yeasts and molds), 6 surfaces sampled in total on areas A and B.
- 3rd step : Spraying of the disinfectant product under test on the defined "Test" surfaces (noted surface E of 100 cm<sup>2</sup>) of each of the area A and B.
- 4th step : Fresh preparation (on each test day) of two bacterial suspensions of *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniae* ATCC 4352 to obtain a final theoretical concentration of between 1.10<sup>4</sup> and 5.10<sup>4</sup> CFU / ml.
- 5th step : Inoculation with the freshly prepared reference strains of the "test" and "control" surfaces at different times, after deposition of the disinfectant (T0 to T72 hours). Spraying with a nebulizer of the bacterial suspensions which are deposited homogeneously on a surface of 100 cm<sup>2</sup>, surfaces noted "E" and "T" of the two zones A (with *S. aureus*) and B (with *K. pneumoniae*).
- 6th step : After drying of the artificially inoculated surfaces and an action time of 15 minutes, duplicate samples using CT3P contact agar (with neutralizer) are taken in parallel on each of the surfaces "T" and "E" of areas A and B.
- Incubation of the contact agars for 3 days at 30°C +/- 1°C.

## Results of the evaluation of the natural contamination of the surface used for the test

6 controls by contact agar of 25 cm<sup>2</sup> on each zone A and B, results in CFU / 25 cm<sup>2</sup>.

Surface control area A (C – 1 to 3)	Surface control area B (C – 3 to 6)
2	5
5	1
1	6
<b>Average : 3 CFU / 25 cm<sup>2</sup></b>	<b>Average : 4 CFU / 25 cm<sup>2</sup></b>

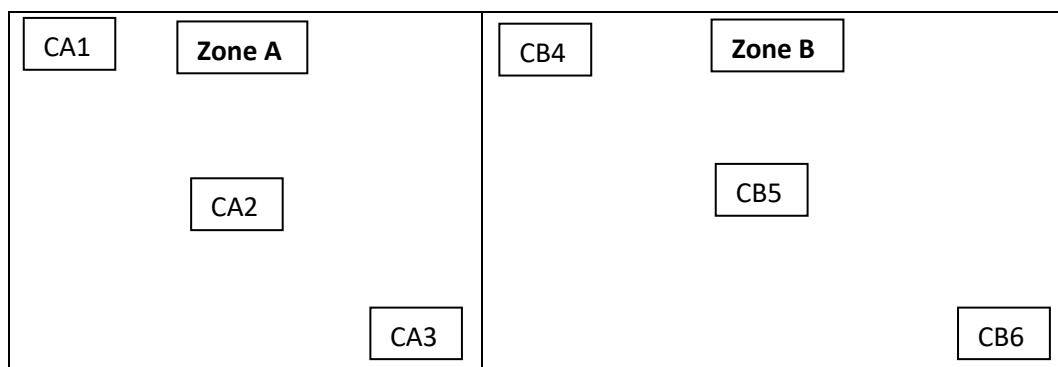
### Interpretation and comments :

The "natural" biocontamination of the surface before artificial seeding by the germs described above are everywhere quite low, here between 3 and 4 CFU / 25 cm<sup>2</sup>. By comparison, these surfaces comply with the GMP criteria for ISO 5 classrooms, less than 5 CFU / 25 cm<sup>2</sup>.

This surface is "naturally" not very contaminated and allows to carry out the tests in an optimal way.

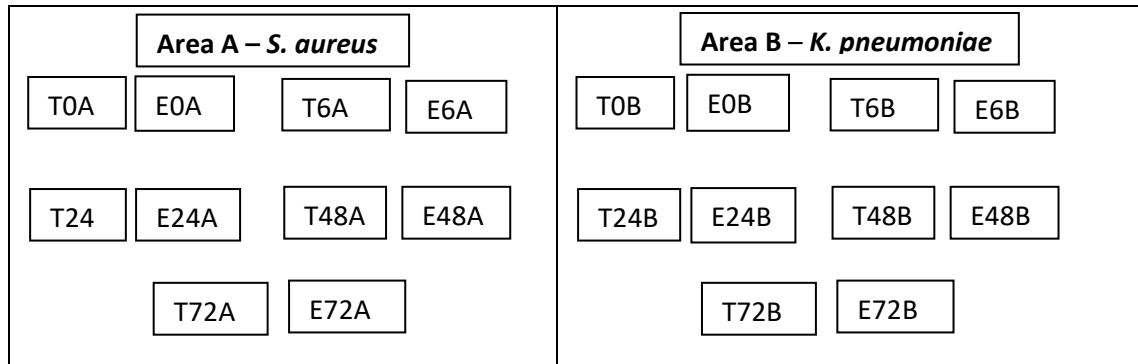
### Sampling plan for "control" surfaces before artificial addition of germs

#### CT3P contact agar sampling, 10 seconds with applicator - 3 samples per area



## Sampling plan for surfaces with artificial inoculation, treated (E) and untreated (T)

CT3P contact agar sampling, 10 seconds with applicator - 2 samples per 100 cm<sup>2</sup> surface



