

## UMICORE ZINC ALLOYING AND CHEMICALS

*c/o M. P. Verbiest*

Watertorenstraat, 33  
**B-2250 OLEN**

<b>your notice of</b>	<b>your ref.</b>	<b>our ref.</b>	<b>date</b>
	Request dated 10-02-05	YR/bt/05/499	Chaineux, 02 June 2005

Dear M. Verbiest,

Please, find enclosed the test report Nr 5127, concerning the tests results of microbiology performed according to your request dated 10 Februari 2005.

The invoice of 1117.99 - €(exclusive VAT) will be sent to you by our Office in Centexbel-Brussels.

If you need more information, please contact us.

Best regards.

Yvette Rogister  
Order responsible



071-T

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# UMICORE ZINC ALLOYING AND CHEMICALS

*c/o M. P. Verbiest*

Watertorenstraat, 33  
**B-2250 OLEN**

<b>your notice of</b>	<b>your ref.</b>	<b>our ref.</b>	<b>date</b>
	Request dated 10-02-05	YR/bt/05/499	Chaineux, 02 June 2005

## Analysis Report Nr. 5127

Required test(s) :

**Assessment of the antimicrobial activity of immobilized antimicrobial agents under dynamic contact conditions**

Sampling by : UMICORE ZINC ALLOYING AND CHEMICALS

Description of the sample(s) :

Identification number	Information given by the customer	Date of receipt
V500283	ZnO for bactericide property testing : test G	10-02-2005
V500284	ZnO for bactericide property testing : test H	10-02-2005
V500285	ZnO for bactericide property testing : test I	10-02-2005

Yvette Rogister  
Order responsible

For further information please contact our sectorial adviser : Marc Gochel.

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Reference : V500283 - ZnO for bactericide property testing : test G  
V500284 - ZnO for bactericide property testing : test H  
V500285 - ZnO for bactericide property testing : test I

<p style="text-align: center;"><b>Assessment of the antimicrobial activity of immobilized antimicrobial agents under dynamic contact conditions</b></p>
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Date of ending the test : 27-05-2005

## 1. Method

### **ASTM E 2149-01 – Standard test method for determining the antimicrobial activity of immobilized antimicrobial agents under dynamic contact conditions**

#### **Scope :**

- To assess the antibacterial activity of fibres treated with a non-leaching antimicrobial under dynamic contact. This dynamic shake flask test was developed to overcome difficulties in using classical test methods to evaluate substrate-bound antimicrobials.

#### **Method and test principle :**

- Treated and untreated samples are weighed and shaken in a concentrated bacterial suspension for a one hour contact time at ambient temperature. Other contact time may be used ; for example : 24 hours.

##### Strain normally used :

*Klebsiella pneumoniae* - ATCC 4352 (Gram -)

Other aerobic organisms may be used for example : *Staphylococcus aureus* (Gram+)

- The suspension is serially diluted both before and after contact and cultured. The number of viable organisms in the suspension is determined and the percent reduction (when measuring cfu/ml) or the Death Rate Constant (when calculating mean Log<sub>10</sub>) is calculated based on initial count.

Applied formula :

$$\text{Reduction \% (cfu/ml)} = \frac{B - A}{B} \times 100$$

**Death Rate Constant** (mean log<sub>10</sub> density) = B - A

where A = CFU / ml (or mean log<sub>10</sub> density of bacteria ) for the flask containing the treated substrate after the specified contact time

B = “0” contact time CFU / ml (or mean log<sub>10</sub> density of bacteria ) for the flask before the addition of the substrate

Effectiveness : We note an antibacterial activity when higher reduction percents are obtained on treated samples compared to untreated samples (control flasks).

The method is intended for materials having a reduction percent activity of 50 % to 100 % for the specified contact time.

Remark : Normally, the flasks containing the strain alone and the “Controls” (untreated) after specified contact time should give percent reduction within 15% of original count.

If it is not, the percent reduction of organisms from treated sample must be calculated directly compared to the untreated sample.

B is replaced by D = CFU/ml (or mean log<sub>10</sub> density of bacteria ) for the flask containing untreated substrate after the specified contact time.

## 2. Results

### **“Shake Flask Method” according to the ASTM E 2149-01**

Deviations to the standard :

- The sample tested is not a textile product treated with an antibacterial product but a powder which must have an antibacterial effect.
- As the powder is in the suspension, it is sure that the powder is still present in the Petri dishes and continues to act during the determination of the remaining viable bacteria after contact time (suspension is serially diluted and cultured in Petri dishes during 24 hours at 37°C) . So the activity measured is the result of the contact in the flask during 1 hour and also in the Petri dishes during 24 hours. In order to know exactly the effect after 1 hour contact time, we must normally neutralize the effect of the antibacterial agent during the determination of the remaining viable bacteria. That can be done by adding a neutralizing agent in the culture media. A neutralization agent is specific for one type of antibacterial product and preliminary tests must be performed to prove the neutralization effect.
- Due to that problem we have considered as “0” contact time, the concentration of the bacteria in the flask at the beginning of the test
- No “untreated sample” has been supplied by the customer.

