Could hydrogen peroxide decontamination affect the results of a sterility test?

A *case study* of hydrogen peroxide penetration through the load packaging

M. Luisa Bernuzzi, *R&D Manager*
MBR@fedegari.com

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Package Integrity Verification

USP 39, General Chapter 1208 Sterility testing - Isolators Systems
Package Integrity Verification

«Some materials are affected by decontaminating agents which can result in inhibition of microbial growth.
Of concern are the penetration of decontaminating agents into product containers or, in general, any material that could come in contact with product, media or dilution fluids used in the sterility test.
Its the responsibility of the operator to verify that containers, media and suppliers are unaffected by the decontamination process.»

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4-MOL2-VACIT5	CITOCARTIN 40mg/ml 1:200 molteni cartucho	Carpules	1-AMCARA or 1-AMCARAG (cylindrical ampoules)	8,8 x 63
VFCLI300	CLINDAMICINA 300mg/2ml vial	Liquid vial	1-VI2	16,75 x 33,5
VFCLI600	CLINDAMICINA 600mg/4ml vial	Liquid vial	1-VI10	23,25 x 46
VFCX1GR	CLOXACILINA 1g	Powder	1-VIMOLDII	23,5 x 58,8
VFCX500	CLOXACILINA 500mg	Powder	1-VIMOLDE	23 x 46,8
8-CLO	**CLOXACILINA SÓDICA**	Powder	1-VI100FI	49 x 104,5
VADKT	DEXKETOPROFENO 50MG ampolla	Ampoule	Ampoule 2ml	10,75 x 57
VADI	DICLOFENACO INY.ampolla	Ampoule	Ampoule 2ml	
DIEPP	DISOLV.EPOPROSTENOL 0,5mg FI 50ml	Infuser bottle	1-VI50FI	
DIAPI10	DISOLVENTE AGUA INYECCION 10ml	Ampoule	Ampoule 10ml	
DIAPI2	DISOLVENTE AGUA INYECCION 2 ml	Ampoule	Ampoule 2ml	
DIAPI3_2	DISOLVENTE AGUA INYECCION 3,2ml	Ampoule	Ampoule 4ml	
DIAPI3_5	DISOLVENTE AGUA INYECCION 3,5ml	Ampoule	Ampoule 4ml	
DIAPI4	DISOLVENTE AGUA INYECCION 4 ml	Ampoule	Ampoule 4ml	17,25 x 57
DIAPI5	DISOLVENTE AGUA INYECCION 5 ml	Ampoule	Ampoule 5ml	17,25 x 57
DIAPI6	DISOLVENTE AGUA INYECCION 6ml	Ampoule	Ampoule 10ml	17,25 x 87
DICNC4	DISOLVENTE CEFONICID 4% 2,5 ml	Ampoule	Ampoule 4ml	17,25 x 57
DICLNA	DISOLVENTE CLORURO SODICO 1ml	Ampoule	Ampoule 2ml	10,75 x 57
DICNC4	DISOLVENTE LIDOC. CEFONICID 4ml	Ampoule	Ampoule 4ml	17,25 x 57
DILI4	DISOLVENTE LIDOCAINA 0,5% 4ml	Ampoule	Ampoule 4ml	17,25 x 57
DILI2	DISOLVENTE LIDOCAINA 0,5%2ml	Ampoule	Ampoule 2ml	10,75 x 57
DILI1%2	DISOLVENTE LIDOCAINA 1% 2ml.	Ampoule	Ampoule 2ml	10,75 x 57
DILI1.3,5	DISOLVENTE LIDOCAINA 1% 3,5ml.	Ampoule	Ampoule 4ml	17,25 x 57
DILI1%4	DISOLVENTE LIDOCAINA 1% 4ml	Ampoule	Ampoule 4ml	17,25 x 57
DILI1%5	DISOLVENTE LIDOCAINA 1% 5ml	Ampoule	Ampoule 5ml	17,25 x 57
DILI2,5	DISOLVENTE LIDOCAINA 25mg/2,5ml (10%)	Ampoule	Ampoule 4ml	17,25 x 57
VLEPP05	EPOPROSTENOL 0,5mg liofilizado	Liophylized vial	1-VI10	23,25 x 46

