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STANDARD METHODOLOGY FOR THE EXAMINATION OF THE MICROBIOLOGICAL EFFICIENCY OF POINT OF USE SANITISATION METHODS

MODULE THREE **THE CHALLENGE TEST - POU**

Effectiveness of Sanitisation Methods in Removing Pathogens

V2 May 2018

MODULE THREE – THE CHALLENGE TEST – POU

The Challenge Test is intended to allow manufacturers of "Point of Use watercoolers" (from now POU) to provide their customers with a method of machine sanitisation (cleaning and disinfection) that has been proven to work to Watercoolers Europe (from now on WE) standards, even when a POU is heavily contaminated with pathogenic bacteria.

This test involves the deliberate contamination of POU with "*Pseudomonas aeruginosa*" and the undertaking of a Full Sanitisation. Once the sanitisation is completed, water not contaminated with *Pseudomonas aerugnosa* is loaded into the POU and a *Pseudomonas aeruginosa* test from the water dispensed after the sanitisation is performed in order to prove the sanitisation method efficiency. Water drawn from the POU is tested, rather than an internal water contact surface being swabbed.

SCOPE

- The aim is NOT to demonstrate that POU treatments (carbon filters, Reverse Osmosis, UV, decalcification, etc.) are able to remove or prevent the contamination with *Pseudomonas aeruginosa*, but proving that an efficient sanitisation of the POU under test is achievable when the manufacturer's or other WE member instructions and recommended sanitisation methods are followed when a POU is contaminated microbiologically.
- Easing the identification of causes, remedies and responsibilities concerning microbiological contamination of POU in the field.

BENEFITS OF STANDARDISED TEST METHODOLOGIES

- **MODULE THREE - POU**

Demonstrates on both quantitative and qualitative basis that a pathogen infected POU can be successfully sanitised.

Being aware of the great variability of tests carried out by POU manufacturers throughout Europe, it is considered essential to have a test which ensures that sanitisation of these machines is common for all WE members.

WE REQUIREMENTS

MODULE THREE-POU

This module may be submitted by manufacturers but not restricted to other WE members as the mandatory Module for WE Supplier members, or by those intending to show at WE Trade Shows. Other than that, the Challenge Test - POU is an optional test, except in those countries where the National Association's Code of Practice may require it.

NOTES:

1) Testing and Certification indicating that products have attained the WE standards must be undertaken by approved and accredited third party test facilities.

2) Certification does not imply, or grant WE approval or endorsement of the product tested. Strict guidelines relate to the use of such Certification in advertising and marketing material.

3) POU and equipment manufacturers who consider that they are unable to execute the present test on their equipment or with their products, should submit an alternative proposal (before commencing any testing) to the WE Standards & Technical Committee who will determine if the alternative protocol is acceptable.

SANITISATION METHODS

a) *Cleaning*

The objective is to physically remove as much scaling and bio-film as possible. This can be by:

- Use of a descaling agent
- Use of a detergent
- Physical cleaning using brushes and/or cloths

Descaling agents are especially effective and simultaneously achieve a reasonable elimination of bacteria, whilst cleaning hard to access areas

b) Disinfection

Materials may include the use of:

- Chlorine compounds
- Hydrogen Peroxide (H_2O_2)
- Peracetic acid (PAA) and others Peroxides
- Ozone (including permanently fitted ozonation devices)
- Steam (including internal steam generating devices)
- Hot water

c) One Step Sanitisation

- Replacement by pre-sanitised or disposable components

METHODS CLAIMING REDUCTION OF FREQUENCY OF SANITISATIONS

Equipment/materials claimed to reduce the need for Full Sanitisations below those specified in the WE Code of Practice of 4-2 per year include:

- Antibacterial plastics
- In-place heating devices
- In-place ozonisation devices

NOTE: Use of antibacterial materials for water contact surfaces or ozonisation devices must comply with any existing National Legislation.

METHODOLOGY

MODULE THREE: THE CHALLENGE TEST - POU

The Challenge Test - POU involves contamination of POU with "*Pseudomonas aeruginosa*" before undertaking sanitisation using a method provided by the interested WE member. *Pseudomonas aeruginosa* is allowed to re-grow for a period after sanitisation in order to test the ability of the organism to continue to contaminate the POU after the sanitisation. The water dispensed by the POU is tested, rather than a swab of an internal water contact surface.