**Sample : V500283**General information :

Weight of the powder : 0.5 g → Concentration of the powder in the flask : 1 %

Test liquid medium : 50 ml of a phosphate buffer containing bacteria

Count of bacteria in the flask at the beginning of the test : +/-  $2 \cdot 10^5$  CFU/ml

For each sample, the procedure is repeated 3 times. For each replicate, we carried out two sets of dilutions and numerations.

Contact time : 1 hour

Micro-organism tested : *Staphylococcus aureus* (ATCC 6538)

Agitation : Wrist action shaker CAT S 50

Table 1 : Results obtained for sample V500283 with *Staphylococcus aureus* (compared to the 0 contact time). Contact time : 1 hour .

Strain concentration in the suspension :  $1.2 \cdot 10^5$  CFU /ml

	<u><b>Trial 1</b></u> <u>(CFU/ml)</u>	<u><b>Trial 2</b></u> <u>(CFU/ml)</u>	<u><b>Trial 3</b></u> <u>(CFU/ml)</u>
<b>“0” contact time (B)</b>	$1.3 \cdot 10^5$ $1.2 \cdot 10^5$		
<u><b>Mean</b></u> <u>(log<sub>10</sub>)</u>	<u><b><math>1.25 \cdot 10^5</math></b></u> <u><b>5.1</b></u>		
<b>Treated sample – V500283</b> <b>“1 hour contact time” (A)</b>	0 0	0 9	0 4
<u><b>Mean</b></u> <u>(log<sub>10</sub>)</u>	<u><b>0</b></u> <u><b>1</b></u>	<u><b>5</b></u> <u><b>0.7</b></u>	<u><b>2</b></u> <u><b>0.3</b></u>
<b>Strain alone</b> <b>“0” contact time</b>	$1.1 \cdot 10^5$ $1.2 \cdot 10^5$		
<u><b>Mean</b></u> <u>(log<sub>10</sub>)</u>	<u><b><math>1.1 \cdot 10^5</math></b></u> <u><b>5.0</b></u>		
<b>Strain alone</b> <b>“1 hour contact time”</b>	$1.4 \cdot 10^5$ $1.2 \cdot 10^5$	$1.1 \cdot 10^5$ $1.3 \cdot 10^5$	
<u><b>Mean</b></u> <u>(log<sub>10</sub>)</u>	<u><b><math>1.3 \cdot 10^5</math></b></u> <u><b>5.1</b></u>	<u><b><math>1.2 \cdot 10^5</math></b></u> <u><b>5.1</b></u>	
	<u><b>Reduction percent (%)</b></u> <u><b>(compared to the “0” contact time)</b></u>		
<u><b>Treated – V500283</b></u>	100	99.99	99.99
	<u><b>Constant Rate Death</b></u> <u><b>(compared to the “0” contact time)</b></u>		
<u><b>Treated – V500283</b></u>	( 5.1)	4.4	4.8

Conclusion : The powder used at 1 % has a very strong activity against *Staphylococcus aureus*. The strain is very stable after 1 hour contact time (flask “strain alone”)

Performed in the microbiological lab under the responsibility of Yvette Register

**Sample :V500284****General information :**

Weight of the powder : 0.5 g → Concentration of the powder in the flask : 1 %

Test liquid medium : 50 ml of a phosphate buffer containing bacteria

Count of bacteria in the flask at the beginning of the test : +/- 2 10<sup>5</sup> CFU/ml

For each sample, the procedure is repeated 3 times. For each replicate, we carried out two sets of dilutions and numerations.

Contact time : 1 hour

Micro-organism tested : *Staphylococcus aureus* (ATCC 6538)

Agitation : Wrist action shaker CAT S 50

Table 2 : Results obtained for sample V500284 with *Staphylococcus aureus* (compared to the 0 contact time). Contact time : 1 hour .

