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Summary of Microbiological Test Data (Alcohol Foam)

ISSUE 3

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Deb Group Worldwide

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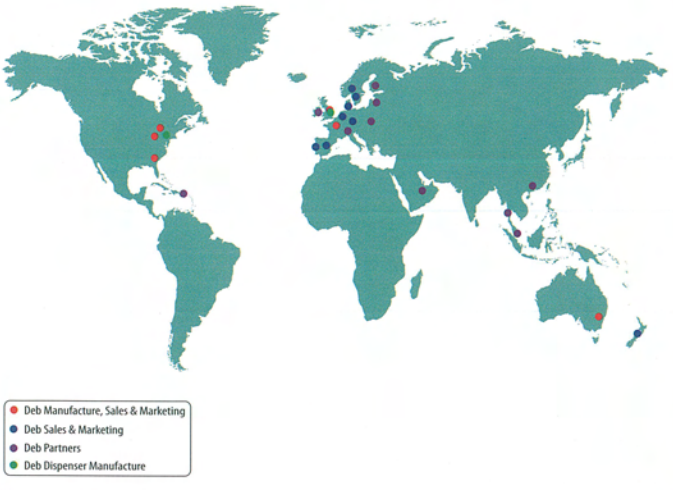

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EN1276

Objective

This European Standard is a quantitative suspension test and is used for the evaluation of bactericidal activity of a product.

General Study Information

Protocol:	EN 1276 Draft for revision (May 2002)
Test House:	HygCen GmbH Schwerin, Germany
Date of Report:	07/11/2006
Report Ref:	SN5656 EN 1276
Test Product:	Foaming Alcohol AFS 6510
Study Dates:	07/06/2006 - 07/08/2006

Summary of Test Conditions

Test Product:	Foaming Alcohol AFS 6510
Product Test Concentrations:	80%, 50% (v/v end concentrations)
Test Temperature:	20 ± 1°C
Neutraliser/Rinsing Liquid:	3.0% polysorbate 80 + 3.0% saponine + 0.1% histidine +0.1% cysteine (TSHC)
Interfering substance:	Without loading
Counting procedure:	Pour plate method
Incubation:	48h at 36 ± 1°C
Test Strains:	*Staphylococcus aureus* ATCC 6538 *Enterococcus hirae* ATCC 10541 *Escherichia coli* ATCC 10536 *Pseudomonas aeruginosa* ATCC 15442
Contact Time:	1.0 minute

Test Results

According to EN 1276 Draft for revision (May 2002), Foaming Alcohol possesses bactericidal activity in 1 minute at 20°C for the referenced strains *Staphylococcus aureus, Enterococcus hirae, Escherichia coli & Pseudomonas aeruginosa* when diluted at 80% (v/v).

Study Conclusion

Foaming Alcohol has been shown to possess bactericidal activity.

RAPID GERMICIDAL ACTIVITY
SALMONELLA TYPHIMURIUM

Objective

The objective of this testing was to produce data that provides basic information on rate-of-kill of Foaming Alcohol against *Salmonella typhimurium*.

General Study Information

Protocol:	Time Kill Test Assay for Antimicrobial Agents
Test House:	ATS Labs Eagan Minnesota, USA
Date of Report:	09/29/2006
Report Ref:	A04224
Test Product:	Foaming Alcohol AFS 6510 Lot 113
Study Dates:	08/29/2006 - 09/29/2006

Summary of Test Conditions

Test Product:	Foaming Alcohol AFS 6510
Dilution:	Ready to use
Exposure Temperature:	21°C
Organic Soil Load:	5% fetal bovine serum
Test Organism:	*Salmonella typhimurium* ATCC 6539
Exposure Time:	15 seconds

Test Results

Foaming Alcohol demonstrated >99.999% or >5.8 log reduction of *Salmonella typhimurium* ATCC 6539 after a 15 second contact time at 21°C.

Study Conclusion

Foaming Alcohol demonstrated effectiveness >5.8 log reduction) by undiluted application against *Salmonella typhimurium* after a contact time of 15 seconds.

EN1275
CANDIDA ALBICANS

Objective

This European Standard is a quantitative suspension test and is used for the evaluation of fungicidal activity of a product.

