Septalkan®

Septalkan Lingettes (Toallitas)

Detergente desinfectante

con secado húmedo
Sin alcohol

Superficies, Equipamiento y

Dispositivos médicos
no esterilizables,
no sumergibles

Listo para uso

Expediente técnica

Alkapharm

Juntos contra el riesco de infeccion

Dossier Septalkan / 2021-04

SODEL - ISO 9001/13485
190 rue René Barthélemy, Pôle d'activités de l'Espérance, 14100 Lisieux, France.
TEL: +33 (0)2 31 31 10 50.
www.sodel-sa.eu - www.alkapharm.fr

Septalkan®

Septalkan Lingettes (Toallitas)

Detergente desinfectante

sin alcohol
con secado húmedo

Superficies, Equipamiento y
Dispositivos médicos
no esterilizables,
no sumergibles

Listo para uso

Noticia técnica

Alkapharm
Juntos contra el riesco de infeccion

SODEL - ISO 9001/13485
190 rue René Barthélemy, Pôle d'activités de l'Espérance, 14100 Lisieux, France.
TEL: +33 (0)2 31 31 10 50.
www.sodel-sa.eu - www.alkapharm.fr

Índice

Declaración de conformidad 3

Presentación 4

Ámbito de utilización 4

Características 4

Presentaciones comerciales 5

Uso 6

Información reglamentaria 6

Modo de empleo 6

Precauciones de empleo 7

Manipulación - Puesta en servicio - Almacenamiento 7

Vertido accidental - Eliminación 7

Datos físico-químicos 8

Composición 8

Características fisicoquímicas 8

Datos microbiológicos 9

Ensayos de determinación de la actividad bactericida 9

Ensayos de determinación de la actividad levuricida y fungicida 13

Ensayos de determinación de la actividad virucida 16

Ensayos de determinación de la actividad esporicida 19

Datos de compatibilidad 20

Informes o certificados de compatibilidad 20

Tanto los informes de los ensayos de microbiología y compatibilidad, como la ficha de datos de seguridad se proporcionarán por separado a pedido del interesado o en el archivo técnico.

Declaración de conformidad

SODEL garantiza y declara que el producto **Sept**alkan cumple los requisitos esenciales de la Directiva 93/42/CEE del 14 de junio de1993 relativa a los Dispositivos Médicos.

SODEL garantiza y declara que el producto **Sept**alkan **Lingettes (Toallitas)** cumple los requisitos esenciales de la Directiva 93/42/CEE del 14 de junio de1993 relativa a los Dispositivos Médicos.

Estos productos están en la clase IIb, de conformidad con la regla 15 del Anexo IX de la Directiva 93/42/CEE modificada por el Directiva 2007/47/CE que define que: "Todos los productos que se destinen específicamente a la desinfección de productos sanitarios se incluirán en la clase IIa, a no ser que estén destinados específicamente a la desinfección de productos invasivos, en cuyo caso se incluirán en la clase IIb".

CE 0459
2013

SODEL declara que el producto **Sept**alkan y **Sept**alkan **Lingettes (Toallitas)** no contiene:

- ninguna sustancia derivada de sangre humana;
- ninguna sustancia que, utilizada por separado, pueda ser considerada como un medicamento, en el sentido del artículo 1º de la Directiva 2001/83/CE;
- tejidos de origen animal;
- ftalatos;
- látex.

Septalkan y **Sept**alkan **Lingettes (Toallitas)** también son productos biocidas según el reglamento (UE) N° 528/2012 DEL PARLAMENTO EUROPEO Y DEL CONSEJO del 22 de mayo de 2012.

Septalkan es una preparación líquida TP2 y TP4.
Septalkan **Lingettes (Toallitas)** es una toallita pre-impregnado con una preparación líquida TP2 y TP4.

Presentación

⚠ En la presente guía técnica, el término **Sept**alkan designa indistintamente sus dos presentaciones comerciales: **Sept**alkan o **Sept**alkan **Lingettes (Toallitas)**. Las particularidades de cada presentación se indicarán explícitamente.

Ámbito de utilización

- **Sept**alkan se utiliza para la limpieza y desinfección en húmedo de las superficies de dispositivos médicos invasivos y no invasivos, reutilizables, no esterilizables por métodos convencionales y no desinfectables por inmersión debido a su tamaño, su fabricación o materiales constitutivos.
  - Dispositivos médicos invasivos:
    sondas endocavitarias envainadas entre cada paciente (solo de limpieza), endoscopios y fibroscopios flexibles envainadas, etc.
  - Dispositivos médicos no invasivos:
    sondas de ecografía, estetoscopios, termómetros, glucómetros, incubadoras (con toallitas y en ausencia del recién nacido), respiradores, superficies de los generadores de hemodiálisis, etc.
- **Sept**alkan se utiliza para la limpieza y desinfección con pulverización y con secado húmedo de todas las superficies, en el marco del control de riesgos microbiológicos asociados al medio ambiente.

  Septalkan es particularmente adecuado para ser utilizado en los servicios de exploración funcional, laboratorios, centros de transfusión y diálisis, servicios de neonatología y unidades móviles de atención sanitaria: ambulancias, servicio de incendios y auxilio, etc.

Características

- **Sept**alkan es una preparación detergente desinfectante sin alcohol.
- **Sept**alkan está formulado con menos de 0,1% de alcohols, sin EDTA, sin colorantes and sin perfumes.
- **Sept**alkan (spray) es bactericida, levuricida, activo en *Aspergillus fumigatus*, activo en Adenovirus, Norovirus, Rotavirus, virus de la Hepatitis C, virus de la Hepatitis B, VIH y virus de la Vaccine (modelo de virus envueltos como Coronavirus), y esporicida (*Bacillus subtilis*, *Clostridium difficile*).

  Septalkan **Lingettes (Toallitas)** es bactericida, levuricida, activo en Adenovirus, Norovirus, Rotavirus, virus de la Hepatitis C, virus de la Hepatitis B, VIH y virus de la Vaccine (modelo de virus envueltos como Coronavirus).

Presentaciones comerciales

Referencia	Envase	Embalaje
Septalkan		
ALKE182	Frasco pulverizador de 750 mL	12 frascos
Septalkan Lingettes (Toallitas)		
ALK0165	Bolsa “Flow Pack” de 100 toallitas (180x200mm)	12 bolsas

Uso

Información reglamentaria

- Como dispositivo médico de Clase IIb y biocida TP2/TP4, el etiquetado de **Sept**alkan cumple con los requisitos del anexo I de la Directiva 93/42/CEE y del artículo 69 del Reglamento (UE) no 528/2012. Incluye el manual de instrucciones y lleva el número de lote y la fecha de caducidad de cada fabricación, en la forma:

LOT XXXXXXXXXXXX

AAAA.MM

o

Produit : (01) ALKXXXX
Lot : (10) 6SXXNYYYY
DLU : (17) AAAAMM

- Toda la información sobre seguridad está reunida en la hoja de datos de seguridad consultable en el sitio web www.alkapharm.fr o disponible si se solicita previamente.
- Esta información es la que figura en la etiqueta del producto **Sept**alkan.

Información reglamentaria conforme al reglamento (CE) n°1272/2008 y sus modificaciones	
Símbolos de peligro	-
Menciones de peligro	Esta mezcla no presenta ningún peligro físico. Esta mezcla no presenta ningún peligro para la salud, excepto eventuales valores límite de exposición profesional. Esta mezcla no presenta ningún peligro para el medio ambiente.
Consejos de prudencia	P102 Mantener fuera del alcance de los niños. P501 Eliminar el contenido / contenedor como residuo no peligroso.

Modo de empleo

Septalkan está listo para usar.

Pulverizador de 750 ml

1. Pulverizar **Sept**alkan hasta cubrir toda la superficie a tratar (0,43g/cm² según NF T 72-281) o sobre un paño no tejido limpio.
2. Limpiar a fondo la superficie.
3. Dejar actuar el producto 5 minutos. No enjaguar (excepto en el caso de superficies en contacto directo con alimentos).
4. Cambiar de paño para cada equipo.

Toallitas

1. Sacar una toallita **Sept**alkan de la bolsa.
2. Limpiar minuciosamente la superficie a tratar.
3. Dejar actuar el producto 5 minutos. No enjaguar (excepto en el caso de superficies en contacto directo con alimentos).
4. Cambiar de toallita para cada equipo.
5. Cerrar bien la bolsa después de usarla.

Precauciones de empleo

- **Sept**alkan no presenta ningún peligro en particular para los usuarios, siempre que se respete el protocolo antes mencionado.

Manipulación - Puesta en servicio - Almacenamiento

- **Sept**alkan se deberá conservar en su embalaje y con el etiquetado original, que describe el modo de uso y las precauciones de empleo y seguridad.
- **Sept**alkan se deberá conservar bien cerrado, en un lugar fresco alejado de toda fuente de calor.
- Si el producto, en condiciones accidentales y durante un tiempo prolongado, estuviese a proximidad de una fuente intensa de calor, es preferible que no se vuelva a utilizar, ya que sus cualidades, propiedades y características podrían haberse deteriorado.
- La caducidad de **Sept**alkan es de 3 años a partir de la fecha de fabricación.

Caducidad
35 meses después de la fecha de fabricación. Indicada en la etiqueta de la siguiente manera: AAAA.MM (Año-Mes)

- Cierre el envase inmediatamente después de usarlos para preservar las cualidades, propiedades y características de **Sept**alkan.

Vertido accidental - Eliminación

- Es necesario recuperar con precaución el contenido de los envases dañados y eliminar los recipientes.
- El producto vertido deberá ser absorbido por un soporte inerte.
- Los residuos recuperados se eliminarán a través del canal adecuado.