Strain concentration in the suspension : 1.2 10<sup>5</sup> CFU /ml

	<b><u>Trial 1</u></b> <b><u>(CFU/ml)</u></b>	<b><u>Trial 2</u></b> <b><u>(CFU/ml)</u></b>	<b><u>Trial 3</u></b> <b><u>(CFU/ml)</u></b>
<b>“0” contact time (B)</b>	1.3 10 <sup>5</sup> 1.2 10 <sup>5</sup>		
<b><u>Mean</u></b> <b><u>(log<sub>10</sub>)</u></b>	<b><u>1.25 10<sup>5</sup></u></b> <b><u>5.1</u></b>		
<b>Treated sample – V500284</b> <b>“1 hour contact time” (A)</b>	0 0	0 0	0 0
<b><u>Mean</u></b> <b><u>(log<sub>10</sub>)</u></b>	<b><u>0</u></b> <b><u>/</u></b>	<b><u>0</u></b> <b><u>/</u></b>	<b><u>0</u></b> <b><u>/</u></b>
<b>Strain alone</b> <b>“0” contact time</b>	1.1 10 <sup>5</sup> 1.2 10 <sup>5</sup>		
<b><u>Mean</u></b> <b><u>(log<sub>10</sub>)</u></b>	<b><u>1.1 10<sup>5</sup></u></b> <b><u>5.0</u></b>		
<b>Strain alone</b> <b>“1 hour contact time”</b>	1.4 10 <sup>5</sup> 1.2 10 <sup>5</sup>	1.1 10 <sup>5</sup> 1.3 10 <sup>5</sup>	
<b><u>Mean</u></b> <b><u>(log<sub>10</sub>)</u></b>	<b><u>1.3 10<sup>5</sup></u></b> <b><u>5.1</u></b>	<b><u>1.2 10<sup>5</sup></u></b> <b><u>5.1</u></b>	
	<b><u>Reduction percent (%)</u></b> <b><u>(compared to the “0” contact time)</u></b>		
<b><u>Treated – V500284</u></b>	100	100	100
	<b><u>Constant Rate Death</u></b> <b><u>(compared to the “0” contact time)</u></b>		
<b><u>Treated – V500284</u></b>	(5.1)	(5.1)	(5.1)

Conclusion : The powder used at 1 % has a very strong activity against *Staphylococcus aureus*. The strain is very stable after 1 hour contact time (flask “strain alone”)

Performed in the microbiological lab under the responsibility of Yvette Register

**Sample :V500285****General information :**

Weight of the powder : 0.25 g → Concentration of the powder in the flask : 0.5 %

Test liquid medium : 50 ml of a phosphate buffer containing bacteria

Count of bacteria in the flask at the beginning of the test : +/- 2 10<sup>5</sup> CFU/ml

For each sample, the procedure is repeated 3 times. For each replicate, we carried out two sets of dilutions and numerations.

Contact time : 1 hour

Micro-organism tested : *Staphylococcus aureus* (ATCC 6538)

Agitation : Wrist action shaker CAT S 50

Table 3 : Results obtained for sample V500285 with *Staphylococcus aureus* (compared to the 0 contact time). Contact time : 1 hour .

Strain concentration in the suspension : 1.2 10<sup>5</sup> CFU /ml

	<b><u>Trial 1</u></b> <b><u>(CFU/ml)</u></b>	<b><u>Trial 2</u></b> <b><u>(CFU/ml)</u></b>	<b><u>Trial 3</u></b> <b><u>(CFU/ml)</u></b>
<b>“0” contact time (B)</b>	1.3 10 <sup>5</sup> 1.2 10 <sup>5</sup>		
<b><u>Mean</u></b> <b><u>(log<sub>10</sub>)</u></b>	<b><u>1.25 10<sup>5</sup></u></b> <b><u>5.1</u></b>		
<b>Treated sample – V500285</b> <b>“1 hour contact time” (A)</b>	0 0	0 0	0 0
<b><u>Mean</u></b> <b><u>(log<sub>10</sub>)</u></b>	<b><u>0</u></b> <b><u>/</u></b>	<b><u>0</u></b> <b><u>/</u></b>	<b><u>0</u></b> <b><u>/</u></b>
<b>Strain alone</b> <b>“0” contact time</b>	1.1 10 <sup>5</sup> 1.2 10 <sup>5</sup>		
<b><u>Mean</u></b> <b><u>(log<sub>10</sub>)</u></b>	<b><u>1.1 10<sup>5</sup></u></b> <b><u>5.0</u></b>		
<b>Strain alone</b> <b>“1 hour contact time”</b>	1.4 10 <sup>5</sup> 1.2 10 <sup>5</sup>	1.1 10 <sup>5</sup> 1.3 10 <sup>5</sup>	
<b><u>Mean</u></b> <b><u>(log<sub>10</sub>)</u></b>	<b><u>1.3 10<sup>5</sup></u></b> <b><u>5.1</u></b>	<b><u>1.2 10<sup>5</sup></u></b> <b><u>5.1</u></b>	
	<b><u>Reduction percent (%)</u></b> <b><u>(compared to the “0” contact time)</u></b>		
<b><u>Treated – V500285</u></b>	100	100	100
	<b><u>Constant Rate Death</u></b> <b><u>(compared to the “0” contact time)</u></b>		
<b><u>Treated – V500285</u></b>	(5.1)	(5.1)	(5.1)

Conclusion : The powder used at 0.5 % has a very strong activity against *Staphylococcus aureus*. The strain is very stable after 1 hour contact time (flask “strain alone”)

**Analysis Report Nr «rapport»**

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