DETERMINATION OF THE DECONTAMINATION PROCESS OF SURFACES BY THE APPLICATION OF THE HIGH LEVEL DISINFECTANT *ADANTIUM PLUS* WITH *HYGIENIO MACHINE*

FINAL REPORT

REPORT N° 5/2014

Client :

FIS & DM S.r.l.
Strada di Tavernolo, 2
05100 Terni (TR) Italy

Pages number: 18

COMPLIANCE STATEMENT

In relation to the conducted study, it is stated that the following report describes in faithfully and truthfully way the procedures we adopted and the results we obtained.
Tools and procedures are certified in accordance with:

A) ACCREDIA- UNI EN ISO/ IEC 17025:2005 " General requirements for the testing and calibration Laboratories competence" (Attachment III).

B) IQNet / DQS- Certification ISO 9001:2008 (Attachment IV).

C) HEALTHCARE INSTITUTE-Approval of the production and/or sterilization quality assurance system (Attachment V).

D) UNI EN ISO 4833-1:2013 : Horizontal Method for the microorganisms count. Part 1: Colonies count at 30 °C with the deep insemination technique.

E) ISO/TS 19036:2006/Amd 1:2009 Measurement uncertainty for low counts.

F) UNI EN ISO 15883-1 e 2:2009**-** Washing and disinfection equipment.

The study was coordinated by the working group:

U. Sciamannini, Ph.D ..... Date: 29/10/2014

(*Study Director*)

A. Sciamannini, A.D. ..... Date: 29/10/2014

(*General Administrator*)

In collaboration with:

Dr. S. Saveri *Senior Chemist FIS&DM Srl*

Dr L. Alzalamira *Biologist Jacaroni Diagnostic Centre*

Dr. L.Molinari *Biologist Jacaroni Diagnostic Centre*

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1. CLIENT DATA

FIS & DM S.r.l.
Strada di Tavernolo, 2
05100 Terni (TR) Italy

2. TEST LABORATORY

JACARONI Diagnostic Centre s.r.l.
Via I Maggio, 60
05100 Terni (TR) Italy
Operator: Dr. Marchetti

3. TEST RELATED DATE

Object of the study is the determination of the disinfection effectiveness of the *ADANTIUM PLUS* disinfectant though the use of the *HYGIENIO sanitizing.*

Responsible laboratories of the execution of the tests are ACCREDIA certificate (Accreditation number: 0820 Rev.2 Deadline: 2016-05-05- Attachment III).

Tests were conducted on the 14th October 2014 at the production office of FIS & DM Srl.

The tests of bacterial contamination has been performed on flat surfaces of Inox steel.

4. REFERENCE DOCUMENTS

4.1 International law.

For the protocol described in this relation, reference was made to the international law below specified.

- **Farmacopea Europea** – Current edition
- **UNI EN ISO 4833:2004** – Horizontal method for microorganisms count – Colonies count at 30 °C technique
- **UNI EN 15883-1:2009 –** Washing and disinfection tools - Part 1: General requirements, terms, definitions and tests
- **UNI EN 15883-2:2009** - Washing and disinfection tools - Parte 2: Requirements and tests for washing and disinfection tools for chirurgic tools, anaesthetic equipment, hollow bodies, floors, containers, utensils, glassware, etc., using thermal disinfection.

5. PURPOSE

The validation consists in the valuation of the efficiency of the washing/decontamination process, obtained by the synergic use of the "ADANTIUM PLUS" disinfectant, vaporized through the use of HYGIENIO disinfectant device in order to locate and estimate the presence/absence of vital microorganisms in certain condition of culture .

6 TEST DATA

6.1 HYGIENIO device description

Hygienio is an innovative system for the hot application of the disinfectant, it quickly allows disinfecting surfaces and material of every kind at a high level and with the reuse oh spaces and equipment.