Datos físico-químicos

Composición

Composición detergente
Tensioactivos catiónicos y no iónicos, Desinfectantes. Contiene menos de 0,1% de alcohol.
Composición biocida
Preparación liquida lista para usar TP2/TP4. Toallita preimpregnado con una preparación líquida lista para usar TP2/TP4. CLORURO DE DIDECILDIMETILAMONIO (CAS: 7173-51-5) a 0,25 g/kg. CLORURO DE ALQUIL (C12-16) BENCILDIMETILAMONIO (CAS: 68424-85-1) a 0,95 g/kg.

Características fisicoquímicas

Aspecto	Líquido fluido incoloro
Olor	Sin perfume (olor característico)
Densidad	1,00 ± 0,01
pH	7,00 ± 0,25

Datos microbiológicos

Ensayos de determinación de la actividad bactericida

Norma NF EN 13727+A1 (diciembre 2013)

Condiciones del ensayo	
Cepas:	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Enterococcus hirae*
Sustancias interferentes:	Condiciones de suciedad: albúmina bovina 3 g/l y eritrocitos de corderos a 3 ml/l
Tiempo de contacto:	5 minutos +/- 10 sec.
Temperatura:	20°C +/- 1°C

Informe de ensayo n°034-1 REA 15 del 03/08/2015
Laboratorio SCIENTIS

Conclusión: De conformidad con la norma EN 13727+A1, el producto F2563 (Septalkan) lote n°2013.03.01.70, listo para uso, posee una actividad bactericida en 5 minutos a 20°C, en condiciones de suciedad, sobre las 3 cepas bacterianas de referencia.

Norma NF EN 14561 (marzo de 2007)

Condiciones del ensayo	
Cepas:	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Enterococcus hirae*
Sustancias interferentes:	Condiciones de suciedad: albúmina bovina 3g/l y eritrocitos de corderos a 3ml/l
Tiempo de contacto:	5 minutos
Temperatura:	20°C

Informe de ensayo n° 061F02-2012-01 del 13/07/2012
Laboratorio APEX Bio Solutions

Conclusión: De conformidad con la norma EN 14561 (marzo de 2007), el producto «aerosol desinfectante sin alcohol de pH neutro» (Septalkan), lote n°2163F2563A, posee una actividad bactericida en las tres cepas bacterianas de referencia al emplearse puro durante 5 minutos de contacto a 20°C, en condiciones de suciedad (3 g/l de albúmina bovina y 3ml/l de eritrocitos de corderos finales).

Norma NF EN 1276 (marzo de 2010)

Condiciones del ensayo	
Cepas:	*Pseudomonas aeruginosa* *Escherichia coli* *Staphylococcus aureus* *Enterococcus hirae*
Sustancias interferentes:	Condiciones de suciedad: albúmina bovina 3g/l
Tiempo de contacto:	5 minutos +/- 10 sec.
Temperatura:	20°C +/- 1°C

Informe de ensayo n° RE 14359-2 del 18/11/2014
Laboratorio MIDAC

Conclusión: De conformidad con la norma europea EN 1276 (marzo de 2010), el producto Septalkan lote 2013 03 01 70 presenta una actividad bactericida en las condiciones de ensayo obligatorias: condiciones de suciedad - 5 minutos de contacto a 20º C +/- 1°C - con una concentración en el ensayo del 10 % (v/v) en las cuatro cepas probadas.

Norma NF EN 13697 (junio de 2015)

Condiciones del ensayo	
Cepas:	*Pseudomonas aeruginosa* *Escherichia coli,* *Staphylococcus aureus* *Enterococcus hirae*
Sustancias interferentes:	Condiciones de suciedad: albúmina bovina 3g/l
Tiempo de contacto:	1 minuto +/- 10 sec.
Temperatura:	18-25°C +/- 1°C

Informe de ensayo n° RE 16250-3 del 30/11/2016
Laboratorio MIDAC

Conclusión: De conformidad con la norma NF EN 13697 (junio de 2015), el producto Septalkan presenta una actividad bactericida en las superficies, probado con una concentración del 100% (v/v) en las siguientes condiciones de ensayo: 1 minuto de contacto a 20°C, condiciones de suciedad (3g/l de albúmina bovina).

Norma europea EN 16615 (mayo de 2015)

Condiciones del ensayo	
Cepas:	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Enterococcus hirae*
Sustancias interferentes:	Condiciones de suciedad: albúmina bovina 3g/l y eritrocitos de corderos a 3ml/l
Tiempo de contacto:	5 minutos +/- 15 sec.
Temperatura:	20°C +/- 2.5°C

Informe de ensayo n° RE 16088-4 del 21/06/2016
Laboratorio MIDAC

Conclusión: De conformidad con la norma NF EN 16615 (mayo de 2015), el producto "SEPTALKAN toallita – flow pack" – lote 2015 06 03 5201 presenta una actividad bactericida en las superficies en 5 minutos de contacto a 20°C, en condiciones de suciedad (3g/l de albúmina bovina y 3ml/l de eritrocitos de corderos).

Norma NF T 72-281 (mayo de 2009)

Condiciones del ensayo	
Cepas:	*Pseudomonas aeruginosa* *Escherichia coli* *Staphylococcus aureus* *Enterococcus hirae*
Tiempo de pulverización:	3 pulverizaciones (5 sec.) a 20cm
Peso:	1,35g
Superficie de contacto:	12,56 cm² (disco de acero inoxidable con un diámetro de 2 cm)
Tiempo de contacto:	5 minutos
Temperatura:	21°C

Informe de ensayo n°156B11-2012 F2563 del 05/10/2012
Laboratorio APEX Bio Solutions

Conclusión: Los ensayos muestran que el producto F2563 (Septalkan), lote nº2163F2563A, presenta una actividad bactericida por vía aérea, de conformidad con la norma NF T 72-281 (mayo de 2009), en una dosis de 3 pulverizaciones manuales y 5 minutos de contacto.

Norma NF T 72-281 (Mayo de 2009) equipado con pulverización + limpie con toallitas después del contacto con el producto

Condiciones del ensayo	
Cepas:	*Pseudomonas aeruginosa* *Escherichia coli* *Staphylococcus aureus* *Enterococcus hirae*
Tiempo de pulverización:	3 pulverizaciones (5 sec.) a 20cm
Peso:	1,41g
Número de limpieza:	3 : volver-go, con una toallita estéril
Superficie de contacto:	12,56 cm² (disco de acero inoxidable con un diámetro de 2 cm)
Tiempo de contacto:	5 minutos de contacto con la toallita
Temperatura:	21,6°C

Informe de ensayo n°156B11-201-01 del 05/10/2012
Laboratorio APEX Bio Solutions

Conclusión: Los ensayos muestran que el producto F2563 (Septalkan), lote nº2163F2563A, presenta una actividad bactericida por vía aérea, de conformidad con la norma NF T 72-281 (mayo de 2009), en una dosis de 3 pulverizaciones manuales y 5 minutos de contacto, con la limpieza con toallita.

Ensayos de determinación de la actividad levuricida y fungicida

Norma NF EN 13624 (noviembre de 2013)

Condiciones del ensayo	
Cepas:	*Candida albicans*
Sustancias interferentes:	Condiciones de suciedad: albúmina bovina 3g/l y eritrocitos de corderos a 3ml/l
Tiempo de contacto:	5 minutos
Temperatura:	20°C +/- 1°C

Informe de ensayo n°037-1 REA 15 CI del 30/08/2015
Laboratorio SCIENTIS

Conclusión: De conformidad con la norma EN 13624 (noviembre de 2013), el producto F2563 (Septalkan) lote n°2013.03.01.70, listo para uso, posee una actividad levuricida en 5 minutos a 20°C, en condiciones de suciedad, sobre la cepa de referencia *Candida albicans*.

Norma NF EN 14562 (septiembre de 2006)

Condiciones del ensayo	
Cepas:	*Candida albicans*
Sustancias interferentes:	Condiciones de suciedad: albúmina bovina 3g/l y eritrocitos de corderos a 3ml/l
Tiempo de contacto:	5 minutos
Temperatura:	20°C

Informe de ensayo n° 061F02-2012-02 del 13/07/2012
Laboratorio APEX Bio Solutions

Conclusión: De conformidad con la norma EN 14562 (septiembre de 2006), el producto «aerosol desinfectante sin alcohol de pH neutro» (Septalkan), lote n°2163F2563A, posee una actividad levuricida en la cepa de referencia *Candida albicans* al emplearse puro durante 5 minutos de contacto a 20°C, en condiciones de suciedad (3g/l de albumina bovina y 3ml/l de eritrocitos de corderos finales).

Norma NF EN 1650 (julio de 2013)

Condiciones del ensayo	
Cepas:	*Candida albicans*
Sustancias interferentes:	Condiciones de suciedad: albúmina bovina 3g/l
Tiempo de contacto:	5 minutos +/- 10 sec.
Temperatura:	20°C +/- 1°C

Informe de ensayo n° RE 14357-3 del 18/11/2014
Laboratorio MIDAC

Conclusión: De conformidad con la norma NF EN 1650 (julio de 2013), el producto Septalkan lote 2013.03.01.70 presenta una actividad levuricida probada con una concentración del 80% (v/v) en las siguientes condiciones de ensayo: 5 minutos de contacto a 20ºC, en condiciones de suciedad (3g/l de albúmina bovina), con respecto a *Candida albicans* CIP 4872.

