

## ANALYTICAL REPORT

**Register numbers: 112394 to 112397 &  
112399 to 112402**

EBWA

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Ending date: 28.11.2005

Physical and microbiological analysis of water samples for the sanitization testing of 4 different watercooler models, manufactured by EBWA members, after a simulation of use in this laboratory's premises for 21 to 23 days, as specified in the "Minutes of the EBWA (Technical) Standards Committee meetings 13/05 and 03/06/2005, V2 Draft, 6th of September 2005", from now "EBWA *Pseudomonas aeruginosa* Test".

Characteristics of the water used in the analysis (pH, hardness, conductivity) were checked to see if it met the EBWA *Pseudomonas aeruginosa* test requirements. Four bottles were contaminated with *Pseudomonas aeruginosa* strain: NCTC 10660 PHLS until a concentration of over 100 cfu/250 ml was reached. The watercoolers were then contaminated with this water of known concentration of *Pseudomonas aeruginosa* and sanitized by Laboratory's personnel following manufacturers instructions or by manufacturers' staff. Two weeks later, new, sterilized bottles of the same batch (Register numbers: 112399 to 112402) were placed on the watercoolers to check the efficiency of the sanitisation.

### Dispensers used to do the essay:

Kind of watercooler: Fixed reservoir

Materials in contact with water: Stainless steel reservoir

Kind of watercooler: Disposable reservoir

Materials in contact with water: 18/10 Polished Stainless steel reservoir

Kind of watercooler: Disposable reservoir

Materials in contact with water: PLC

Kind of watercooler: Direct chill

Materials in contact with water: Stainless steel

From now they will be named "Type 1", "Type 2", "Type 3" and "Type 4" in order to preserve the confidentiality of the results.

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**Water used in the essay:**

Aigua de Sant Aniol natural mineral water bottled in new polymeric material bottles of 18.9 L. Batch number 5272.

Aigua de Sant Aniol natural mineral water contains the chemical composition specified in the EBWA *Pseudomonas aeruginosa* test, as it is shown in the table below:

Results obtained in the bottle Reference number 112394 before contamination:

PARAMETER	Reference Number 112394	EBWA'S REQUIREMENTS
pH at 20°C	7,58	>7
Conductivity at 20°C	515 µS/cm	>100 µS/cm
Hardness	30'2°F	≥22'4 °F

Results obtained in the bottles Reference number 112394 to 112397 before contamination:

PARAMETER	Results obtained:					EBWA'S REQUIREMENTS
	112394	112395	112396	112397	Units	
<i>Pseudomonas aeruginosa</i>	< 1	< 1	< 1	< 1	cfu/250mL	< 1 cfu/250mL

**Sample description:**

Once the absence of *Pseudomonas aeruginosa* were checked, this bottles *Pseudomonas aeruginosa* free were contaminated with known concentration of *Pseudomonas aeruginosa* strain: NCTC 10660 PHLS. Several counts were done on each contaminated bottle water in order to reach the desirable concentration.

When contamination was over 100 cfu/250mL in all four bottles, the bottles were collocated in the watercoolers and they were plugged to start the pouring simulation of the contaminated water into the coolers.

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Results obtained in the contaminated bottles Reference number 112394 to 112397 after contamination:

PARAMETER	Results obtained:					EBWA's REQUIREMENTS
	112394	112395	112396	112397	Units	
<i>Pseudomonas aeruginosa</i>	> 450	128	> 450	> 450	cfu/250mL	> 100 cfu/250mL

To do the essay it has been simulated the watercoolers' use emptying during minimum of 21 days (only working days), 1L of Agua de Sant Aniol natural mineral water: 250 mL from the natural temperature water tap and 250 mL from the cold water tap twice a day.

Microbiological analyses were done to check development of the introduced contamination at different stages of the test: start ( $t_0$ ), 7<sup>th</sup> day ( $t_7$ ), 14<sup>th</sup> day ( $t_{14}$ ).

On the sanitization day ( $t'_0$ ), *Pseudomonas aeruginosa* analyses of the bottle and the cooler were done to check non-detection of *Pseudomonas aeruginosa* (according to *Pseudomonas aeruginosa* Method UNE-EN 12780:2003) in the new bottle and in the cooler at  $t'_0$ , and a last microbiological analysis was done on the 7<sup>th</sup> day after sanitization ( $t'_7$ ).

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#### CHECKING OF CONTAMINATION

$t_0$ : Start of the test; 250 ml of water from each tap were sampled to count *Pseudomonas aeruginosa*.

$t_7$ : Seven days after the test and pouring simulation start, 250 ml of water from each tap were sampled in the afternoon to count *Pseudomonas aeruginosa*.

$t_{14}$ : On the fourteenth day, a *Pseudomonas aeruginosa* count was done on each tap of the four coolers.

#### CHECKING OF SANITISATION

$t'_0$ : Immediately after plugging the new water sterile bottles, samples of 250 mL from each tap of the 4 coolers were taken in order to check the absence of *Pseudomonas aeruginosa* at  $t_0$  after sanitisation.