Thanks to the HYGIENIO system it is possible to obtain a two-phase mixture (disinfectant + steam) that in contact with a generic surface condenses, allowing a continuous and homogeneous disinfectant treatment. The steam jets, full of disinfectant particles, invade, stinking to them, floors, walls, furniture and objects of every shape and material and no-solution of continuity material, that is, without leaving micro-spaces where bacteria, fungus and mildew that would normally continue to proliferate.

6.2ADANTIUM PLUS disinfectant description

ADANTIUM PLUS is a modern, concentrated synthetic preparation for simultaneous high-level cleansing and disinfection aimed at completely decontaminating Biomedical Devices in hospitals, according to current safety laws.

Adantium® Plus contains a new synthetic salt which, alone or resuspended with formula, is able to effectively hinder the nucleasic activity of the RNase present in plasma, preserving the added synthetic RNA from degradation (see the study of Prof. Pistello on the clinical validation).

Adantium® Plus, furthermore protects the cellular RNA from degradation through RNase A.

In conclusion, on the basis of the experimental proof obtained and considering the high resistance to the physical-chemical inactivation of the RNase, Adantium® Plus has an effective inhibitory role on these nucleases which, in a biological environment, has an important role in curbing/inactivating viral replication.

Active ingredients: Diphedac®, Complex surfactant (consisting of Alkyl-dimethyl-benzyl ammonium chloride.), Solvents Isopropylic Alchol and Surfactants.

6.3 Sampling plane

To conduct the bacteriological test, have been effected:

- A sample through sterile swab, after contamination of the surfaces;
- A sample through sterile swab, successive at HYGIENIO/*Adantium Plus* treatment, after contact with the contaminated surfaces;

7. MATERIALS AND METHODS USED.

7.1 Culture fields and tools of bacterial determination

Culture fields used for the detection of the contaminant charge:

Field

Type of field: Plate Count Agar (PCA)
Manufacturing company: Biogenetics
Site: 118480/073
Deadline: 01/12/15

atcc Shackles used for the contamination

Salmonella ATCC 14028
Escherichia coli ATCC 25922
Staphylococcus aureus ATCC 25923
Listeria monocytogenes ATCC 11

Every field already cited is sterile. The preparation of the fields has been conducted following the producer indications and/or according to the referring method.

Features of the equipment used for the test:

Incubator at 30°C

Inventory number:01
builder: TERMOSTABIL Kt
Model: K2 TE
Measure and test field: 20°C-70°C, used at 30°C ± 1°C

Fridge - freezer

Inventory number:04
Builder: INDESIT
Modell: TAAN 3V
Measurement and test field: fridge used from +2°C / +8°C

Fridge

Inventory number:06
Builder: KELVINATOR
Model: KG 101
Measurement and test field: fridge used from +2°C / +5°C

Colonies count

Inventory number:16
Builder: STUART
Model: SC6
Measurement and test field: na

Logger date (inside the incubator)

Inventory number:09
Serial number:0526-0116
Builder: Escort Data Logging Systems Ltd, New Zealand
Model: 62 D 32
Measurement and test field: used at 30°C ± 1°C

Logger date (inside the fridge 4)

Inventory number:21
Serial number: MI-BH-358-082
Builder: Escort Data Logging Systems Limited
Model: MI-0E-D-2-L
Measurement and test field: - 40°C/+65°C used between 2°C e 5°C

8. OPERATIVE PROCEDURES FOR THE MICROBIOLOGICAL COUNT

8.1 Execution methods of the biological analysis

A 600 x 400 mm inox steel surface has been contaminated with the ATCC bacterial inoculum (above described).

The first swab has been withdrawn (sample number: 01).

The surface has been treated with vapour jet generated by Hygienio containing *ADANTIUM PLUS* disinfectant diluited at 2,5% for a period of about 60 seconds.

After a period of 5 minutes has been dragged the second swab (sample number: 07) in the right side of the contaminated surface.