The prescribed testing method is aimed at proving that pathogenic contamination of the POU can be eliminated from the model of POU under test having applied to it the sanitisation method specified by the interested WE member.

In order to allow adequate time for the pathogenic micro-organism to develop a biofilm in each POU, a simulation of normal usage is maintained over a 14 day period. During this time 250 ml of water is drawn from each tap of each POU

twice a day (in the morning and in the afternoon). During weekends and public holidays it is acceptable that this regular dispensing is interrupted.

THE PATHOGEN: *PSEUDOMONAS AERUGINOSA*

The chosen pathogen is *Pseudomonas aeruginosa* for the following reasons:

- Cultivation in water is easy and fast.
- Biofilm is formed.
- It is difficult to eliminate; so if the sanitisation method works to eliminate it, an equivalent result can be expected with other pathogens.
- Detection by a qualified laboratory is easy.
- Although it is a microorganism which is not present in the current european legislation regarding water intended for human consumption, standards of some countries (France: "*Surveillance microbiologique de l'environnement dans les établissements de santé (Air, eaux et surfaces), Comité Technique National des Infections Nosocomiales*") are including it as a microorganism of choice due to the formation of biofilm and its pathogenic potential.

ACCEPTABLE STRAINS

Any wild-type strain *Pseudomonas aeruginosa* isolated from water is highly recommended and could be acceptable if growth of 5 logs cfu in 250mL is ensured at day 14. An example of an acceptable strain is:

- Nutrient Agar from Laboratorio Dr Oliver Rodés (LDOR), S.A. of El Prat de Llobregat in Spain, wild strain Collection SS40. (See report 19.5.05)

A) WATER TO BE USED

a) Best biofilm development results were obtained by LDOR with a mineral water with more than 90mg of Calcium per litre. This specification is strongly recommended.

b) In order to represent the most challenging conditions, and also to have a standard water which makes the tests comparable, it is recommended that commercially available un-ozonated bottled water with a calcium content in excess of 90mg/l, a minimum TDS (total dissolved solids) and in excess of 150 mg/l and a Langelier Index (L.I.) ≥ +0.5 at 20°C is used for the tests.

c) Before undertaking any tests, an analysis of the water to be used should be submitted to the WE Standards & Technical Committee for their approval.

NOTE: Water with residual traces of disinfectant (as ozone, chlorine, and others…) it is NOT acceptable.

B) POU TO BE TESTED

a) Three (3) of each model of Cold only, or Cook & Cold POU supplied by the manufacturer. Only cook and cold water systems will be considered as a possible test.
b) For Cook & Hot POU, and Cold & Hot POU, it has been demonstrated that hot water systems that reach ≥ 50ºC are able to remove *Pseudomonas aeruginosa* contamination due to high temperatures and not because of the sanitisation procedure.
c) POU of different body types but identical water contact surfaces are classed as of the same model for these purposes.
d) POU treatments do not have to be in place or switched on.

C) TESTING FACILITY

In order to be acceptable to the EBWA, laboratories to be used must be

a) able to demonstrate an ability to undertake the test work required in the following areas:
i) Technical ability and experience.
ii) Adequate space to store and test the numbers of POU and bottles of water needed for the trials.
iii) A test facility in Europe. If the facility is outside Europe, the laboratory must be approved by the WE Standards & Technical Committee before testing begins.
b) ISO/IEC 17025 accredited to do testing of *Pseudomonas aeruginosa* in water by a National or international body:

(e.g. NATA (Australia), BMWA (Austria), BELTEST (Belgium), INMETRO (Brazil), HKAS (China), CAI (Czech Republic), DANAK (Denmark), EAK (Estonia), FINAS (Finland), COFRAC (France), DACH or DAP, or DATech (Germany), ESYD (Greece), INAB (Ireland), ISRAC (Israel), SINAL (Italy), LATAK (Latvia), LA (Lithuania), RVA (Netherlands), LANZ (New Zealand, NA (Norway), PCA (Poland), IPAC (Portugal), RENAR (Romania), SAC/spring (Singapore), SNAS (Slovakia), SA (Slovenia), SANAS (South Africa), ENAC (Spain), SWEDAC (Sweden), SAS (Switzerland), TURKAK (Turkey), UKAS (United Kingdom) and A2LA (USA)).