Norma NF EN 13697 (noviembre de 2001)

Condiciones del ensayo	
Cepas:	*Candida albicans*
Sustancias interferentes:	Condiciones de suciedad: albúmina bovina 3g/l
Tiempo de contacto:	5 minutos +/- 10 sec.
Temperatura:	21°C +/- 1°C

Informe de ensayo n° RE 14363-3 del 18/11/2014
Laboratorio MIDAC

Conclusión: De conformidad con la norma europea EN 13697 (noviembre de 2001), el producto Septalkan lote 2013.03.01.70, presenta una actividad levuricida probada con una concentración del 10% (v/v) en las siguientes condiciones de ensayo: 5 minutos de contacto a 21ºC, en condiciones de suciedad (3g/l de albúmina bovina), con respecto a *Candida albicans*.

Norma europea EN 16615 (mayo de 2015)

Condiciones del ensayo	
Cepas:	*Candida albicans*
Sustancias interferentes:	Condiciones de suciedad: albúmina bovina 3g/l y eritrocitos de corderos a 3ml/l
Tiempo de contacto:	5 minutos +/- 15 sec.
Temperatura:	20°C +/- 2.5°C

Informe de ensayo n° RE 16088-3 del 15/07/2016
Laboratorio MIDAC

Conclusión: De conformidad con la norma NF EN 16615 (mayo de 2015), el producto "SEPTALKAN toallita – flow pack" lote 2015 06 03 5201 presenta una actividad levuricida en las superficies en 5 minutos de contacto a 20°C, en condiciones de suciedad (3g/l de albúmina bovina y 3ml/l de eritrocitos de corderos).

Norma NF T 72-281 (mayo de 2009)

Condiciones del ensayo	
Cepas:	*Candida albicans*
Tiempo de pulverización:	3 pulverizaciones (5 sec.) a 20cm
Peso:	1,35g
Superficie de contacto:	12,56 cm² (disco de acero inoxidable con un diámetro de 2 cm)
Tiempo de contacto:	5 minutos
Temperatura:	21°C

Informe de ensayo n°156B11-2012 F2563 del 05/10/2012
Laboratorio APEX Bio Solutions

Conclusión: Los ensayos muestran que el producto F2563 (Septalkan), lote nº2163F2563A, presenta una actividad levuricida por vía aérea, de conformidad con la norma NF T 72-281 (mayo de 2009), en una dosis de 3 pulverizaciones manuales y 5 minutos de contacto.

Norma NF T 72-281 (Mayo de 2009) equipado con pulverización + limpie con toallitas después del contacto con el producto

Condiciones del ensayo	
Cepas:	*Candida albicans*
Tiempo de pulverización:	3 pulverizaciones (5 sec.) a 20cm
Peso:	1,41g
Número de limpieza:	3 : volver-go, con una toallita estéril
Superficie de contacto:	12,56 cm² (disco de acero inoxidable con un diámetro de 2 cm)
Tiempo de contacto:	5 minutos de contacto con la toallita
Temperatura:	21,6°C

Informe de ensayo n°156B11-201-01 del 05/10/2012
Laboratorio APEX BIO SOLUTIONS

Conclusión: Los ensayos muestran que el producto F2563 (Septalkan), lote nº2163F2563A, presenta una actividad levuricida por vía aérea, de conformidad con la norma NF T 72-281 (mayo de 2009), en una dosis de 3 pulverizaciones manuales y 5 minutos de contacto, con la limpieza con toallita.

Norma NF T 72-281 (mayo de 2009) adecuado para toallitas preimpregnadas en el *Aspergillus fumigatus*

Condiciones del ensayo	
Cepas:	*Aspergillus fumigatus*
Superficie de contacto:	12,56 cm² (disco de acero inoxidable con un diámetro de 2 cm)
Tiempo de contacto:	5 minutos
Temperatura:	21°C

Informe de ensayo n°032B02-2013-01 del 18/10/2013
Laboratorio APEX Bio Solutions

Conclusión: El protocolo del ensayo (adaptado según las condiciones de uso del producto en el terreno) permite obtener una reducción de 6.03 log en el disco después de la acción mecánica ejercida por la limpieza manual con una toallita preimpregnada de producto Septalkan y 5 minutos de contacto; o una reducción superior a la prevista por el protocolo clásico de la norma NF T 72-281 (reducción de al menos 4log).

Ensayos de determinación de la actividad virucida

Norma NF EN 14476+A1 (enero de 2007) en el Adenovirus

Condiciones del ensayo	
Cepa:	Adenovirus de tipo 5
Sustancias interferentes:	Condiciones de suciedad: albúmina bovina 3g/l y eritrocitos de corderos a 3ml/l
Tiempo de contacto:	5 minutos
Temperatura:	20°C +/- 1°C

Informe de ensayo n°201V8-2012 del 05/10/2012
Laboratorio APEX Bio Solutions

Conclusión: Los ensayos realizados han demostrado que el producto F2563 (Septalkan) empleado puro tiene una actividad virucida sobre el Adenovirus, en aplicación de la metodología de la norma NF EN 14476+A1, a los 5 minutos de contacto a 20°C, en condiciones de suciedad.

Norma NF EN 14476+A1 (enero de 2007) en el Norovirus murino

Condiciones del ensayo	
Cepa:	Norovirus murino
Sustancias interferentes:	Condiciones de suciedad: albúmina bovina 3g/l y eritrocitos de corderos a 3ml/l
Tiempo de contacto:	5 minutos
Temperatura:	20°C +/- 1°C

Informe de ensayo n°306V36-2010 del 13/01/2011 (Reeditado en 01/02/2013)
Laboratorio APEX Bio Solutions

Conclusión: Los ensayos realizados han demostrado que Septalkan, utilizado con una concentración del 100%, posee una actividad virucida sobre el Norovirus murino, en aplicación de la metodología de la norma NF EN 14476+A1, a los 5 minutos de contacto a 20°C, en condiciones de suciedad.

Norma NF EN 14476+A1 (enero de 2007) en el Rotavirus bovino

Condiciones del ensayo	
Cepa:	Rotavirus bovino
Sustancias interferentes:	Condiciones de suciedad: albúmina bovina 3g/l y eritrocitos de corderos a 3ml/l
Tiempo de contacto:	5 minutos
Temperatura:	20°C +/- 1°C

Informe de ensayo n°326V40-2010 del 28/01/2010 (Reeditado en 01/02/2013)
Laboratorio APEX Bio Solutions

Conclusión: Los ensayos realizados han demostrado que Septalkan, utilizado con una concentración del 100%, posee una actividad virucida sobre el rotavirus bovino, en aplicación de la metodología de la norma NF EN 14476+A1, a los 5 minutos de contacto a 20°C, en condiciones de suciedad.

Norma NF EN 14476+A1 (enero de 2007) en el PRV (virus modelo Hepatitis B)

Condiciones del ensayo	
Cepa:	Pseudo Rabies Virus (PRV)
Sustancias interferentes:	Condiciones de suciedad: albúmina bovina 3g/l y eritrocitos de corderos a 3ml/l
Tiempo de contacto:	5 minutos
Temperatura:	20°C +/- 1°C

Informe de ensayo n°201V12-2012 del 03/10/2012
Laboratorio AEX Bio Solutions

Conclusión: Los ensayos realizados han demostrado que:

- el producto F2563 (Septalkan) (lote n°2463F2563A) empleado al 100% posee una actividad virucida sobre el virus de la Pseudo Rabies Virus (virus suidherpes de tipo 1- PRV), en aplicación de la metodología de la norma NF EN 14476+A1, a los 5 minutos de contacto a 20°C, en condiciones de suciedad.
- el virus PRV se usa como un modelo para probar la eficacia de los desinfectantes en el virus de la hepatitis B (VHB), el producto F2563 es eficaz en PRV en las condiciones de ensayo, sino que también es eficaz en los virus VHB durante 5 minutos de contacto a 20°C en condiciones de suciedad.

Norma NF EN 14476+A1 (enero de 2007) en el VDVB (virus modelo Hepatitis C)

Condiciones del ensayo	
Cepa:	Bovin Viral Diarrhea Virus (BVDV)
Sustancias interferentes:	Condiciones de suciedad: albúmina bovina 3g/l y eritrocitos de corderos a 3ml/l
Tiempo de contacto:	5 minutos
Temperatura:	20°C +/- 1°C

Informe de ensayo n°201V13-2012 del 28/10/2011
Laboratorio AEX Bio Solutions

Conclusión: Los ensayos realizados han demostrado que:

- el producto F2563 (Septalkan) (lote n°2463F2563A) empleado al 100% posee una actividad virucida sobre el virus de la diarrea viral bovina (VDVB), en aplicación de la metodología de la norma NF EN 14476+A1, a los 5 minutos de contacto a 20°C, en condiciones de suciedad.
- el virus DVB se usa como un modelo para probar la eficacia de los desinfectantes en el virus de la hepatitis C (VHC), el producto F2563 es eficaz en VDVB en las condiciones de ensayo, sino que también es eficaz en los virus VHC durante 5 minutos de contacto a 20°C en condiciones de suciedad.

Norma NF EN 14476+A1 (enero de 2007) en el Virus de Inmunodeficiencia Humana (VIH)

Condiciones del ensayo	
Cepa:	VIH-1
Sustancias interferentes:	Condiciones de suciedad: albúmina bovina 3g/l y eritrocitos de corderos a 3ml/l
Tiempo de contacto:	5 minutos
Temperatura:	20°C +/- 1°C

Informe de ensayo n°201V11-2012 del 25/10/2012
Laboratorio APEX Bio Solutions

Conclusión: Los ensayos realizados han demostrado que el producto F2563 (Septalkan) lote n°2463F2563A empleado puro posee una actividad virucida sobre el virus de inmunodeficiencia humana (VIH), en aplicación de la metodología de la norma NF EN 14476+A1, a los 5 minutos de contacto a 20°C, en condiciones de suciedad.