8.2 Test execution mode

Microorganisms at 30°C

It was taken a sterile Petri plate and it was transferred through a sterile pipette 1 ml of the initial suspension (dilution 10-1). Starting by the initial suspension, we have obtained some scalar dilutions until we arrived at the 10-4 dilution.
From every dilution has been withdrawn 1 ml, putting it in a Petri plate, where inside it was stilled for inclusion the PCA. Once solidified, the plates have been put to incubate at 30°C per 72 h ± 3 h. Then plates were been read through the use of colonies count, calculating the number of units forming colonies for ml.

8.3 Colonies count mode

The laboratory for the numeration techniques uses a plate for the dilution for almost two successive dilutions. After the period of necessary incubation, the colonies number is calculated.

MICROORGANISMS AT 30°C

These test methods include a maximum counting of colonies on the plate, equal to 300.

- For plates that don't contain colonies, the Laboratory expressed the result as <10 ufc per cm^2.

- For plates that contain a number of colonies between 1 and 3 on every sow plate, the Laboratory expresses the result as < 4 x d ufc per cm^2.

- For plates that contain a number of colonies between 4 and 300, the Laboratory applies, only after to have verified the proportionality of dilutions, the formula of the general case of ISO 7218, that is $N = \sum C / V \times 1{,}1 \times d$

where:

ΣC is the sum of the total number of colonies that were counted on every plate;
V is the volume of the inoculum stilled on every plate, in ml;
d is the dilution corresponding at the first considered dilution (d = 1 when referring to the liquid product not diluted of the sample test).

9. RESULTS

The results obtained are resumed in the following table and brought back in the certificate presented in the attachments (ALLEGATI VI- X)

MICROBIOLOGIC COUNT (UFC/ml)	
RESULTS	**Pre Treatment**
Total microbial charge control swab, control N01	$2,8 \times 10^3$ (UFC/cm^2)
	Post Treatment
Total microbial charge control swab, control N07 after a contact time of 5 minutes	< 1 (UFC/cm^2)

10. CONCLUSIONS

Results obtained with disinfection Hygienio- *ADANTIUM PLUS* system were very satisfactory, underlining the efficiency of the treatment that, during the contact time of 5 minutes, reconditions surfaces.

The test on microorganisms research turn out to be positive, satisfying completely the set goals.

As it can be noted from the test reports of the microorganisms count, the process reaches the highest efficiency in 5 minutes of contact, destroying completely the bacterial charge.

The achievement of this reduction in the time of a few minutes, let you continue quietly normal activities ambulatory.

This result is useful to the valuation of a economic return, in the field of Health operations, since it involves a reduction of the economic expense on materials, professional operators and mainly on the risk management.

11. ATTACHMENTS

I. Bacteria test photos

II. CERTIFICATION ACCREDIA LABORATORY JACARONI- " General requirements for the test and calibration laboratories competency".

III. IQNet / DQS- Certification ISO 9001:2008 US LABS Srl.

IV. INSTITUTE OF HEALTH-Approval of the system of warranty of production quality and/or sterilization of the US LABS Srl.