D) STORAGE OF WATER

a) Bottled water used throughout the test period should be from the same source and supplier with no variability other than bottling date or production batch codes.
b) Identical batch codes should be used simultaneously on all POU under test and control.
c) The water should be stored in a cool (15˚C-25°C) dark place, away from polluting or contaminating substances.**E) SANITISATION**

a) This must be undertaken in accordance with the methodology and materials supplied by the POU or device manufacturer.
b) Staff undertaking sanitisation must either be trained by a qualified representative of the POU manufacturer or the manufacturer must provide trained staff to undertake the sanitisation at the appointed time.
c) The third party testing facility representative must supervise the entire sanitisation operation.
d) All replacement water contact components used for sanitisation shall be supplied in sealed packs and only handled with clean disposable gloves by the person qualified to execute the sanitisation.

F) SUMMARY OF REQUIREMENTS

a) 3 POU of each model to be tested.
b) 5 Gallon bottles (18,9L) of mineral water (10 bottles approximately).
c) 2 tanks, with 100 litres of volume per tank. (Tank #1 and Tank #2).
d) 2 boost pumps for liquids. (Pump #1 and Pump#2).
e) Polypropylene connectors, fittings, reducers, valves, etc. (Ex: John Guest®).
f) Disposable tubes to make a water circuit.

G) TESTING

The test procedure module has 5 steps, described in more detail below:

Step 1: Fill tank #1 with mineral water contaminated with *Pseudomonas aeruginosa*.

Step 2: Connect the 3 POU to the tank and simulate 14 days of field use.

Step 3: Sanitise the 3 POU with a method specified by manufacturer.

Step 4: Fill tank #2 with mineral water free of *Pseudomonas aeruginosa*. Plug into the water system a new water circuit free of *Pseudomonas aeruginosa.*

Step 5: Test for the absence of *Pseudomonas aeruginosa* in 250 ml of samples of water drawn from each POU tap.

STEP 1
Preparing tank #1 and pump #1:

1. Fill the tank #1 with bottles of mineral water. Necessary volume depends on POU internal tanks and length of tubes circuit.

2. Inoculate *Pseudomonas aeruginosa* into the tank with a concentration that guarantees a minimum count of 10^5 cfu in 250mL at day 14. We recommended a minimum theoretical 10^3cfu/250mL inoculum. (contaminated with a 5x10^5 cfu to 2x10^6cfu inoculum in for example 80L of water).

3. The concentration of contamination must be submitted by the laboratory in order to compare the evolution of the contamination.

STEP 2
Contaminating POU:

1. Interconnect POU (with no carbon filters, and treatments switched off) to tank #1 and pump #1, with polypropylene pieces and compatible tubes.

2. In order to ensure that the contaminated water is in contact with all POU internal components, 250 mL of contaminated water should be drawn off from each tap.

3. The POU are NOT to be plugged into the electrical supply because the bacteria best develops at room temperature (20 to 30°C). If the taps require that the electrical supply is switched on to allow them to open, do this for only the minimum time required to open and draw-off water and then unplug the POU from the electrical supply.

4. After a 3 day period, a *Pseudomonas aeruginosa* count performed on water samples drawn off from the taps of each of the 3 POU. If necessary, the machine can be plugged into the electrical supply long enough to allow for this.

5. The level of contamination of each water sample after 3 days must be at a minimum 10^3 cfu / 250 mL. If that level has not been attained, the POU has to be re-contaminated (go back to Step 1.2 and re-inoculate bacteria into the tank).

6. Simulation of normal use is maintained for the period of 14 days by drawing off 250 mL samples of water from each tap of each POU twice a day (in the morning and in the afternoon). During weekends and public holidays regular dispensing may be interrupted.
   The level of contamination of each POU must be controlled in order to guarantee the evolution of it.