EXAMPLE OF TESTING MATERIALS

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Testing materials: examples

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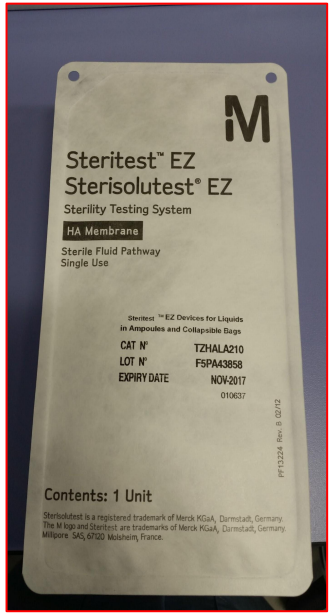
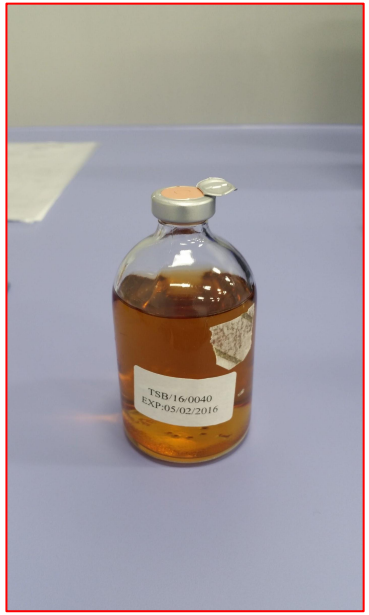
Testing materials: examples





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The study

A customer of us required our support for «A Package Integrity Verification» which involved all the samples and consumables he had inside his Sterility Test Isolator.

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Package Integrity Verification study

After running a decontamination cycle we evaluated residuals of Hydrogen Peroxide on the surface of the samples treated and inside the containers.

A chemical evaluation always represents the first step to perform.

If residuals of the chemical agent are found, a microbiological investigation has to be arranged.

The microbial investigation was not the scope of our job.

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Test protocol

- Running a decontamination cycle
- Evaluating residuals

  - on the surface of the containers

  - in the head space

  - in the liquid contained
- Analysing the results obtained
- Defining with the Customer the corrective actions to be implemeted

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Consumables and equipments

Dräger X-am® 5100 Single gas detector

MQuant™ Peroxide Test 1.10011.0001

Drager sensor H_2O_2 LC

Drager Polytron 7000 LC

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Consumable and equipments

Hydrogen Peroxide residuals investigation

On the surface

Dräger X-am® 5100
Single gas detector

Inside the liquid

MQuant™ Peroxide
Test 1.10011.0001

In the empty space

Drager Polytron 7000
LC

Consumables and equipments

Drager Polytron 7000 LC to investigate residuals inside sample containers.

Dräger Polytron 7000 *and its low concentration* ***sensor*** *are used to detect residuals inside the container.* ***A FKM or a PTFE tube*** *(connected with the Dräger intake), immediately after the cycle, is inserted into the container and the concentration in the head space is read on Dräger display.*

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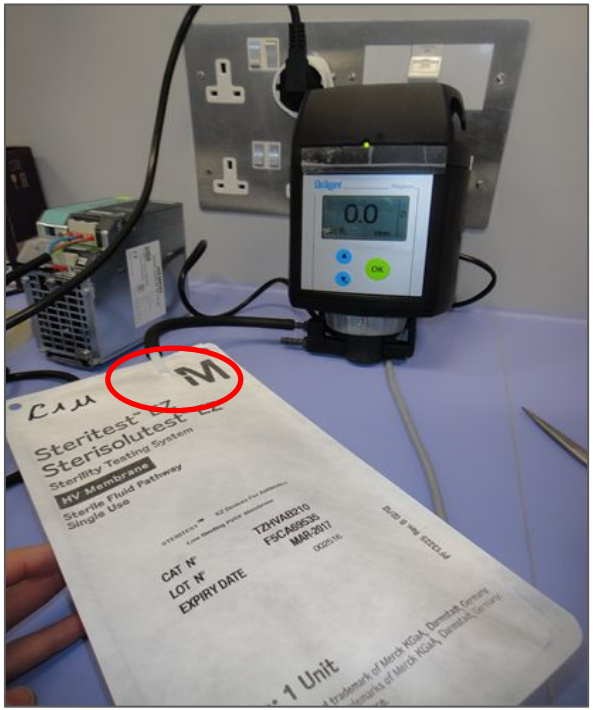
Consumables and equipments



Drager Polytron 7000 LC

to investigate residuals inside sample containers.