General Study Information

Protocol:	EN 1275 (March 2006)
Test House:	HygCen GmbH Schwerin, Germany
Date of Report:	07/11/2006
Report Ref:	SN 5656 DIN EN 1275 *C. albicans*
Test Product:	Foaming Alcohol AFS 6510
Study Dates:	07/06/2006 - 07/08/2006

Summary of Test Conditions

Test Product:	Foaming Alcohol AFS 6510
Product Test Concentrations:	80%, 50% (v/v end concentrations)
Diluent:	Distilled water
Test Temperature:	20 ± 1°C
Inactivation Method:	Dilution - neutralization
Test Strain:	*Candida albicans* ATCC 10231
Contact Time:	1.0 minute
Counting procedure:	Pour plate method
Incubation:	30 ± 1°C for 48h
Neutraliser or Rinsing Liquid:	3.0% polysorbate 80 + 3.0% saponine + 0.1% histidine + 0.1% cysteine (TSHC)

Test Results

According to EN 1275 Foaming Alcohol possesses yeasticidal activity at 20°C in 1 minute for the referenced strain *Candida albicans* when diluted at 80% (v/v).

Study Conclusion

Foaming Alcohol has been shown to possess yeasticidal activity.

EN1275
ASPERGILLUS NIGER

Objective

This European Standard is a quantitative suspension test and is used for the evaluation of fungicidal activity of a product.

General Study Information

Protocol:	EN 1275 (March 2006) (modified method)
Test House:	HygCen GmbH Schwerin, Germany
Date of Report:	07/24/2006
Report Ref:	SN 5656 DIN EN 1275 *A.niger* 90%
Test Product:	Foaming Alcohol AFS 6510
Study Dates:	07/18/2006 - 07/22/2006

Summary of Test Conditions

Test Product:	Foaming Alcohol AFS 6510
Product Test Concentration:	90% (v/v end concentration - modified method)
Diluent:	Distilled water
Test Temperature:	20 ± 1°C
Inactivation Method:	Dilution - neutralization
Test Strain:	*Aspergillus niger* ATCC 16404
Contact Time:	1.0 minute
Counting procedure:	Pour plate method
Incubation:	30 ± 1°C for 96h
Neutraliser or Rinsing Liquid:	3.0% polysorbate 80 + 3.0% saponine + 0.1% histidine + 0.1% cysteine (TSHC)

Test Results

According to EN 1275 Foaming Alcohol possesses fungicidal activity at 20°C in 1 minute for the referenced strain *Aspergillus niger* when diluted at 90% (v/v).

Study Conclusion

Foaming Alcohol has been shown to possess fungicidal activity.

HUMAN INFLUENZA VIRUS A (H3N2)

Objective

The objective of this study was to evaluate the virus-inactivating properties of the hand/skin sanitizer Foaming Alcohol against human influenza virus A using a quantitative suspension assay according to EN14476: 2005(1).

General Study Information

Protocol:	EN 14476:2005
Test House:	Mikrolab GmbH Bremen, Germany
Date of Report:	10/07/2006
Report Ref:	E06ML344hl
Test Product:	Foaming Alcohol AFS 6510 Lot 113
Study Dates:	07/28/2006 - 10/07/2006

Summary of Test Conditions

Test Product:	Foaming Alcohol AFS 6510
Product Test Concentration:	80.0% and 10.0%
Test Temperature:	20 ± 0.5°C
Contact Times:	0.5, 1.0, 2.0 and 5.0 minutes
Interfering Substance:	PBS
Procedure to stop action of disinfectant:	Immediate dilution
Diluent:	Water of standardized hardness (10.0% solution)
Virus Strain:	Human influenza virus A/ Panama/2007/99 (H3N2)

Test Results

Foaming Alcohol (80.0%) was able to inactivate human influenza virus A after 30s in a quantitive suspension test. At that time, no human influenza virus was detectable. The reduction factor was ≥4.38.

Study Conclusion

Foaming Alcohol demonstrated effectiveness by undiluted application against human influenza virus A after a contact time of 30 seconds. Therefore, Foaming Alcohol can be declared as virucidal against human influenza virus A.