7. On Day 14, a *Pseudomonas aeruginosa* count should be undertaken on water drawn from each tap of each of the 3 POU.
   The level of contamination measured from samples taken from each tap after the 14 day period must be at least 10^5 cfu / 250 mL. This ensures the *Pseudomonas aeruginosa* is still vigorous. If the count reaches this level, proceed to Step 3. If the level of contamination after 14 days on a sample taken from any POU is lower, it will be necessary to go back to Step 1.2 and re-inoculate bacteria into the tank. 3 days later, after this new inoculation, a *Pseudomonas aeruginosa* count has to be done on each tap of the POU The count must be at least ≥10^5 cfu / 250 ml before you can proceed to Step 3.

8. If the level of contamination on a sample taken from any POU is still lower than 10^5 cfu/250mL you must return to Step 1, and repeat procedure with new *Pseudomonas aeruginosa* contaminated tank.

STEP 3
Sanitising As Specified by Manufacturer

1. Undertake the sanitisation method specified by the manufacturer of the POU. This sanitisation method must be the one specified in the manufacturer's manual supplied with the POU when distributed to users or the method officially notified by the manufacturer to its clients.

2. The sanitisation method should specify which POU models (made by the same manufacturer) have been tested with, and therefore use, that particular sanitisation method.

3. Laboratory staff undertaking sanitisation must either be trained by a qualified representative of the POU manufacturer or the manufacturer must provide trained staff to undertake the sanitisation at the appointed time. Should the manufacturer's staff undertake sanitisation then qualified laboratory staff must supervise the operation.

4. In case of any difference in the methodology of sanitisation between the written manufacturer's manual and the procedure actually applied, the manufacturer's manual must be modified accordingly, and a new manual distributed to all clients of the manufacturer.

5. Manufacturer's sanitisaton manual must include date of issue and number of version.

STEP 4
Preparing tank #2, pump #2, and new water circuit.

1. Fill the tank #2 with bottles of mineral water not contaminated with *Pseudomonas aeruginosa*. Necessary volume depends on POU internal tanks and length of tubes circuit.

2. Interconnect POU, tank #2 and pump #2, with new polyproplylene pieces and compatible tubes, Ensure that it is not possible contamination with *Pseudomonas aeruginosa.*

3. Take water samples from the tank #2 and pump #2 to check the no detection of *Pseudomonas aeruginosa* in 250 mL. The tests results must be 0 cfu/250ml.

STEP 5
Test for the no detection of *Pseudomonas aeruginosa*

1. Immediately after re-filling the POU with mineral water not contaminated with *Pseudomonas aeruginosa*. Water samples of 250 ml should be drawn from each tap of the POU and the no detection of *Pseudomonas aeruginosa* (t'0) verified. The test result must be 0 cfu/250ml.

H) RESULTS EXPECTED MODULE THREE-B - THE CHALLENGE TEST FOR POU

A pass is registered only when all the results from each tap of each POU under test are 0 cfu/250ml on each tap at t'0.

WHERE t ' means Time and t'0 means Day Zero.

t'0 ≥ 1 cfu/250ml - FAIL
t'0 = 0 cfu/250ml – PASS

NOTES

Note 1: The test result will be valid for each model of POU with identical water contact surfaces as the ones tested. “Identical models” is defined here as, “two different looking POU whose surface materials and design of all parts in contact with water are the same”.

Note 2: The test result will be valid only for the sanitising method tested for Module Three-B. Each new sanitising method would then have to be fully tested to be certified in the Module.

Note 3: The result of the level of contamination must be given by the laboratory in exact counts with two significant figures, in order to compare the evolution of contamination. (Ie 28 cfu/ 250 ml or 1.0E+03 cfu/250ml or $1.0x10^3$/250 ml.)

Note 4: Manufacturers, at their own discretion, may extend the test period in Step 5 from 0 to 14 days if they wish to demonstrate extended performance of their POU and/or sanitisation method.

I) SUBMISSION OF RESULTS

Results from all test POU with full details of the methodology used should be submitted to the WE Standards & Technical Committee accompanied by full details and relevant accreditation(s) of the laboratory used. This information will only be examined by members of the WE Standards & Technical Committee and will be kept strictly confidential.

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