Norma UNI EN 16777 (enero de 2019) en el Virus de la Vaccine cepa Ankara

Condiciones del ensayo	
Cepa:	Vaccinia virus
Sustancias interferentes:	Condiciones de suciedad: albúmina bovina 3g/l y eritrocitos a 3ml/l
Tiempo de contacto:	5 minutos
Temperatura:	Entre 18°C +/- 1°C y 25°C +/- 1°C

Informe de ensayo n°20/000242970 del 30/06/2020
Laboratorio APEX Bio Solutions

Conclusión: Según la norma UNI EN 16777: 2019, en las condiciones de las pruebas aplicadas, el producto ensayado tiene una actividad virucida (R ≥ 4) contra el Vaccinia virus.

Ensayos de determinación de la actividad esporicida

Norma NF T 72-281 (mayo de 2009)

Condiciones del ensayo	
Cepas:	*Esporas de Bacillus subtilis*
Tiempo de pulverización:	2 pulverizaciones (2 sec.) a 20cm
Peso:	0.397g
Superficie de contacto:	50,24 cm² (disco de acero inoxidable con un diámetro de 4 cm)
Tiempo de contacto:	5 minutos
Temperatura:	25.5°C

Informe de ensayo n°152B17-2014-01 del 16/09/2014
Laboratorio APEX Bio Solutions

Conclusión: Los ensayos muestran que el producto SEPTALKAN, lote nº2013030170, presenta una actividad esporicida en el *Bacillus subtilis* a por vía aérea, de conformidad con la norma NF T 72-281 (mayo de 2009), en una dosis de 2 pulverizaciones manuales y 5 minutos de contacto.

Norma NF T 72-281 (Mayo de 2009) equipado con pulverización + limpie con toallitas después del contacto con el producto

Condiciones del ensayo	
Cepas:	*Esporas de Clostridium difficile*
Tiempo de pulverización:	2 pulverizaciones (4 sec.) a 20cm
Peso:	0.505g
Número de limpieza:	3 : volver-go
Superficie de contacto:	12,56 cm² (disco de acero inoxidable con un diámetro de 2 cm)
Tiempo de contacto:	5 minutos de contacto con la toallita
Temperatura:	22°C

Informe de ensayo n° 032B03-2013-01 del 19/06/2013 (Reeditado en 14/11/2013)
Laboratorio APEX Bio Solutions

Conclusión: El producto SEPTALKAN presenta una actividad esporicida en *Clostridium difficile*, dispersando una dosis de dos pulverizaciones, de conformidad con el protocolo del ensayo y las condiciones previstas en la norma NF T 72-281:
- En el soporte después de ser limpiado,
- En la toallita tras 5 minutos de contacto con el producto limpiado.

El protocolo del ensayo (adaptado según las condiciones de uso del producto en el terreno) permite obtener una reducción de 5.18log en el disco después de la acción mecánica ejercida por la limpieza manual con una toallita seca y estéril; o una reducción superior a la prevista por el protocolo clásico de la norma NF T 72-281 (reducción de al menos 3log).

Este protocolo permite asimismo obtener una reducción >3log en la toallita, después de limpiar los discos y tras 5 minutos de contacto con el producto SEPTALKAN.

Datos de compatibilidad

En vista de los estudios de compatibilidad de materiales y los comentarios sobre el producto, **Sept**alkan se considera compatible con la mayoría de los materiales en el mercado

Informes o certificados de compatibilidad

- **Placa de plexiglás y sonda de ultrasonido:**

Condiciones del ensayo		
Material:	Placa de plexiglás (x3)	Sonda de ultrasonido
Tiempo de contacto:	Inmersión durante 24h (T=24h) Rocíe + limpie con una toallita una vez al día durante 30 días (T=30d)	10 limpiezas por día durante 30 días (T=30d)
Observación:	Comparación de la superficie bajo el Microscopio óptico a T=0 y T=24h / T=30d	Comparación de la superficie bajo el Microscopio óptico a T=0 y T=24h / T=30d

Informe de ensayo n° IAC-R12-0934-A02 del 26/09/2012
Intertek

Conclusión:

- Placa de plexiglás:
  Se retira una película protectora de la placa y tiene micro arañazos en la superficie.
  Después de 24 horas de inmersión, no hay diferencia significativa.
  Después de 30 días de limpieza con una toallita o rociando la solución sobre la placa y luego limpiando con una toallita, se observa un aumento en los rasguños.
  En conclusión, la solución es compatible con el material, y los arañazos se deben a la limpieza repetitiva con la toallita de prueba.

- Sonda de ultrasonido:
  En T=0, la sonda tiene algunos defectos en la superficie: la superficie no es lisa y contiene polvo y perlas.
  Después de 30 días, la superficie contiene menos cuentas y tiene irregularidades superficiales muy leves.
  En conclusión, la solución es compatible con la sonda de ultrasonido.

- **Test Prueba de compatibilidad de diferentes materiales:** según los resultados de las pruebas realizadas por K-PLAN Innovation microbiology en noviembre de 2015, los siguientes materiales son compatibles con la solución SEPTALKAN lote n ° 2014.12.01.61:
  Aluminio, aluminio anodizado, aluminio con recubrimiento en polvo, juntas, acabados cromados, poliestireno de impacto, aleación de zinc, acero endurecido, acero galvanizado, madera recubierta con la capa superior, espuma aislante PS, tarjeta electrónica, caucho, PMMA, espuma de poliuretano, cubierta de cable + cable de cobre, plástico , correas de tela, dibond, imán, tecnopolímero (poliamida, fibra de vidrio, plata, cerámica inorgánica), neopreno, polipropileno, TPE, PVC flexible y transparente.

  Los siguientes materiales son moderadamente compatibles con el lote SEPTALKAN n ° 2014.12.01.61 porque se oscurecen pero siguen siendo mecánicamente funcionales:
  Aisi 303 acero inoxidable y acero.

FICHA DE DATOS DE SEGURIDAD

(Reglamento REACH (CE) n° 1907/2006 - n° 2015/830)

SECCIÓN 1 : IDENTIFICACIÓN DE LA SUSTANCIA O LA MEZCLA Y DE LA SOCIEDAD O LA EMPRESA

1.1. Identificador del producto

Nombre del producto : SEPTALKAN

Código del producto : FS CLP2563

1.2. Usos pertinentes identificados de la sustancia o de la mezcla y usos desaconsejados

Detergente desinfectante para limpiar y desinfectar superficies y dispositivos médicos con un paño húmedo.

1.3. Datos del proveedor de la ficha de datos de seguridad

Denominación Social : SODEL - Gamme ALKAPHARM.

Dirección : 190 Rue René Barthélémy - Pôle d'activités de l'Espérance.14100.LISIEUX.FRANCE.

Teléfono : +33 (0)2 31 31 10 50. Fax : +33 (0)2 31 31 80 60.

www.sodel-sa.eu / www.alkapharm.fr

1.4. Teléfono de emergencia : +33 (0)1 45 42 59 59.

Sociedad/Organismo : INRS / ORFILA http://www.centres-antipoison.net.

SECCIÓN 2 : IDENTIFICACIÓN DE LOS PELIGROS

2.1. Clasificación de la sustancia o de la mezcla

En conformidad con el reglamento (CE) n° 1272/2008 y sus adaptaciones.

Esta mezcla no presenta peligro físico. Consulte las recomendaciones acerca de los demás productos presentes en el lugar.

Esta mezcla no supone un peligro para la salud a excepción de posibles valores límites de exposición profesional (véanse los apartados 3 y 8).

Esta mezcla no es peligrosa para el medioambiente. No existe ninguna amenaza conocida ni previsible para el medioambiente en las condiciones normales de uso.

2.2. Elementos de la etiqueta

La mezcla es un producto detergente de uso biocida (ver sección 15).

La mezcla se utiliza en forma de pulverización.

La mezcla es un producto detergente desinfectante para el dispositivos médicos.

En conformidad con el reglamento (CE) n° 1272/2008 y sus adaptaciones.

Consejos de prudencia - Carácter general :

P102 Mantener fuera del alcance de los niños.

Consejos de prudencia - Eliminación :

P501 Eliminar el contenido / contenedor como residuo no peligroso.

2.3. Otros peligros

La mezcla no contiene “Sustancias extremadamente preocupantes » (SVHC) >= 0,1% publicadas por el Organismo Europeo de Productos Químicos (ECHA) según el artículo 57 del REACH : http://echa.europa.eu/fr/candidate-list-table

La mezcla no responde a los criterios aplicables a las mezclas PBT ni vPvB en conformidad con el anexo XIII de la reglamentación REACH (CE) n° 1907/2006.

SECCIÓN 3 : COMPOSICIÓN/INFORMACIÓN SOBRE LOS COMPONENTES

3.2. Mezclas

Ninguna substancia responde a los criterios estipulados en el anexo II del reglamento REACH (EC) n° 1907/2006

SECCIÓN 4 : PRIMEROS AUXILIOS

De forma general, en caso de duda o si persisten los síntomas, llamar siempre a un médico

NO hacer ingerir NUNCA nada a una persona inconsciente.

4.1. Descripción de los primeros auxilios

En caso de proyecciones o de contacto con los ojos :

Lávese a fondo con agua limpia y fresca durante 15 minutos manteniendo los párpados separados.

Si tiene dolor, enrojecimiento o malestar visual, consulte a un oftalmólogo.

En caso de proyecciones o de contacto con la piel :

En caso de manifestación alérgica, consulte a un médico.