HERPES SIMPLEX VIRUS (TYPE 1)

Objective

The purpose of this study was to evaluate the antiviral properties of Foaming Alcohol against Herpes simplex virus type 1 when exposed (in suspension) for the specified exposure periods.

General Study Information

Protocol:	Modification of ASTM E1052
Test House:	ATS Labs Eagan, Minnesota, USA
Date of Report:	09/01/2006
Report Ref:	A04173
Test Product:	Foaming Alcohol AFS 6510 Lot 113
Study Dates:	08/09/2006 - 09/01/2006

Summary of Test Conditions

Test Product:	Foaming Alcohol AFS 6510
Dilution Tested:	Ready to use
Exposure Temperature:	34°C
Organic Soil Load:	5% fetal bovine serum
Virus:	Herpes simplex virus type 1 Strain F(1), ATCC VR-733
Exposure Time:	30 seconds

Test Results

Under the above test conditions, Alcohol Foam demonstrated a ≥99.99994% reduction in the stock virus titer. The log reduction in viral titer was ≥6.25$\log_{10}$.

Study Conclusion

Foaming Alcohol demonstrated effectiveness by undiluted application against Herpes simplex virus type 1 after an exposure time of 30 seconds.

HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

Objective

The purpose of this study was to evaluate the antiviral properties of Foaming Alcohol against Human Immunodeficiency Virus type 1 when exposed (in suspension) for the specified exposure time.

General Study Information

Protocol:	Modification of ASTM E1052
Test House:	ATS Labs Eagan, Minnesota, USA
Date of Report:	09/01/2006
Report Ref:	A04172
Test Product:	Foaming Alcohol AFS 6510 Lot 113
Study Dates:	08/08/2006 - 09/01/2006

Summary of Test Conditions

Test Product:	Foaming Alcohol AFS 6510
Dilution Tested:	Ready to use
Exposure Temperature:	34.0°C
Organic Soil Load:	10% fetal bovine serum
Virus:	Human Immunodeficiency Virus type 1, Strain HTLV-111B
Exposure Time:	30 seconds

Test Results

Under the above test conditions, Foaming Alcohol demonstrated a ≥99.99% reduction in the stock virus titer. The log reduction in viral titer was ≥4.0$\log_{10}$.

Study Conclusion

Foaming Alcohol demonstrated effectiveness by undiluted application against Human Immunodeficiency Virus type 1 after an exposure time of 30 seconds.

HEPATITIS A VIRUS

Objective

The purpose of this study was to evaluate the antiviral properties of Foaming Alcohol against Hepatitis A virus (in suspension) for the specified exposure period.

General Study Information

Protocol:	Modification of the Standard Test Method for Efficacy of Virucidal Agents Intended for Special Applications (ASTM E1052)
Test House:	ATS Labs Eagan, Minnesota, USA
Date of Report:	09/01/2006
Report Ref:	A04176
Test Product:	Foaming Alcohol AFS 6510 Lot 113
Study Dates:	08/08/2006 - 09/01/2006

Summary of Test Conditions

Test Product:	Foaming Alcohol AFS 6510
Dilution Tested:	Ready to use
Exposure Temperature:	35°C
Organic Soil Load:	5% fetal bovine serum
Virus Strain:	Hepatitis A virus, Strain HIM-175
Exposure Time:	30 seconds

Test Results

Under the above test conditions, Foaming Alcohol demonstrated 99.7% reduction in the stock virus titer. The log reduction in viral titer was 2.5 $\log_{10}$.

Study Conclusion

In the presence of 5% fetal bovine serum organic soil load, Foaming Alcohol demonstrated a 99.7% reduction in viral titer following a 30 second exposure time to Hepatitis A virus.

FELINE CALICIVIRUS
SURROGATE OF NOROVIRUS

Objective

The objective of this study was to determine the virus-eliminating effectiveness of Foaming Alcohol against norovirus, using feline calicivirus (FCV) as a surrogate. This virus was chosen because there is no cell culture method for noroviruses available and there are many similarities between both members of the family Caliciviridae.

The method of test chosen was in vivo, using the fingerpads of seven volunteers.