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Consumables and equipments

MQuant™ Peroxide Test for aqueous solutions and organic solvents.

Measuring range / color-scale graduation
0.5 - 2 - 5 - 10 - 25 mg/l H_2O_2

O_2^{2-}

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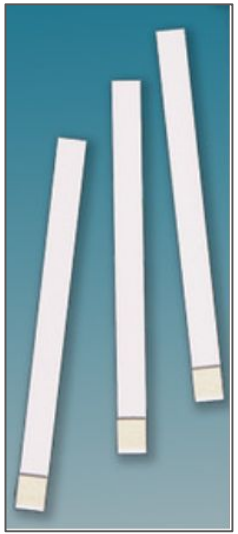
Consumables and equipments

MQuant™ Peroxide Test



The reaction zone of the Peroxide strip is immersed in the aqueous solution contained for 1 second. We allow excess liquid to run off via the long edge of the strip into an absorbent paper towel and after 15 sec we compare the color obtained with the color scale provided by the strip supplier.

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Consumables and equipments

Dräger X-am® 5100 Single gas detector for Hydrogen Peroxide

for surfaces

We locate the sensor next to the surface to investigate and we read the concentration on its display.

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Samples and analysis to be performed

According to the sample, we performed different analysis.

Samples for the investigation on the *surface* are called "A".

Samples for the investigation in the *aqueous solution* are called "B".

Samples for the investigation in the "*head space*" are called "C".

Samples "A" are analysed by an operator, "B" ones by another operator and "C" ones by another one, in order not to waste time and to obtain accurate results.

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Number of containers

Each test requires a container: to perform different chemical tests, we need several containers of the same sample.

In order to assure repeatability of the results, each investigation will be performed using two containers of the same sample.

I.e.: two containers of sample "X" for checking the amount in the aqueous solution, two containers of sample "X" for checking the amount on the surface….

Running a cycle….

Samples grouped into three groups: “A” in the center, “B” on the left, “C” on the right side of the isolator.

Decontamination cycle at 700 ppm for 30 minutes

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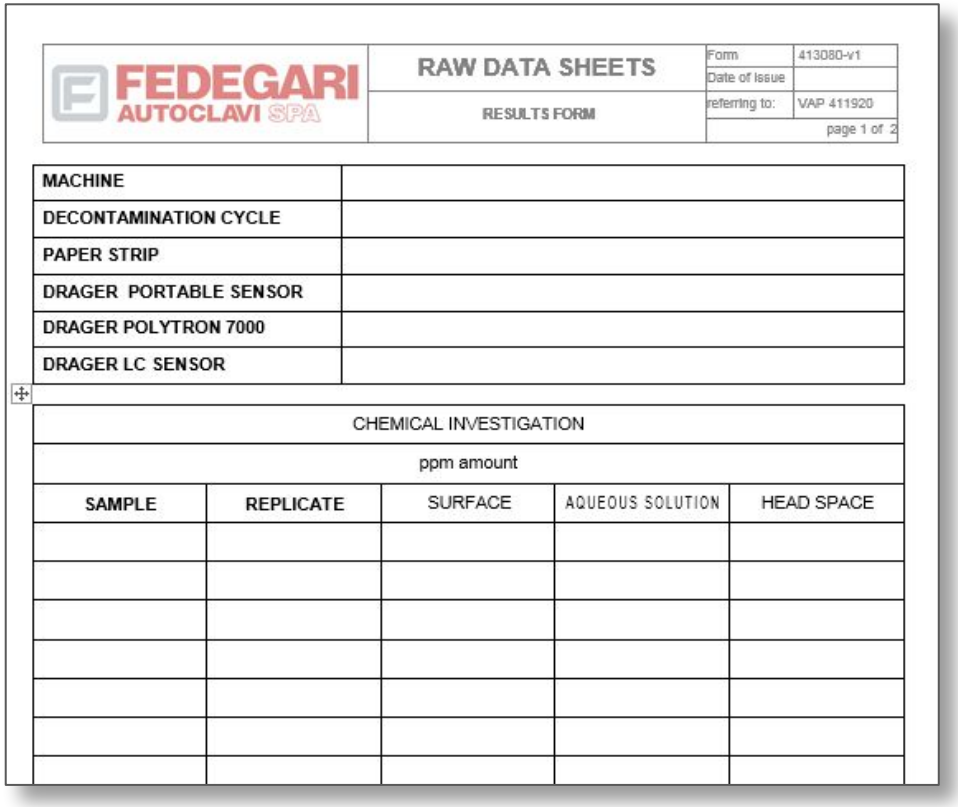
Collecting Raw Data