V. TEST REPORT JACARONI DIAGNOSTIC 3656/2014.
VI. TEST REPORT JACARONI DIAGNOSTIC 3662/2014.

ATTACHMENTS

ATTACHMENT I

Photo Bacterial Test

Foto 1: Bacterial Contamination of surface

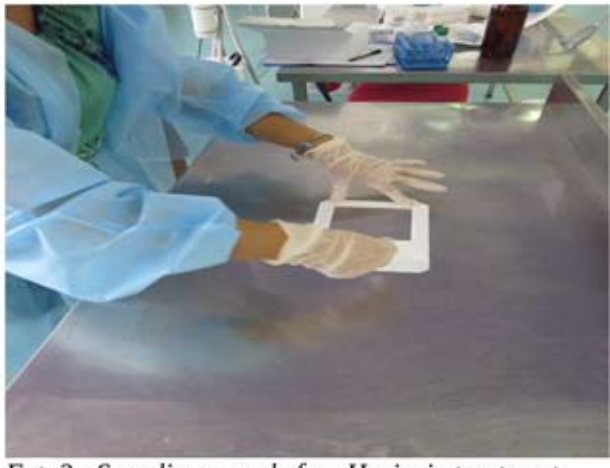

Foto2 : Sampling swap before Hygienio treatment

Foto 3: Hygienio-Adantium Plus tratment

Foto 4: Sampling swap at 5 minuts tratment

ATTACHMENT II

ACCREDIA
L'ENTE ITALIANO DI ACCREDITAMENTO

Membro degli Accordi di Mutuo Riconoscimento EA, IAF e ILAC
Signatory of EA, IAF and ILAC Mutual Recognition Agreements

ilac-MRA

CERTIFICATO DI ACCREDITAMENTO
Accreditation Certificate

Accreditamento n°
Accreditation n°
0820 Rev. **2**

Si dichiara che
We declare that
Laboratorio Settore di Microbiologia Alimentare dello Jacaroni Centro Diagnostico S.r.l.
Appartenente all'ente:
JACARONI CENTRO DIAGNOSTICO S.r.l.
Sede:
Via I Maggio 60 - 05100 Terni TR

è conforme ai requisiti della norma
meets the requirements of the standard
UNI CEI EN ISO/IEC 17025:2005 "Requisiti generali per la competenza dei Laboratori di prova e taratura"
EN ISO/IEC 17025:2005 "General Requirements for the Competence of Testing and Calibration Laboratories" standard

quale / *as*
Laboratorio di Prova
Testing Laboratory

L'accreditamento attesta la competenza tecnica del Laboratorio relativamente allo scopo riportato nelle schede allegate al presente certificato. Le schede possono variare nel tempo. I requisiti gestionali della ISO/IEC 17025:2005 (sezione 4) sono scritti in un linguaggio idoneo all'attività dei laboratori di Prova, sono conformi ai principi della ISO 9001:2008 ed allineati con i suoi requisiti applicabili.
Il presente certificato non è da ritenersi valido se non accompagnato dalle schede allegate e può essere sospeso o revocato in qualsiasi momento nel caso di inadempienza accertata da parte di ACCREDIA.
La vigenza dell'accreditamento può essere verificata sul sito WEB (www.accredia.it) o richiesta direttamente ai singoli Dipartimenti .

The accreditation certifies the technical competence of the laboratory limited to the scope detailed in the attached Enclosure. The scope may vary in the time. The management system requirements in ISO/IEC 17025:2005 (Section 4) are written in a language relevant to Testing laboratories operations and meet the principles of ISO 9001:2008 and are aligned with its pertinent requirements.
The present certificate is valid only if associated to the annexed schedule, and can be suspended or withdrawn at any time in the event of non fulfilment as ascertained by ACCREDIA.
The in force status of the accreditation may be checked in the WEB site (www.accredia.it) or on direct request to appointed Department.

Data di 1ª emissione *1st issue date* **2008-05-07**	Data di modifica *Modification date* **2012-03-29**	Data di scadenza *Expiring date* **2016-05-05**
Il Direttore Generale *The General Director* (Dr. Filippo Trifiletti)	Il Direttore di Dipartimento *Department Director* (Dr.ssa Silvia Tramontin)	Il Presidente *The President* (Cav. del Lav. Federico Grazioli)

ACCREDIA
Sede operativa e legale: Via Guglielmo Saliceto, 7/9 | 00161 Roma - Italy | Tel. +39 06 8440991 | Fax +39 06 8841199

ATTACHMENT III

CERTIFICATO

THE INTERNATIONAL CERTIFICATION NETWORK

CERTIFICATE

IQNet and
DQS GmbH Deutsche Gesellschaft zur Zertifizierung von Managementsystemen
hereby certify that the company

US LABS S.r.l.