- Made under licence of European Label System® MSDS software from InfoDyne - http://www.infodyne.fr -

En caso de ingestión :

Consultar a un médico y mostrarle la etiqueta.

4.2. Principales síntomas y efectos, agudos y retardados

No hay datos disponibles.

4.3. Indicación de toda atención médica y de los tratamientos especiales que deban dispensarse inmediatamente

No hay datos disponibles.

SECCIÓN 5 : MEDIDAS DE LUCHA CONTRA INCENDIOS

No inflamable.

5.1. Medios de extinción

Medios de extinción apropiados

Polvo químico, dióxido de carbono y otros gases son adecuados para la extinción de pequeños incendios.

5.2. Peligros específicos derivados de la sustancia o la mezcla

Un incendio produce frecuentemente un espeso humo negro. La exposición a los productos de descomposición puede conllevar riesgos para la salud

No respirar los humos

En caso de incendio, se puede formar :

- monóxido de carbono (CO)
- dióxido de carbono (CO2)

5.3. Recomendaciones para el personal de lucha contra incendios

El personal de intervención deberá estar equipado de aparatos de protección respiratoria autónomos aislantes

SECCIÓN 6 : MEDIDAS EN CASO DE VERTIDO ACCIDENTAL

6.1. Precauciones personales, equipo de protección y procedimientos de emergencia

Remitirse a las medidas de protección enumeradas en las rúbricas 7 y 8

Para el personal de primeros auxilios

El personal de intervención contará con equipos de protección individual apropiado (Consultar la sección 8).

6.2. Precauciones relativas al medio ambiente

Contener y recoger las fugas con materiales absorbentes no combustibles, como por ejemplo : arena, tierra, vernicular, tierra de diatomeas en bidones para la eliminación de los residuos

Impedir el vertido en alcantarillas o cursos de agua.

6.3. Métodos y material de contención y de limpieza

Limpiar preferentemente con un detergente y evitar la utilización de disolventes

6.4. Referencia a otras secciones

No hay datos disponibles.

SECCIÓN 7 : MANIPULACIÓN Y ALMACENAMIENTO

Las prescripciones relativas a los lugares de almacenamiento se aplican a las zonas de trabajo donde se manipula la mezcla.

7.1. Precauciones para una manipulación segura

Lavarse las manos después de cada utilización.

Prevención de incendios :

Prohibir el acceso a las personas no autorizadas

Equipos y procedimientos recomendados :

Para la protección individual, consultar la sección 8.

Observar las precauciones indicadas en la etiqueta, así como las normativas de la protección de seguridad y prevención de riegos laborales.

Equipos y procedimientos prohibidos :

Está prohibido fumar, comer y beber en los lugares donde se utiliza la mezcla.

7.2. Condiciones de almacenamiento seguro, incluidas posibles incompatibilidades

No hay datos disponibles.

Almacenamiento

Manténgase fuera del alcance de los niños.

Almacenar a una temperatura entre 5°C y 35°C.

Embalaje

Conservar siempre en embalaje original.

- Made under licence of European Label System® MSDS software from InfoDyne - http://www.infodyne.fr -

7.3. Usos específicos finales

No hay datos disponibles.

SECCIÓN 8 : CONTROLES DE EXPOSICIÓN/PROTECCIÓN INDIVIDUAL

8.1. Parámetros de control

No hay datos disponibles.

8.2. Controles de la exposición

Medidas de protección individual, tales como los equipos de protección individual

Utilizar equipos de protección individual limpios y en buen estado.

Almacenar los equipos de protección individual en un lugar limpio, lejos de la zona de trabajo.

Durante la utilización, no comer, beber ni fumar. Quitarse y lavar la ropa contaminada antes de volver a utilizarla. Proporcionar una ventilación adecuada, sobre todo en los lugares cerrados.

- Protección de ojos / rostro

Evitar el contacto con los ojos

Usar lentes de seguridad no es obligatorio. Si sus protocolos recomiendan usarlos, use protección para los ojos diseñada contra salpicaduras de líquidos.

Estos protectores oculares deben cumplir con la norma NF EN166.

- Protección de las manos

Usar guantes protectores apropiados en caso de contacto prolongado o reiterado con la piel.

- Protección corporal

Después del contacto con el producto, habrá que lavar todas las partes del cuerpo que se hayan contaminado.

SECCIÓN 9 : PROPIEDADES FÍSICAS Y QUÍMICAS

9.1. Información sobre propiedades físicas y químicas básicas

Información general

Estado Físico :	Líquido Fluido
Color	incoloro
Olor :	característico

Información importante en relación con la salud, la seguridad y el medio ambiente :

pH :	7.00 .
	Neutro
Punto/intervalo de ebullición :	No concernido.
Intervalo de Punto de inflamación :	No concernido.
Presión de vapor (50°C) :	No concernido.
Densidad :	1.00 +/- 0.01 g/mL
Solubilidad en agua :	Disoluble.
Viscosidad :	v < 7 mm2/s (40°C)
Punto/intervalo de fusión :	No concernido.
Temperatura de autoinflamación :	No concernido.
Punto/intervalo de de descomposición :	No concernido.

9.2. Otros datos

No hay datos disponibles.

SECCIÓN 10 : ESTABILIDAD Y REACTIVIDAD

10.1. Reactividad

No hay datos disponibles.

10.2. Estabilidad química

Esta mezcla es estable en las condiciones de manipulación y de almacenamiento recomendadas en la sección 7.

10.3. Posibilidad de reacciones peligrosas

No hay datos disponibles.

10.4. Condiciones que deben evitarse

Evitar :

- la congelación

10.5. Materiales incompatibles

- Made under licence of European Label System® MSDS software from InfoDyne - http://www.infodyne.fr -

10.6. Productos de descomposición peligrosos

La descomposición térmica puede provocar/formar :

- monóxido de carbono (CO)
- dióxido de carbono (CO2)

SECCIÓN 11 : INFORMACIÓN TOXICOLÓGICA

11.1. Información sobre los efectos toxicológicos

No hay datos disponibles.

11.1.1. Sustancias

No hay ninguna información toxicológica disponible sobre las sustancias.

11.1.2. Mezcla

Corrosión cutánea/irritación cutánea :

Corrosividad :	Ningún efecto observado.

SECCIÓN 12 : INFORMACIÓN ECOLÓGICA

12.1. Toxicidad

12.1.2. Mezclas

No hay ninguna información disponible sobre la toxicidad acuática de la mezcla.

12.2. Persistencia y degradabilidad

Tensioactivos en esta preparación cumplan con las normas de biodegradabilidad, tal como se definen en el Reglamento (CE) n ° 648/2004 sobre detergentes.

Los datos para justificar esta afirmación están a disposición de las autoridades competentes de los Estados miembros y se pondrá a disposición a petición suya, bien a petición de un fabricante de detergentes.

12.3. Potencial de bioacumulación

No hay datos disponibles.

12.4. Movilidad en el suelo

No hay datos disponibles.

12.5. Resultados de la valoración PBT y mPmB

No hay datos disponibles.

12.6. Otros efectos adversos

No hay datos disponibles.

SECCIÓN 13 : CONSIDERACIONES RELATIVAS A LA ELIMINACIÓN

Se debe realizar una gestión apropiada de los residuos de la mezcla y/o de su envase en conformidad con las disposiciones de la directiva 2008/98/CE.

13.1. Métodos para el tratamiento de residuos

No verter en las alcantarillas ni en los cursos de agua

Residuos :

La gestión de los residuos se realiza sin poner en peligro la salud humana y sin perjudicar el medioambiente, y en especial, sin crear riesgos para el agua, el aire, el suelo, la fauna o la flora.

Reciclarlos o eliminarlos según la legislación en vigor, de preferencia por un gestor de residuos o una empresa autorizada.

No contaminar el suelo o el agua con los residuos, y no eliminarlos en el medio ambiente.

Envases contaminados :

Vaciar completamente el envase. Conservar la(las) etiqueta(s) en el envase.

Entregar a un gestor autorizado.

SECCIÓN 14 : INFORMACIÓN RELATIVA AL TRANSPORTE

Preparación exenta de la clasificación y del etiquetado Transporte

14.1. Número ONU

-

14.2. Designación oficial de transporte de las Naciones Unidas

-

- Made under licence of European Label System® MSDS software from InfoDyne - http://www.infodyne.fr -

14.3. Clase(s) de peligro para el transporte

-

14.4. Grupo de embalaje

-

14.5. Peligros para el medio ambiente

-

14.6. Precauciones particulares para los usuarios

-

SECCIÓN 15 : INFORMACIÓN REGLAMENTARIA

15.1. Reglamentación y legislación en materia de seguridad, salud y medio ambiente específicas para la sustancia o la mezcla

-Información relativa a la clasificación y al etiquetado que figura en la sección 2:

Se han tenido en cuenta las siguientes reglamentaciones:

Reglamento (CE) n° 1272/2008 modificado por la normativa (UE) n° 2017/776 (ATP 10)

-Información relativa al embalaje:

No hay datos disponibles.

- Disposiciones particulares :

No hay datos disponibles.

- Etiquetado de los detergentes (Reglamento CE n° 648/2004,907/2006) :

- inferior al 5 % : tensioactivos catiónicos
- inferior al 5 % : tensioactivos no iónicos
- desinfectantes.

- Etiquetado de los biocidas (Reglamento 1896/2000, 1687/2002, 2032/2003, 1048/2005, 1849/2006, 1451/2007 y Directiva 98/8/CE) :

Nombre	CAS	%	Tipo de producto
CLORURO DE DIDECILDIMETILAMONIO	7173-51-5	0.25 g/kg	02 04
ALKYL (C12-16) DIMETHYLBENZYL AMMONIUM CHLORIDE	68424-85-1	0.95 g/kg	02 04

Tipo de producto 2 : Desinfectantes y alguicidas no destinados a la aplicación directa a personas o animales.