FEDEGARI AUTOCLAVI SPA	RAW DATA SHEETS	Form	413080-v1
		Date of Issue	
	RESULTS FORM	referring to:	VAP 411920
			page 1 of 2

MACHINE	
DECONTAMINATION CYCLE	
PAPER STRIP	
DRAGER PORTABLE SENSOR	
DRAGER POLYTRON 7000	
DRAGER LC SENSOR	

CHEMICAL INVESTIGATION				
ppm amount				
SAMPLE	REPLICATE	SURFACE	AQUEOUS SOLUTION	HEAD SPACE

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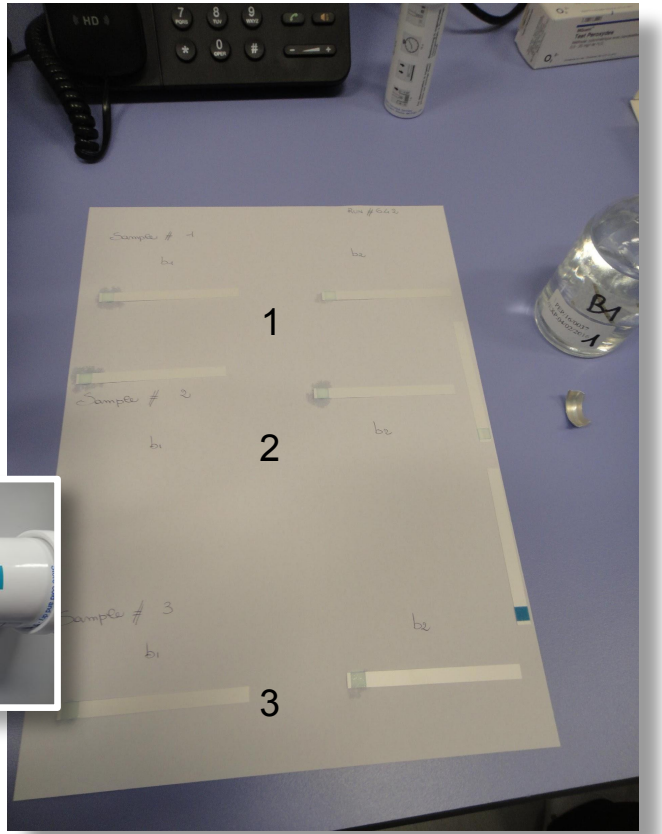
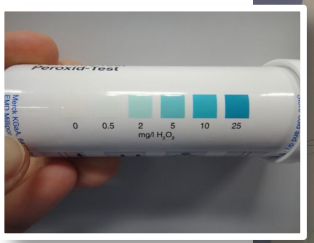
Chemical investigation for liquid





Paper strips of samples 1, 2, 3; a blar and a positive control (after the contact with a 30% H_2O_2 aqueous solution); the slight blue color of the blank is caused by the presence of the positive control next to it.

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Results obtained: Crimped bottles

	SURFACE (A)		AQUEOUS SOLUTION (B)	HEAD SPACE (C)
	Stopper	Glass		
Test A1	0 ppm	0 ppm		
Test A2	0 ppm	0 ppm		
Test B1			L.T. 0.5 ppm	
Test B2			L.T. 0.5 ppm	
Test C1				0 ppm
Test C2				0 ppm

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Results obtained: Millipore dilutor device

	SURFACE (A)		HEAD SPACE (C)
	Tyvek	Plastic	
Test A1	0.8 ppm	0.1 ppm	
Test A2	2.4 ppm	0 ppm	
Test C1			3.8 ppm
Test C2			4 ppm

If the concentration read by the sensor is a high concentration, it takes several minutes, after the test, to clean the sensor reaching again 0 ppm. The analyses can't be consecutive.