## Tests with artificial contamination

### *Tests on area A surfaces (S. aureus) - Results in CFU / 25 cm<sup>2</sup>*

Time in hours after application of disinfectant T = control area      E = test area	Area A <i>S. aureus</i> (average of the tests in double CFU / 25 cm <sup>2</sup> )	Percentage reduction
T0 (total bacteria) (Avg. 2 samples)	80	100 %
E0 (total bacteria) (Avg. 2 samples)	0	
T6 h (total bacteria) (Avg. 2 samples)	75	100 %
E6 h (total bacteria) (Avg. 2 samples)	0	
T24 h (total bacteria) (Avg. 2 samples)	268	100 %
E24 h (total bacteria) (Avg. 2 samples)	0	
T48 h (total bacteria) (Avg. 2 samples)	273	100 %
E48 h (total bacteria) (Avg. 2 samples)	0	
T72 h (total bacteria) (Avg. 2 samples)	92	100 %
E72 h (total bacteria) (Avg. 2 samples)	0	
<b>Average percentage reduction</b>		<b>Avg. : 100 %</b>

**Tests on area B surfaces (*K. pneumoniae*) - Results in CFU / 25 cm<sup>2</sup>**

Time in hours after application of disinfectant T = control area      E = test area	Area B <i>K. pneumoniae</i> (average of the tests in double CFU / 25 cm <sup>2</sup> )	Percentage reduction
T0 (total bacteria) (Avg. 2 samples)	149	100 %
E0 (total bacteria) (Avg. 2 samples)	0	
T6 h (total bacteria) (Avg. 2 samples)	72	100 %
E6 h (total bacteria) (Avg. 2 samples)	0	
T24 h (total bacteria) (Avg. 2 samples)	133	> 99 % (99.2 %)
E24 h (total bacteria) (Avg. 2 samples)	1	
T48 h (total bacteria) (Avg. 2 samples)	38	100 %
E48 h (total bacteria) (Avg. 2 samples)	0	
T72 h (total bacteria) (Avg. 2 samples)	45	100 %
E72 h (total bacteria) (Avg. 2 samples)	0	
<b>Average percentage reduction</b>		<b>Avg. : 99,8 %</b>

**Interpretation and comments:** the quantities of germs deposited on the surfaces are in accordance with expectations. For both strains, their number on the "control" surfaces is always higher than 10 CFU, while remaining countable (< 300) for a surface of 25 cm<sup>2</sup>. Artificial bacterial plating on these surfaces is correctly performed. The count values of the "control" surfaces (not treated with the disinfectant) are usable.

Concerning the test surfaces (E), the results obtained generally indicate a total elimination of the two bacteria deposited (*Staphylococcus aureus* and *Klebsiella pneumoniae*) on the surfaces treated with the disinfectant.

**Conclusion:** The disinfectant product showed efficacy on both bacterial strains tested. The disinfectant reduces the number of viable bacteria of the *Staphylococcus aureus* species by 100%, and the number of viable bacteria of *Klebsiella pneumoniae* by 99.2 to 100%. This can be observed as soon as the product is applied to the surface and for up to 72 hours, for a contact time of 15 minutes.



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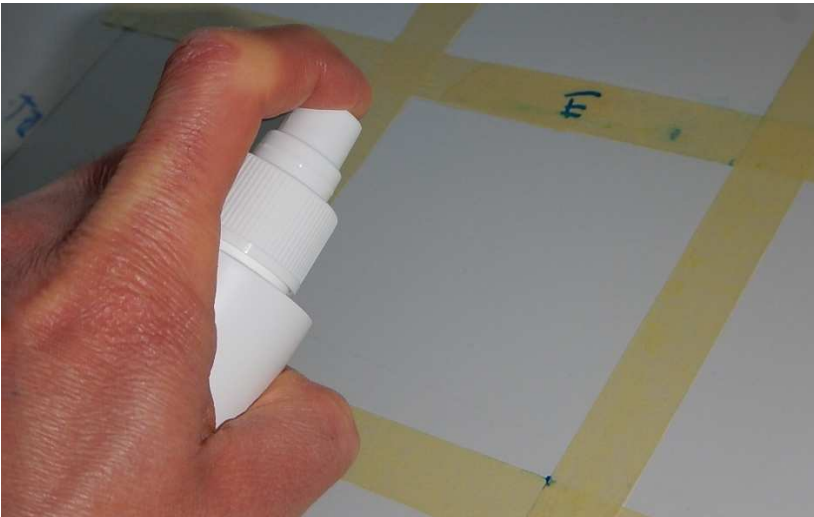
Plan-les-Ouates, April 9, 2021

### Photos of the preparation of the tests

Work surface (example zone A)



Deposit of disinfectant on test surfaces marked "E"



Preparation of bacterial suspensions of *S. aureus* and *K. pneumoniae* in sprayers



Photo of CT3P boxes used for surface sampling and of the applicator



Example of surface sampling



**Photos of the count-tact plates after sampling and incubation for 3 days at 30°C**

Count-tact « T » et « E » Area A – T0



Count-tact « T » et « E » Area B – T0



Count-tact « T » et « E » Area A – T6h



Count-tact « T » et « E » Area B – T6h



Count-tact « T » et « E » Area A – T48h



Count-tact « T » et « E » Area B – T48h



Count-tact « T » et « E » Area A – T72h



Count-tact « T » et « E » Area B – T72h