Strada di Recentino, 7
05100 Terni
Italy

has implemented and maintains a **Quality Management System**.

Scope:
Research, development, production and selling of products for disinfection and sterilization

Through an audit, documented in a report, it was verified that the management system fulfills the requirements of the following standard:

ISO 9001 : 2008

Date of certification 2011-12-02

Valid until 2014-12-01

Registration Number: 428803 QM08

Michael Drechsel
Managing Director of DQS GmbH
President of IQNet

IQNet Partners*:

AENOR *Spain* AFNOR Certification *France* AIB-Vinçotte International *Belgium* ANCE *Mexico* APCER *Portugal* CCC *Cyprus* CISQ *Italy* CQC *China* CQM *China* CQS *Czech Republic* Cro Cert *Croatia* DQS Holding GmbH *Germany* DS *Denmark* ELOT *Greece* FCAV *Brazil* FONDONORMA *Venezuela* ICONTEC *Colombia* IMNC *Mexico* Inspecta Certification *Finland* IRAM *Argentina* JQA *Japan* KFQ *Korea* MSZT *Hungary* Nemko AS *Norway* NSAI *Ireland* PCBC *Poland* Quality Austria *Austria* RR *Russia* SII *Israel* SIQ *Slovenia* SIRIM QAS International *Malaysia* SQS *Switzerland* SRAC *Romania* TEST St Petersburg *Russia* TSE *Turkey* YUQS *Serbia*

IQNet is represented in the USA by: AFNOR Certification, CISQ, DQS Holding GmbH and NSAI Inc.

* The list of IQNet partners is valid at the time of issue of this certificate. Updated information is available under www.iqnet-certification.com

Istituto Superiore di Sanità

Organismo Notificato N° 0373 - Sezione Presso il Dipartimento AMPP
Notified Body N° 0373 Unit relating to the Department AMPP

APPROVAZIONE DEL SISTEMA DI GARANZIA DELLA QUALITA' DELLA PRODUZIONE E/O DELLA STERILIZZAZIONE

secondo l'Allegato V della Direttiva Europea 93/42/CEE e successive modificazioni
(recepita in Italia con il DLgs n. 46 del 24.02.1997 e successive modificazioni)

APPROVAL OF QUALITY ASSURANCE SYSTEM FOR PRODUCTION AND/OR STERILIZATION

according to Annex V of EC Directive 93/42/EEC and following modifications
(transposed in Italy by the DLgs n. 46 issued on 24.02.1997 and following modifications)

L'Istituto Superiore di Sanità, Organismo Notificato n° 0373, certifica che il sistema di garanzia della qualità della produzione e/o della sterilizzazione attuato da
The Istituto Superiore di Sanità, Notified Body n° 0373, certifies that the quality assurance system for the production and/or sterilization enforced by

U.S. LABS S.r.l.

SEDE LEGALE/ REGISTERED OFFICE: Str. di Recentino, 7 – 05100 Terni (TR) Italia

per il dispositivo/i
for the device/s

Dispositivi destinati al trattamento di dispositivi medici, non sterili *(vedi allegato/ see annex)*

è conforme ai requisiti applicabili della Direttiva Europea 93/42/CEE e successive modificazioni.
Is in compliance with the applicable requirements of EC Directive 93/42/EEC and following modifications.