The holding time between the two analyses was 5 minutes. In spite of it, the second reading confirmed the first result obtained. Tyvek® can be considered a barrier against hydrogen peroxide evaporation.

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Results obtained: Syringes

	SURFACE (A)		AQUEOUS SOLUTION (B)
	Stopper	Glass	
Test A1	3.8 ppm	6 ppm	
Test A2	1.8 ppm	7 ppm	
Test B1			L.T. 0.5 ppm
Test B2			L.T.0.5 ppm

The measurement was performed on the syringe surface, with the paper label.

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Results obtained: Syringes

	SURFACE (A)		AQUEOUS SOLUTION (B)
	Stopper	Glass	
Test A1	0.4 ppm	0.4 ppm	
Test A2	0.2 ppm	0.3 ppm	
Test B1			L.T. 0.5 ppm
Test B2			L.T. 0.5 ppm

The measurement was performed on the syringe surface, without the paper label.

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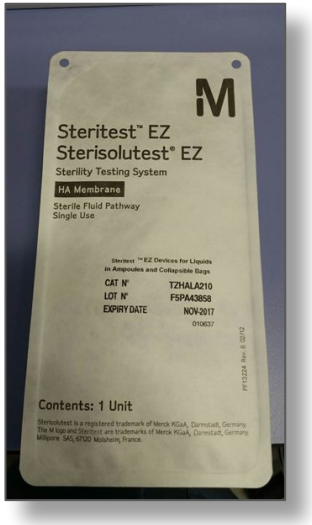
Results obtained: Millipore Steritest canister device HA membrane



	SURFACE (A)		HEAD SPACE (C)
	Tyvek	Plastic	
Test A1	9 ppm	0.3 ppm	
Test A2	6 ppm	0.3 ppm	
Test C1			2.4 ppm
Test C2			3 ppm

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Millipore Steritest canister device HA membrane

The most critical part of the canister is the plastic part next to the filtration membrane. After measuring the concentration inside the canister we opened it and we read 5 ppm next to the membrane.

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Results obtained: Syringe pack

	SURFACE (A)		HEAD SPACE (C)
	Tyvek	Plastic	
Test A1	16 ppm	0.3 ppm	
Test A2	20 ppm	0.4 ppm	
Test C1			1.9 ppm
Test C2			0.1 ppm *

If the concentration read by the sensor is a high concentration, it takes several minutes, after the test, to clean the sensor reaching again 0 ppm. The analyses can't be consecutive.

The holding time between the two analyses was 5 minutes. (*)The second reading didn't confirm the first result obtained. The cellulosic material the syringe pack is made with doesn't represent a strong barrier against Hydrogen peroxide evaporation.

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Results obtained: Scharlau TSA 55 mm Rodac contact plates

	SURFACE (A)	HEAD SPACE (C)
Test A1	0 ppm	
Test C1, External bag		0 ppm
Test C2, Internal bag		0 ppm

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Results obtained: Scharlau TSA 90 mm Petri plates

	SURFACE (A)	HEAD SPACE (C)
Test A1	3.8 ppm	
Test C1, External bag		3.6 ppm
Test C2, Internal bag		0 ppm

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Conclusions

- Hydrogen Peroxide is absorbed by different materials; the worst case ones are represented by plastics, cellulosic materials and Tyvek®.
- The label on the glass surface absorbs hydrogen peroxide; its absence reduces the absorption.
- A microbiological validation is suggested to evaluate if the residuals of hydrogen peroxide can affect the final results.

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Corrective actions

Every sample which shows residuals of hydrogen peroxide needs to be investigated.

There are different approach to proceed:

- to change decontamination cycle recipe in order to reduce H_2O_2 absorption/penetration ;
- to validate the sterility test procedure in spite of the residuals of biocide found.

The former approach entails to analyze again every sample with the chemical procedure described, the latter implies an inoculum of microorganisms according to “Grow Promotion Test” described by Pharmacopoeias.

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Strategy to reduce H_2O_2 absorption/penetration

A “peak cycle” can often solve this problem: higher concentration than a standard cycle (i.e. 1300 ppm vs 700 ppm) but a shorter exposure time (i.e. 5 minutes vs 30 minutes).