Tipo de producto 4 : Alimentos y piensos.

15.2. Evaluación de la seguridad química

No hay datos disponibles.

SECCIÓN 16 : OTRA INFORMACIÓN

Dado que no conocemos las condiciones de trabajo del usuario, las informaciones que figuran en la presente ficha de seguridad se basarán en el estado de nuestros conocimientos y en las normativas tanto nacionales como comunitarias.

La mezcla no debe ser utilizada para otros usos que no sean los especificados en la sección 1 sin haber obtenido previamente instrucciones de manipulación por escrito.

El usuario es totalmente responsable de tomar todas las medidas necesarias para responder a las exigencias de las leyes y normativas locales.

La información indicada en la presente ficha de datos de seguridad debe considerarse como una descripción de las exigencias de seguridad relativas a esta mezcla y no como una garantía de las propiedades de la misma.

Abreviaturas :

PBT : Persistente, bioacumulable y tóxico.

vPvB : Muy persistente y muy bioacumulable.

SVHC : Sustancias extremadamente preocupantes.

Difference Report

Revisión: N°9 (03/08/2018) / Versión: N°2 (03/08/2018)

~~Revisión: N°4 (29/03/2016) / Versión: N°1 (29/03/2016)~~

- Made under licence of European Label System® MSDS software from InfoDyne - http://www.infodyne.fr -

SECCIÓN 1 : IDENTIFICACIÓN DE LA SUSTANCIA O LA MEZCLA Y DE LA SOCIEDAD O LA EMPRESA

1.2. Usos pertinentes identificados de la sustancia o de la mezcla y usos desaconsejados

~~Desinfectar los dispositivos médicos sin alcohol con detergente.~~

Detergente desinfectante para limpiar y desinfectar superficies y dispositivos médicos con un paño húmedo.

SECCIÓN 2 : IDENTIFICACIÓN DE LOS PELIGROS

2.2. Elementos de la etiqueta

La mezcla es un producto detergente desinfectante para el dispositivos médicos.

En conformidad con el reglamento (CE) n° 1272/2008 y sus adaptaciones.

Consejos de prudencia - Eliminación :

P501	Eliminar el contenido / contenedor como residuo no peligroso.

SECCIÓN 4 : PRIMEROS AUXILIOS

En caso de proyecciones o de contacto con los ojos :

Lávese a fondo con agua limpia y fresca durante 15 minutos manteniendo los párpados separados.

Si tiene dolor, enrojecimiento o malestar visual, consulte a un oftalmólogo.

En caso de proyecciones o de contacto con la piel :

En caso de manifestación alérgica, consulte a un médico.

SECCIÓN 7 : MANIPULACIÓN Y ALMACENAMIENTO

Almacenamiento

~~Almacenar lejos de la helada.~~

Almacenar a una temperatura entre 5°C y 35°C.

SECCIÓN 8 : CONTROLES DE EXPOSICIÓN/PROTECCIÓN INDIVIDUAL

- Protección de ojos / rostro

~~Utilizar protecciones oculares diseñadas contra las proyecciones de líquidos~~

~~Antes de cualquier manipulación, es necesario usar gafas de seguridad conformes a la norma EN166.~~

Usar lentes de seguridad no es obligatorio. Si sus protocolos recomiendan usarlos, use protección para los ojos diseñada contra salpicaduras de líquidos.

Estos protectores oculares deben cumplir con la norma NF EN166.

- Protección corporal

~~La ropa del personal debe lavarse con regularidad.~~

SECCIÓN 9 : PROPIEDADES FÍSICAS Y QUÍMICAS

Información general

~~Color : incoloro~~

~~Olor : característico~~

Color	incoloro
Olor :	característico

Información importante en relación con la salud, la seguridad y el medio ambiente :

~~Densidad : = 1~~

Densidad :	1.00 +/- 0.01 g/mL
Viscosidad :	v < 7 mm2/s (40°C)

SECCIÓN 14 : INFORMACIÓN RELATIVA AL TRANSPORTE

~~Transportar el producto de conformidad con las disposiciones del ADR por carretera, del RID por ferrocarril, del IMDG por mar y del ICAO/IATA por aire (ADR 2015 - IMDG 2014 - ICAO/IATA 2015).~~

14.1. Número ONU

-

14.2. Designación oficial de transporte de las Naciones Unidas

-

- Made under licence of European Label System® MSDS software from InfoDyne - http://www.infodyne.fr -

14.3. Clase(s) de peligro para el transporte

-

14.4. Grupo de embalaje

-

14.5. Peligros para el medio ambiente

-

14.6. Precauciones particulares para los usuarios

-

SECCIÓN 15 : INFORMACIÓN REGLAMENTARIA

-Información relativa a la clasificación y al etiquetado que figura en la sección 2:

~~Reglamento (CE) n° 1272/2008 modificado por la normativa (UE) n° 487/2013~~

~~Reglamento (CE) n° 1272/2008 modificado por la normativa (UE) n° 758/2013~~

~~Reglamento (CE) n° 1272/2008 modificado por la normativa (UE) n° 944/2013~~

~~Reglamento (CE) n° 1272/2008 modificado por la normativa (UE) n° 605/2014~~

~~Reglamento (CE) n° 1272/2008 modificado por la normativa (UE) n° 1297/2014~~

Reglamento (CE) n° 1272/2008 modificado por la normativa (UE) n° 2017/776 (ATP 10)

- Etiquetado de los biocidas (Reglamento 1896/2000, 1687/2002, 2032/2003, 1048/2005, 1849/2006, 1451/2007 y Directiva 98/8/CE) :

~~CHLORURE D'ALKYLBENZYL DIMETHYL AMMONIUM~~	~~68424-85-1~~	~~0.95 g/kg~~	~~02 04~~
~~CHLORURE DE DIDECYL DIMETHYLAMMONIUM~~	~~7173-51-5~~	~~0.25 g/kg~~	~~02 04~~
CLORURO DE DIDECILDIMETILAMONIO	7173-51-5	0.25 g/kg	02 04
ALKYL (C12-16) DIMETHYLBENZYL AMMONIUM CHLORIDE	68424-85-1	0.95 g/kg	02 04

SECCIÓN 16 : OTRA INFORMACIÓN

Abreviaturas :

~~ADR : Acuerdo europeo relativo al transporte internacional de mercancías peligrosas por carretera.~~

~~IMDG : International Maritime Dangerous Goods.~~

~~IATA : International Air Transport Association.~~

~~OACI : Organización de Aviación Civil Internacional.~~

~~RID : Regulations concerning the International carriage of Dangerous goods by rail.~~

~~WGK : Wassergefahrdungsklasse (Clase de peligro para el agua).~~

- Made under licence of European Label System® MSDS software from InfoDyne - http://www.infodyne.fr -

FICHA DE DATOS DE SEGURIDAD

(Reglamento REACH (CE) n° 1907/2006 - n° 2015/830)

SECCIÓN 1 : IDENTIFICACIÓN DE LA SUSTANCIA O LA MEZCLA Y DE LA SOCIEDAD O LA EMPRESA

1.1. Identificador del producto

Nombre del producto : SEPTALKAN LINGETTES

Código del producto : FSCLP2563

1.2. Usos pertinentes identificados de la sustancia o de la mezcla y usos desaconsejados

Toallitas impregnadas con un detergente desinfectante para limpiar y desinfectar superficies y dispositivos médicos, mediante limpieza en húmedo.

1.3. Datos del proveedor de la ficha de datos de seguridad

Denominación Social : SODEL - Gamme ALKAPHARM.

Dirección : 190 Rue René Barthélémy - Pôle d'activités de l'Espérance.14100.LISIEUX.FRANCE.

Teléfono : +33 (0)2 31 31 10 50. Fax : +33 (0)2 31 31 80 60.

www.sodel-sa.eu / www.alkapharm.fr

1.4. Teléfono de emergencia : +33 (0)1 45 42 59 59.

Sociedad/Organismo : INRS / ORFILA http://www.centres-antipoison.net.

SECCIÓN 2 : IDENTIFICACIÓN DE LOS PELIGROS

2.1. Clasificación de la sustancia o de la mezcla

En conformidad con el reglamento (CE) n° 1272/2008 y sus adaptaciones.

Esta mezcla no presenta peligro físico. Consulte las recomendaciones acerca de los demás productos presentes en el lugar.

Esta mezcla no supone un peligro para la salud a excepción de posibles valores límites de exposición profesional (véanse los apartados 3 y 8).

Esta mezcla no es peligrosa para el medioambiente. No existe ninguna amenaza conocida ni previsible para el medioambiente en las condiciones normales de uso.

2.2. Elementos de la etiqueta

La mezcla es un producto detergente de uso biocida (ver sección 15).

La mezcla es un producto detergente desinfectante para el dispositivos médicos.

En conformidad con el reglamento (CE) n° 1272/2008 y sus adaptaciones.

Consejos de prudencia - Carácter general :

P102 Mantener fuera del alcance de los niños.

Consejos de prudencia - Eliminación :

P501 Eliminar el contenido / contenedor como residuo no peligroso.

2.3. Otros peligros

La mezcla no contiene “Sustancias extremadamente preocupantes » (SVHC) >= 0,1% publicadas por el Organismo Europeo de Productos Químicos (ECHA) según el artículo 57 del REACH : http://echa.europa.eu/fr/candidate-list-table

La mezcla no responde a los criterios aplicables a las mezclas PBT ni vPvB en conformidad con el anexo XIII de la reglamentación REACH (CE) n° 1907/2006.