General Study Information

Protocol:	ASTM Standard E 1838-02
Test House:	MikroLab GmbH Bremen, Germany
Date of Report:	10/20/2006
Report Ref:	E06ML433F
Test Product:	Foaming Alcohol AFS 6510 Lots 113 and 122

Summary of Test Conditions

Test Product:	Foaming Alcohol AFS 6510
Concentration of Test Product:	Neat
Reference:	Standard Hard Water (200ppm calcium carbonate)
Virus Strain:	FCV F9
Contact Time:	30 sec

Test Results

Foaming Alcohol Lot 113 produced a reduction factor (RF) of 1.11 ± 0.46 and Lot 122 an RF of 1.39 ± 0.38, demonstrating a higher efficacy than Standard Hard Water with an RF of 1.03 ± 0.35.

Study Conclusion

In this study, the reduction factor (RF) served as a test parameter for effectiveness of the product. Foaming Alcohol produced an overall (mean) RF of 1.25 ± 0.44 after an exposure time of 30 seconds and was more effective against the FCV than the reference (p-value .0017).

AVIAN INFLUENZA VIRUS
USING H3N8 AS SURROGATE OF H5N1

Objective

The objective of this study was to evaluate the virus-inactivating properties of the hand/skin sanitizer Foaming Alcohol against avian influenza virus using a quantitative suspension assay according to EN 14476:2005 (1).

General Study Information

Protocol:	EN 14476:2005
Test House:	MikroLab GmbH Bremen, Germany
Date of Report:	10/07/2006
Report Ref:	E06ML344A1
Test Product:	Foaming Alcohol AFS 6510 Lot 113
Study Dates:	07/28/2006 - 10/07/2006

Summary of Test Conditions

Test Product:	Foaming Alcohol AFS 6510
Concentration of Test Product:	80% and 10%
Test Temperature:	20 ± 0.5°C
Interfering Substance:	PBS
Procedure to stop action of disinfectant:	Immediate dilution
Diluent:	Water of standardized hardness (10.0%)
Virus Strain:	Influenza virus A/duck/ Ukraine/1/63 (H3N8)
Contact Times:	0.5, 1.0, 2.0 and 5.0 minutes

Test Results

Foaming Alcohol (80.0%) was able to inactivate avian influenza virus after 30s in a quantitative suspension test. At that time, no avian influenza virus was detectable. The reduction factor was ≥4.25.

Study Conclusion

Foaming Alcohol demonstrated effectiveness by undiluted application against avian influenza virus after a contact time of 30 seconds. Therefore, Foaming Alcohol can be declared as virucidal against avian influenza virus.

RAPID GERMICIDAL ACTIVITY

Objective

The objective of this testing was to assess the in vitro rate of time kill of an antibacterial hand antiseptic product against each microorganism. The purpose of the study was to demonstrate broad and rapid antimicrobial efficacy of the product.

General Study Information

Protocol:	Time Kill Procedure ASTM E2315-03
Test House:	Worldwide Healthcare Inc Brantford, Ontario, Canada
Date of Report:	02/27/2007
Test Product:	AFS 6510 Lot 96
Study Dates:	June – August 2006

Summary of Test Method and Conditions

The test article was brought into contact with a known population of microorganisms for 15 seconds at 25°C ± 2°C. The test article was neutralized at the sampling time (15 seconds) and the surviving microorganisms were enumerated. The percent reduction and $\log_{10}$ reduction from an initial microbial population was calculated.

Test Product:	Foaming Alcohol AFS 6510
Dilution Tested:	Ready to use
Exposure Time:	15 seconds
Exposure Temperature:	25 ± 2°C

Test Organisms

Gram Negative Bacteria
Acinetobacter sp. (ATCC 9957)
Escherichia coli (ATCC 11229)
Escherichia coli (ATCC 25922)
Enterobacter aerogenes (ATCC 13048)
Klebsiella oxytoca (ATCC 15764) Drug resistant
Klebsiella pneumoniae (ATCC 51503) Drug resistant
Pseudomonas aeruginosa (ATCC 915442)
Pseudomonas aeruginosa (ATCC 27853)
Proteus mirabilis (ATCC 7002)
Serratia marcescens (ATCC 14756)
Salmonella choleraesuis (ATCC 10708)