The “killing effect” of the biocide is obtained because of the high concentration but the short exposure time prevents or reduces the absorption inside the primary packaging material.

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Peak cycle for Petri plates in a double plastic bag

	Standard cycle	Peak cycle
Measurement location	**H_2O_2 residues (ppm)**	
External surface	1,1	0,2
Inside 1st bag	11,0	3,3
Inside 2nd bag	4,1	1,3

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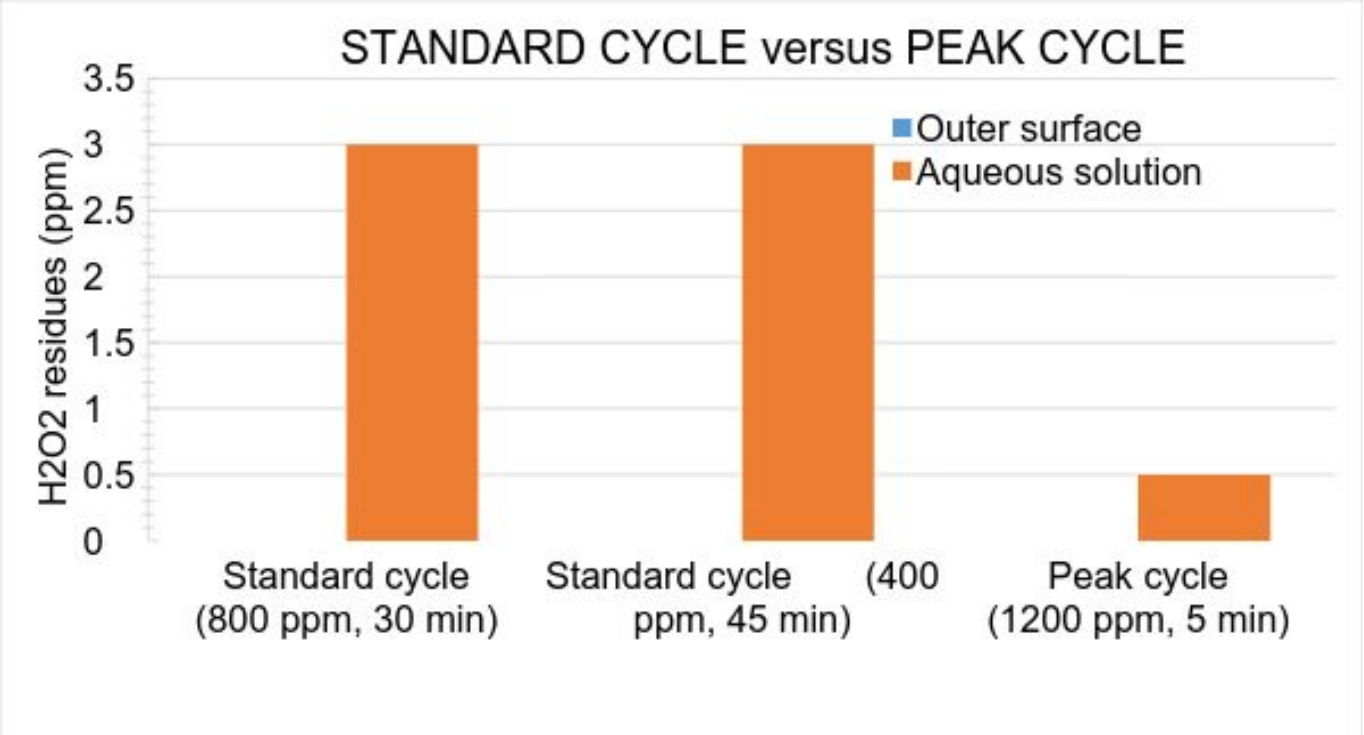
Standard cycle vs Peak Cycle



Microbial validation

After inactivating APIs with an antibiotic activity adding a neutralizing agent, after inoculating samples (the smaller the amount of colony forming units inoculated, the more challenging is the test) with less than 100 CFU, we perform a decontamination cycle and a sterility test.

At the end of the sterility test, after the incubation time required, growth has to be still visible.

A comparison has to be done with a positive control not exposed to a decontamination cycle.

Thank you.

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