$t'_7$ : Seven days later, a new count of *Pseudomonas aeruginosa* was done on 250 mL of water from each tap of the 4 watercoolers ( $t_7$  after sanitization).

#### **Results qualification:**

The results qualification has been made according to the "Minutes of the EBWA (Technical) Standards Committee meetings 13/05 and 03/06/2005, V2 Draft, 6th of September 2005" of the "EBWA *Pseudomonas aeruginosa* Test" as follows:

RESULTS	QUALIFICATION
$\geq 1$ cfu/250ml	UNSATISFACTORY
$< 1$ cfu/250ml	SATISFACTORY

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**Results obtained:**

Reference: "*Pseudomonas aeruginosa* analysis of a water sample from the cooler "Type 1"

Before Sanitisation. Bottle reference number 112394

Time	Tap	Results obtained (cfu / 250 mL)
t <sub>0</sub>	Cook	1000
	Cold	600
t <sub>7</sub>	Cook	540
	Cold	500
t <sub>14</sub>	Cook	150
	Cold	330

After Sanitisation. Bottle reference number 112402

Time	Tap	Results obtained (cfu / 250 mL)	QUALIFICATION
t' <sub>0</sub>	Cook	< 1	SATISFACTORY
	Cold	< 1	SATISFACTORY
t' <sub>7</sub>	Cook	< 1	SATISFACTORY
	Cold	< 1	SATISFACTORY

*Pseudomonas aeruginosa* Analytical method: UNE-EN 12780:2003

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**Results obtained:**

Reference: "Pseudomonas aeruginosa analysis of a water sample from the cooler 'Type 2' "

Before Sanitisation. Bottle reference number 112395

Time	Tap	Results obtained (cfu / 250 mL)
t <sub>0</sub>	Cook	46
	Cold	44
t <sub>7</sub>	Cook	30
	Cold	20
t <sub>14</sub>	Cook	25
	Cold	22

After Sanitisation. Bottle reference number 112400

Time	Tap	Results obtained (cfu / 250 mL)	QUALIFICATION
t' <sub>0</sub>	Cook	< 1	SATISFACTORY
	Cold	< 1	SATISFACTORY
t' <sub>7</sub>	Cook	< 1	SATISFACTORY
	Cold	< 1	SATISFACTORY

*Pseudomonas aeruginosa* Analytical method: UNE-EN 12780:2003

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**Results obtained:**

Reference: "Microbiological analysis of a water sample from the cooler 'Type 3' "

Before sanitisation. Bottle reference number 112396

Time	Tap	Results obtained (cfu / 250 mL)
t <sub>0</sub>	Cook	15000
	Cold	12000
t <sub>7</sub>	Cook	25000
	Cold	16000
t <sub>14</sub>	Cook	3800
	Cold	5000

After Sanitisation. Bottle reference number 112399

Time	Tap	Results obtained (cfu / 250 mL)	QUALIFICATION
t' <sub>0</sub>	Cook	< 1	SATISFACTORY
	Cold	15	UNSATISFACTORY
t' <sub>7</sub>	Cook	< 1	SATISFACTORY
	Cold	50	UNSATISFACTORY

*Pseudomonas aeruginosa* Analytical method: UNE-EN 12780:2003

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**Results obtained:**

Reference: "Microbiological analysis of a water sample from the cooler 'Type 4' "

Before sanitisation. Bottle reference number 112397

Time	Tap	Results obtained (cfu / 250 mL)
t <sub>0</sub>	Cook	850
	Cold	1800
t <sub>7</sub>	Cook	65
	Cold	79
t <sub>14</sub>	Cook	75
	Cold	20

After Sanitisation. Bottle reference number 1123401

Time	Tap	Results obtained (cfu / 250 mL)	QUALIFICATION
t' <sub>0</sub>	Cook	< 1	SATISFACTORY
	Cold	< 1	SATISFACTORY
t' <sub>7</sub>	Cook	< 1	SATISFACTORY
	Cold	< 1	SATISFACTORY

*Pseudomonas aeruginosa* Analytical method: UNE-EN 12780:2003



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Analytical methods:

Parameter	Method
pH	Internal procedure Q-0004. Electrometry.
Conductivity at 20°C	Internal procedure Q-0005. Electrometry.
Hardness	Internal procedure Q-0049. Volumetry.
<i>Pseudomonas aeruginosa</i>	UNE-EN 12780:2003

NOTES:

The expression "cfu" means "colony-forming units".

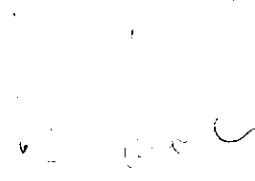
The symbol "<" indicates that the result doesn't go beyond the limits of quantification of the respective analytical method.

The symbol ">" indicates that the result goes beyond the limits of quantification of the respective analytical method.

The present report only bears witness to the tested sample

It is not allowed the partial use of the results appearing in this report without a Laboratory Dr. Oliver Rodés, S.A. written authorisation.

El Prat de Llobregat (Barcelona), 14<sup>th</sup> of Desember 2005

  
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