SECCIÓN 3 : COMPOSICIÓN/INFORMACIÓN SOBRE LOS COMPONENTES

3.2. Mezclas

Ninguna substancia responde a los criterios estipulados en el anexo II del reglamento REACH (EC) n° 1907/2006

SECCIÓN 4 : PRIMEROS AUXILIOS

De forma general, en caso de duda o si persisten los síntomas, llamar siempre a un médico

NO hacer ingerir NUNCA nada a una persona inconsciente.

4.1. Descripción de los primeros auxilios

En caso de proyecciones o de contacto con los ojos :

Lávese a fondo con agua limpia y fresca durante 15 minutos manteniendo los párpados separados.

Si tiene dolor, enrojecimiento o malestar visual, consulte a un oftalmólogo.

En caso de proyecciones o de contacto con la piel :

En caso de manifestación alérgica, consulte a un médico.

- Made under licence of European Label System® MSDS software from InfoDyne - http://www.infodyne.fr -

4.2. Principales síntomas y efectos, agudos y retardados

No hay datos disponibles.

4.3. Indicación de toda atención médica y de los tratamientos especiales que deban dispensarse inmediatamente

No hay datos disponibles.

SECCIÓN 5 : MEDIDAS DE LUCHA CONTRA INCENDIOS

No inflamable.

5.1. Medios de extinción

Medios de extinción apropiados

Polvo químico, dióxido de carbono y otros gases son adecuados para la extinción de pequeños incendios.

5.2. Peligros específicos derivados de la sustancia o la mezcla

Un incendio produce frecuentemente un espeso humo negro. La exposición a los productos de descomposición puede conllevar riesgos para la salud

No respirar los humos

En caso de incendio, se puede formar :

- monóxido de carbono (CO)
- dióxido de carbono (CO2)

5.3. Recomendaciones para el personal de lucha contra incendios

El personal de intervención deberá estar equipado de aparatos de protección respiratoria autónomos aislantes

SECCIÓN 6 : MEDIDAS EN CASO DE VERTIDO ACCIDENTAL

6.1. Precauciones personales, equipo de protección y procedimientos de emergencia

Remitirse a las medidas de protección enumeradas en las rúbricas 7 y 8

Para el personal de primeros auxilios

El personal de intervención contará con equipos de protección individual apropiado (Consultar la sección 8).

6.2. Precauciones relativas al medio ambiente

Impedir el vertido en alcantarillas o cursos de agua.

6.3. Métodos y material de contención y de limpieza

No hay datos disponibles.

6.4. Referencia a otras secciones

No hay datos disponibles.

SECCIÓN 7 : MANIPULACIÓN Y ALMACENAMIENTO

Las prescripciones relativas a los lugares de almacenamiento se aplican a las zonas de trabajo donde se manipula la mezcla.

7.1. Precauciones para una manipulación segura

Lavarse las manos después de cada utilización.

Prevención de incendios :

Prohibir el acceso a las personas no autorizadas

Equipos y procedimientos recomendados :

Para la protección individual, consultar la sección 8.

Observar las precauciones indicadas en la etiqueta, así como las normativas de la protección de seguridad y prevención de riegos laborales.

Equipos y procedimientos prohibidos :

Está prohibido fumar, comer y beber en los lugares donde se utiliza la mezcla.

7.2. Condiciones de almacenamiento seguro, incluidas posibles incompatibilidades

No hay datos disponibles.

Almacenamiento

Manténgase fuera del alcance de los niños.

Almacenar a una temperatura entre 5°C y 35°C.

Embalaje

Conservar siempre en embalaje original.

Conservar solo en el contenedor original.

- Made under licence of European Label System® MSDS software from InfoDyne - http://www.infodyne.fr -

7.3. Usos específicos finales

No hay datos disponibles.

SECCIÓN 8 : CONTROLES DE EXPOSICIÓN/PROTECCIÓN INDIVIDUAL

8.1. Parámetros de control

No hay datos disponibles.

8.2. Controles de la exposición

Medidas de protección individual, tales como los equipos de protección individual

Utilizar equipos de protección individual limpios y en buen estado.

Almacenar los equipos de protección individual en un lugar limpio, lejos de la zona de trabajo.

Durante la utilización, no comer, beber ni fumar. Quitarse y lavar la ropa contaminada antes de volver a utilizarla. Proporcionar una ventilación adecuada, sobre todo en los lugares cerrados.

- Protección de ojos / rostro

Evitar el contacto con los ojos

- Protección de las manos

Usar guantes protectores apropiados en caso de contacto prolongado o reiterado con la piel.

- Protección corporal

Después del contacto con el producto, habrá que lavar todas las partes del cuerpo que se hayan contaminado.

SECCIÓN 9 : PROPIEDADES FÍSICAS Y QUÍMICAS

9.1. Información sobre propiedades físicas y químicas básicas

Información general

Estado Físico :	Líquido Fluido
Olor :	característico

Información importante en relación con la salud, la seguridad y el medio ambiente :

pH :	No concernido.
Punto/intervalo de ebullición :	No concernido.
Intervalo de Punto de inflamación :	No concernido.
Presión de vapor (50°C) :	No concernido.
Densidad :	no precisado.
Solubilidad en agua :	Insoluble.
Punto/intervalo de fusión :	No concernido.
Temperatura de autoinflamación :	No concernido.
Punto/intervalo de de descomposición :	No concernido.
pH de la solución de impregnación :	7.00

9.2. Otros datos

No hay datos disponibles.

SECCIÓN 10 : ESTABILIDAD Y REACTIVIDAD

10.1. Reactividad

No hay datos disponibles.

10.2. Estabilidad química

Esta mezcla es estable en las condiciones de manipulación y de almacenamiento recomendadas en la sección 7.

10.3. Posibilidad de reacciones peligrosas

No hay datos disponibles.

10.4. Condiciones que deben evitarse

Evitar :

- la congelación

10.5. Materiales incompatibles

10.6. Productos de descomposición peligrosos

La descomposición térmica puede provocar/formar :

- monóxido de carbono (CO)
- dióxido de carbono (CO_2)

- Made under licence of European Label System® MSDS software from InfoDyne - http://www.infodyne.fr -

SECCIÓN 11 : INFORMACIÓN TOXICOLÓGICA

11.1. Información sobre los efectos toxicológicos

No hay datos disponibles.

11.1.1. Sustancias

No hay ninguna información toxicológica disponible sobre las sustancias.

11.1.2. Mezcla

Corrosión cutánea/irritación cutánea :

Corrosividad : Ningún efecto observado.

SECCIÓN 12 : INFORMACIÓN ECOLÓGICA

12.1. Toxicidad

12.1.2. Mezclas

No hay ninguna información disponible sobre la toxicidad acuática de la mezcla.

12.2. Persistencia y degradabilidad

Tensioactivos en esta preparación cumplan con las normas de biodegradabilidad, tal como se definen en el Reglamento (CE) n ° 648/2004 sobre detergentes.

Los datos para justificar esta afirmación están a disposición de las autoridades competentes de los Estados miembros y se pondrá a disposición a petición suya, bien a petición de un fabricante de detergentes.

12.3. Potencial de bioacumulación

No hay datos disponibles.

12.4. Movilidad en el suelo

No hay datos disponibles.

12.5. Resultados de la valoración PBT y mPmB

No hay datos disponibles.

12.6. Otros efectos adversos

No hay datos disponibles.

SECCIÓN 13 : CONSIDERACIONES RELATIVAS A LA ELIMINACIÓN

Se debe realizar una gestión apropiada de los residuos de la mezcla y/o de su envase en conformidad con las disposiciones de la directiva 2008/98/CE.

13.1. Métodos para el tratamiento de residuos

No verter en las alcantarillas ni en los cursos de agua

Residuos :

La gestión de los residuos se realiza sin poner en peligro la salud humana y sin perjudicar el medioambiente, y en especial, sin crear riesgos para el agua, el aire, el suelo, la fauna o la flora.

Reciclarlos o eliminarlos según la legislación en vigor, de preferencia por un gestor de residuos o una empresa autorizada.

No contaminar el suelo o el agua con los residuos, y no eliminarlos en el medio ambiente.

Envases contaminados :

Vaciar completamente el envase. Conservar la(las) etiqueta(s) en el envase.

Entregar a un gestor autorizado.

SECCIÓN 14 : INFORMACIÓN RELATIVA AL TRANSPORTE

Preparación exenta de la clasificación y del etiquetado Transporte

14.1. Número ONU

-

14.2. Designación oficial de transporte de las Naciones Unidas

-

14.3. Clase(s) de peligro para el transporte

-

14.4. Grupo de embalaje

-

- Made under licence of European Label System® MSDS software from InfoDyne - http://www.infodyne.fr -

14.5. Peligros para el medio ambiente

-

14.6. Precauciones particulares para los usuarios

-

SECCIÓN 15 : INFORMACIÓN REGLAMENTARIA

15.1. Reglamentación y legislación en materia de seguridad, salud y medio ambiente específicas para la sustancia o la mezcla

-Información relativa a la clasificación y al etiquetado que figura en la sección 2:

Se han tenido en cuenta las siguientes reglamentaciones:

Reglamento (CE) n° 1272/2008 modificado por la normativa (UE) n° 2017/776 (ATP 10)

-Información relativa al embalaje:

No hay datos disponibles.

- Disposiciones particulares :

No hay datos disponibles.

- Etiquetado de los detergentes (Reglamento CE n° 648/2004,907/2006) :

- inferior al 5 % : tensioactivos catiónicos
- inferior al 5 % : tensioactivos no iónicos
- desinfectantes.