Yeasts
Candida albicans (ATCC 10231)
Candida tropicalis (ATCC 750)

Gram Positive Bacteria
Staphylococcus aureus (ATCC 6538)
Staphylococcus aureus (ATCC 29213)
Staphylococcus aureus (ATCC 33591) MRSA
Staphylococcus aureus (ATCC 33592) MRSA
Staphylococcus epidermidis (ATCC 12228)
Staphylococcus hominis (ATCC 51624) MRSH
Staphylococcus haemolyticus (ATCC 29970)
Staphylococcus saprophyticus (ATCC 15305)
Micrococcus luteus (ATCC 7468)
Streptococcus pyogenes (ATCC 19615)
Streptococcus pneumoniae (ATCC 6303)
Enterococcus faecalis (ATCC 29212)
Enterococcus faecium (ATCC 51559) VRE
Enterococcus faecalis (ATCC 51299)
Enterococcus hirae (ATCC 10541)
Listeria monocytogenes (ATCC 19111)

Results and Conclusions

The results show that there were no survivors of each of the 29 test organisms after 15 seconds exposure to the test article AFS6510. Therefore, these results demonstrate that the test article, Foaming Alcohol AFS6510, shows broad and rapid germicidal activity. The efficacy results indicate that all organisms show >99.999% kill.

EN 1500

Objective

This European Standard specifies an in vivo test for assessing hygienic handrubs. The method of test simulates practical conditions for establishing whether a product for hygienic handrub reduces the release of transient flora when rubbed onto the artificially contaminated hands of volunteers.

General Study Information

Protocol:	prEN 1500 (2002-10)
Test House:	HygCen GmbH Schwerin, Germany
Date of Report:	05/09/2006
Report Ref:	2006-05-09 (SN 5445 1)
Test Product:	AFS 6510 Lot 96
Study Dates:	04/27/2006 – 05/09/2006

Summary of Test Conditions

Test Product:	Foaming Alcohol AFS 6510
Dilution test:	Neat
Test Strain:	*Escherichia coli* NCTC 10538
Exposure Time:	15 seconds
Neutralizer:	3.0% polysorbate 80 + 3.0% saponine + 0.1% histidine + 0.1% cysteine
Reference Procedure:	2 x 3ml 60% (v/v) propan-2-ol rubbed on the hands during 60 seconds.
Test Procedure:	2 pumps each of 1.5ml AFS 6510 rubbed on the hands during 15 seconds.

Test Results

The test procedure with AFS 6510 (3ml:15 seconds) resulted in a higher mean reduction factor of 4.65 log than the reference procedure. This difference is statistically significant.

Study Conclusion

The test product Foaming Alcohol AFS 6510 meets the requirements of EN 1500 using 3ml rubbed onto the hands during 15 seconds.

HUMAN ROTOVIRUS

Objective

Activity against this virus was determined using a quantitative suspension test with the method following the guideline of DVV and RKI.

General Study Information

Protocol:	Tests were carried out following the guideline of the Deutsche Vereinigung zur Bekämpfung de Viruskrankheiten e.V. (DVV, German Association for the Control of Virus Diseases) and the Robert Koch-Institute (RKI = Federal Office of Health)
Test House:	MikroLab GmbH Bremen, Germany
Date of Report:	10/07/2006
Report Reference:	E06ML344R
Test Product:	AFS 6510 Lot 113
Study Dates:	07/28/2006 – 10/07/2006

Summary of Test Conditions

Test Product:	Foaming Alcohol AFS 6510
Concentration of Test Product:	Undiluted (80.0%)
Test Temperature:	20 ± 0.5°C
Virus Strain:	Rotavirus strain WA
Contact Times:	0.5, 1.0, 2.0 and 3.0 minutes.
Procedure to stop action of disinfectant:	Immediate dilution or gel filtration

Test Results

Foaming Alcohol was examined undiluted (80.0%). Exposure times were 0.5, 1.0, 2.0 and 3.0 minutes. Foaming Alcohol was active against rotavirus undiluted in both assays after 30 seconds exposure time. The reduction factors were ≥ 5.13 ± 0.52 (1st assay) and ≥ 5.25 ± 0.48 (2nd assay). The mean value was ≥ 5.19 ± 0.35.