Certificato n° *Certificate no.*	**066**	**QPZ**	**1296**	**11**	Addendum n° *addendum no.*	//	//

Data di emissione *Issue date*	**23.09.2011**	Data di validità massima *Maximum expiration date*	**22.09.2016**

Il Responsabile Della Sezione
(Dott.ssa Luciana Gramiccioni)
The Director of the Unit

ATTACHMENT V

MD 02/05-06

Jacaroni Centro Diagnostico s.r.l.
Via I Maggio 60 – Terni
Tel. 0744/428179
Fax 1782234197
www.jacaroni.org
jacaroni@email.it
Partita iva 01296810557

RAPPORTO DI PROVA

N° 3656
Anno: 2014
Pag 1 di 1

Luogo prelievo: FIS & DM S.r.l.	Cliente: FIS & DM S.r.l.
Campionamento eseguito da: Dott.ssa Laila Alzalamira	
Specifica di campionamento: ISO 18593:2004	Indirizzo: Strada di Tavernolo, 2 – Terni (TR)
Trasporto eseguito da: Dott.ssa Laila Alzalamira	

Descrizione del Campione

Tipologia: **TAMPONE SU SUPERFICI DELLE AREE DI LAVORAZIONE**		
Punto di prelievo: Piano in acciaio, contaminato	Data e ora prelievo: **14/10/14 h. 12.45** Numero Campione: **01** Numero lotto/data di produzione: **/**	Data di accettazione: 14/10/14 Temperatura in accettazione: 3,0°C

Riportare le voci applicabili, barrare le *altre*.

Parametro ricercato	Metodo/anno	Risultato di prova	Incertezza	Unità di misura	Data Prova	
					Inizio	Fine
Conta microrganismi a 30° C	UNI EN ISO 4833-1:2013	**$2,8x10^3$**	/	**ufc/cm^2**	14/10/14	17/10/14

I risultati dell'incertezza di misura, quando riportati, per le prove sopra elencate sono stati stimati con un fattore di copertura k = 2, corrispondente ad una probabilità di circa il 95 %.

Note

Il presente rapporto di prova riguarda solo il campione sottoposto a prova, e non può essere riprodotto parzialmente, salvo approvazione scritta del Laboratorio.

Data: 20/10/14

Il Responsabile del Laboratorio
Dr.ssa Alzalamira Laila

ATTACHMENT VI

MD 02/05-06

LAB N° 0820

Jacaroni Centre Diagnostics s.r.l.
Via I Maggio 60 – Terni
Tel. 0744/425179
Fax 1782234197
www.jacaroni.org
jacaroni@email.it
Partita iva 01296810557

RAPPORTO DI PROVA

N° 3662
Anno: 2014
Pag 1 di 1

Luogo prelievo: FIS & DM S.r.l. Campionamento eseguito da: Dott.ssa Laila Alzalamira Specifica di campionamento: ISO 18593:2004 Trasporto eseguito da: Dott.ssa Laila Alzalamira	Cliente: FIS & DM S.r.l. Indirizzo: Strada di Tavernolo, 2 – Terni (TR)

Descrizione del Campione

Tipologia: **TAMPONE SU SUPERFICI DELLE AREE DI LAVORAZIONE**		
Punto di prelievo: Piano in acciaio, sanificato con Hygienio- Adantium Plus, dopo un tempo di contatto di 5 minuti	Data e ora prelievo: **14/10/14 h. 12.45** Numero Campione: **07** Numero lotto/data di produzione: */*	Data di accettazione: 14/10/14 Temperatura in accettazione: 3,0°C

Riportare le voci applicabili, barrare le *altre*.

Parametro ricercato	Metodo/anno	Risultato di prova	Incertezza	Unità di misura	Data Prova	
					Inizio	Fine
Conta microrganismi a 30° C	UNI EN ISO 4833-1:2013	< 1	/	ufc/cm^2	14/10/14	17/10/14

I risultati dell'incertezza di misura, quando riportati, per le prove sopra elencate sono stati stimati con un fattore di copertura k = 2, corrispondente ad una probabilità di circa il 95 %.

Note

Il presente rapporto di prova riguarda solo il campione sottoposto a prova, e non può essere riprodotto parzialmente, salvo approvazione scritta del Laboratorio.

Data: 20/10/14

Il Responsabile del Laboratorio
Dr.ssa Alzalamira Laila