Study Conclusion

Therefore, evaluating Foaming Alcohol the following concentration and exposure time are necessary for inactivation of human rotavirus: **undiluted 30 seconds.**

HUMAN RHINOVIRUS (Type 14)

Objective

The objective of this study was to evaluate the virus-inactivating properties of the hand/skin sanitizer Foaming Alcohol against human rhinovirus type 14 using a quantitive suspension assay following EN 14476: 2007-02.

General Study Information

Protocol:	EN14476:2007-02
Test House:	MikroLab GmbH Bremen, Germany
Date of Report:	05/28/2008
Report Ref:	E07ML499Rh
Test Product:	Foaming Alcohol AFS 6510 D1194
Study Dates:	07/23/2007 – 05/28/2008

Summary of Test Conditions

Test Product:	Foaming Alcohol AFS 6510
Concentration of Test Product:	80% and 10%
Test Temperature:	20 ± 0.5°C
Interfering Substance:	PBS
Procedure to Stop Action of Disinfection:	Immediate dilution, gel filtration and test method following Lycke
Diluent:	Water of standardized hardness (10.0%)
Virus Strain:	Human rhinovirus type 14 (strain 1059)
Contact Times:	0.25, 0.5, 1.0 and 2.0 minutes

Test Results

Foaming Alcohol was able to inactivate human rhinovirus within 30 seconds in a quantitive suspension test. At that time, no human rhinovirus was detectable. The reduction factor was ≥ 4.38 following the method of Lycke.

Study Conclusion

Foaming Alcohol demonstrated effectiveness by undiluted application against human rhinovirus after a contact time of 30 seconds.

Therefore, Foaming Alcohol can be declared as virucidal against human rhinovirus.

CLOSTRIDIUM DIFFICILE
VEGETATIVE CELLS

Objective

The objective of this study was to evaluate the rate-of-kill of an antibacterial hand rub against *Clostridium difficile* (vegetative cells) using an in-vitro time kill method.

General Study Information

Protocol:	In-Vitro Time-Kill Method
Test House:	Bioscience Laboratories Inc. Bozeman, Montana, USA
Date of Report:	06/26/2008
Report Reference:	080512-201
Test Product:	Foaming Alcohol AFS 6510 Lot Number 357-05
Study Dates:	05/27/08 - 06/26/08

Summary of Test Conditions

Test Product:	Foaming Alcohol AFS 6510
Concentration of Test Product:	99% (v/v)
Neutralizer:	Butterfield's Phosphate Buffer Solution with product neutralizers (BPP++)
Test Organism:	*Clostridium difficile* ATTC 9689; vegetative cells
Contact Times:	15, 30 and 60 seconds

Test Results

Foaming Alcohol demonstrated >99.999% kill or >7.0 log reduction after a 15 second contact time.

Summary Conclusion

Foaming Alcohol demonstrated effectiveness (>7.0 log reduction) by undiluted application against *Clostridium difficile* (vegetative cells) after a contact time of 15 seconds.

INFLUENZA A/SWINE
(H1N1)

Objective

The objective of this study was to evaluate the anti-viral properties of Alcohol Foam when challenged with Influenza A/Swine (H1N1).

General Study Information

Protocol:	In-Vitro Time-Kill Method
Test House:	Bioscience Laboratories Inc. Bozeman, Montana, USA
Date of Report:	07/10/2009
Report Reference:	090443-402
Test Product:	Foaming Alcohol AFS 6510 Lot Number 470-04
Study Dates:	05/19/2009 - 07/10/2009

Summary of Test Conditions

Test Product:	Foaming Alcohol AFS 6510
Concentration of Test Product:	90% (v/v)
Neutralizer:	D/E Broth
Test Organism:	Influenza A Virus A/Swine/ IOWA/15/30(H1N1) (ATCC VR-333)
Contact Times:	30, 60 and 120 seconds

Test Results

Foaming Alcohol demonstrated 99.99% kill or 4.0 log reduction after 30, 60 and 120 seconds exposures.

Study Conclusion

Foaming Alcohol reduced the infectivity of Influenza A/Swine (H1N1) by 4.0 log (99.99% reduction) after a contact time of 30 seconds.

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