- Etiquetado de los biocidas (Reglamento 1896/2000, 1687/2002, 2032/2003, 1048/2005, 1849/2006, 1451/2007 y Directiva 98/8/CE) :

Nombre	CAS	%	Tipo de producto
CLORURO DE DIDECILDIMETILAMONIO	7173-51-5	0.25 g/kg	02 04
ALKYL (C12-16) DIMETHYLBENZYL AMMONIUM CHLORIDE	68424-85-1	0.95 g/kg	02 04

Tipo de producto 2 : Desinfectantes y alguicidas no destinados a la aplicación directa a personas o animales.

Tipo de producto 4 : Alimentos y piensos.

15.2. Evaluación de la seguridad química

No hay datos disponibles.

SECCIÓN 16 : OTRA INFORMACIÓN

Dado que no conocemos las condiciones de trabajo del usuario, las informaciones que figuran en la presente ficha de seguridad se basarán en el estado de nuestros conocimientos y en las normativas tanto nacionales como comunitarias.

La mezcla no debe ser utilizada para otros usos que no sean los especificados en la sección 1 sin haber obtenido previamente instrucciones de manipulación por escrito.

El usuario es totalmente responsable de tomar todas las medidas necesarias para responder a las exigencias de las leyes y normativas locales.

La información indicada en la presente ficha de datos de seguridad debe considerarse como una descripción de las exigencias de seguridad relativas a esta mezcla y no como una garantía de las propiedades de la misma.

Abreviaturas :

PBT : Persistente, bioacumulable y tóxico.

vPvB : Muy persistente y muy bioacumulable.

SVHC : Sustancias extremadamente preocupantes.

- Made under licence of European Label System® MSDS software from InfoDyne - http://www.infodyne.fr -

Sodel
HYGIÈNE · DÉSINFECTION · SURODORANTS
Certifié ISO 9001 / 13485
ECOLABEL / ECOCERT

Cibler le risque infectieux
Alkapharm
La marque de la division Santé Sodel

ATTESTATION D'ÉQUIVALENCE

CERTIFICATE OF EQUIVALENCE

CERTIFICADO DE EQUIVALENCIA

Je soussigné, Séverine HUVET, Chargée d'affaires réglementaires, atteste par la présente que les rapports d'essais établis avant 2018 par ou au nom de la société ALKAPHARM peuvent être conservés pour la mise sur le marché des produits **à marque Alkapharm** par la société SODEL.
La composition, la méthode de fabrication et l'usage revendiqué de ces produits ne sont pas modifiés.

*I, Séverine HUVET, Regulatory Affairs Officer, hereby certify that tests reports written before 2018 by or on behalf of ALKAPHARM may be retained for placing on the market of **ALKAPHARM branded** products by SODEL.*
The composition, the method of manufacture and the claimed use of these products are not modified.

Yo, Séverine HUVET, Oficial de Asuntos Regulatorios, certifico que los informes de pruebas escritos antes de 2018 por ALKAPHARM pueden conservarse para la comercialización en el mercado de productos de **marca ALKAPHARM** por parte de SODEL.
La composición, el método de fabricación y el uso reivindicado de estos productos no están modificados.

Pour valoir ce que de droit.

To argue that right.

Para hacer valer ese derecho.

Lisieux,
Le 30 septembre 2018 / September 30, 2018 / 30 de septiembre de 2018

Séverine HUVET
Chargée d'Affaires Réglementaires

Siège : Pôle d'Activités de l'Espérance - 190 rue René Barthélémy - BP 94184 - 14104 Lisieux Cedex, France - Tél : +33 (0)2 31 31 10 50 - Fax : +33 (0)2 31 31 80 60 - www.sodel-sa.eu
Site Division Santé : 102 avenue Gaston Roussel - Parc Biocitech - 93230 Romainville, France - Tél : +33 (0)1 41 50 59 80 - Fax : +33 (0)1 48 30 54 31 – www.alkapharm.fr
S.A.S. au capital de 291 000€ - RCS Lisieux 627 150 105 – Siret 627 150 105 00021 – TVA Intra. : FR12 627 150 105 - APE 2041Z – Code produit : 142956

CERTIFICADO DE EQUIVALENCIA

Yo, el abajo firmante Séverine HUVET, Oficial de Asuntos Regulatorios para la compañía SODEL, cuya oficina central se encuentra en 190 rue René Barthélémy 14100 LISIEUX, Francia, certifica que los siguientes códigos o nombres son químicamente equivalentes a la fórmula **Septalkan** en el mercado:

- F2563
- Spray désinfectant sans alcohol pH neutre
- Jus septalkan
- 09PE037/A002

Para hacer valer este derecho.

Lisieux, 10/07/2020

Séverine HUVET
Oficial de Asuntos Regulatorios

sc1ent..1s

TEST REPORT
034-1 REA 15 AN

Seule la version française fait foi / Only the French version is valid

Test according to the NF EN 13727+A1 (December 2013) standard

Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity in the medical area
Test method and requirements (phase 2, step 1)

Test on three re/erenced strains: Pseudomonas aeruginosa, Staphylococcus aureus and ***Enterococcus hirae***
This report includes 4 pages and relates on/y the sample tested.

1. Sample Identification

Name of the product:	**F2563**
Batch number:	2013.03.01.70
Manufacturer:	**ALKAPHARM** Parc Biocitech - 102 avenue Gaston Roussel 93230 Romainville
Date of delivery :	28/07/201
Storage conditions:	Ambient temperature
Product diluent recommended by the manufacturer :	None
Active substance(s):	Didecyldimethylammonium chloride Alkyl dimethyl benzyl ammonium chloride
Appearance of the product:	Clear liquid

II. Completion of the test

111 Subcontractor laboratory identification

SCIENTIS
Parc Biocitech - 102 avenue Gaston Roussel
93230 ROMAINVILLE

Scientis is one of the commission members T72Q - Antiseptics and chemical disinfectants and is involved in a quality approach in accordance with the NF EN ISO/CEi 17025 v2005 standard.

112 Reference of test report

N° 034-1 REA 15 AN delivered on August 31st 2015.

III. Principle of the test

The purpose of the test according to the NF EN 13727+Al (2013) standard is to draw attention to a reduction in the number of microorganisms, when these come into contact with the test product under conditions specified below.

This test conducted under suspension conditions is used to ascertain a bactericidal activity if the reduction rate is 10^5 or more.

IV. Experimental Conditions

NF EN 13727+Al (2013)	**F2563**	Batch: 2013.03.01.70

Test period:	from July 30th, 2015 to August 3rd, 2015	
Test température :	20°C +/-l°C	
Contact time:	**5 minutes +/-10 seconds**	
Test microorganisms:	*Pseudomonas aeruginosa*	CIP 103467
	Staphylococcus aureus	CIP4.83
	Enterococcus hirae	CIP 58.55
Product diluent:	Sterile water for injection	

Diluent used for product test solutions: tryptone sodium chloride sterile solution
Appearance of product test solutions: clear
Dilution neutralization method: technical stop the bactericidal activity

Neutralizer:	Lécithin (3g/l) ; Polysorbate 80 (30g/l) ; Sodium Thiosulfate (5g/l); L-histidin (lg/l); Saponin (30g/l); Tryptone sodium chloride (qsp li)
Pour plate	
Counting agar:	T.S.A .(Tryptone Soya Agar)
Incubation:	37°C +/-l°C for 24h
Interfering substances :	**Dirty conditions** (Bovine albumin 3g/l and sheep erythrocytes 3ml/l)

Product stability with sterile water for injection : stable
Concentrations of the tested product: 10%; 50%; 100%; (80% as final concentration)

V. Results of the test

a. Test suspension

Strain (Collection and number)	*Pseudomonas aeruginosa* CIP 103467		*Staphylococcus aureus* DSM 799		*Enterococcus hirae* CIP 58.55	
Dilution 10^{-6}	>330	>330	324	299	294	274
Dilution 10^{-7}	32	37	34	44	33	22
Test suspension (N)	3.45×10^8		3.19×10^8		2.83×10^8	
Log N	8.54		8.50		8.45	
No= N/10	3.45×10^7		3.19×10^7		2.83×10^7	
Log No	**7.54**		**7.50**		**7.45**	

N is between 1.5×10^8 and 5.0×10^8 CFU/ml (log N is between 8.17 and 8.70)
N_0 is between 1.5×10^7 and 5.0×10^7 CFU /ml (log N_0 is between 7.17 and 7.70)

b. Validation and controls

Strain (Collection and number)	Validation suspension (N_{v0})		Validation suspension for neutralization ($N_v e/1000$)		Control of experimental conditions (A)		Validation of the neutralising agent (B)		Validation of the method (C)	
Pseudomonas aeruginosa CIP 103467	70	71	67	92	98	81	70	65	69	78
	70.S		**79.5**		**89.S**		**67.5**		**73.5**	
Staphylococcus aureus CIP 4.83	78	84	95	92	84	85	91	84	83	74
	81		**93.5**		**84.5**		**87.S**		**78.5**	
Enterococcus hirae CIP 58.55	70	62	61	82	69	64	66	56	78	62
	66		**71.S**		**66.5**		**61**		**70**	

N_v0 is between 30 and 160 CFU /ml, $N_v e/1000$ is between 30 and 160 CFU/ml
A, Band C are greater than or equal to 0.5 x N_{v0}, Bis greater than or equal to 0.0005 x N_{v8}.
The method is validated in the test conditions

c. **Test**

Strain (Collection and number)		Number of viable cells (Na) and reduction rate (R) for the concentrations of product tested					
		10% (v/v)		50% (v/v)		100% (v/v)	
Pseudomonas aeruginosa CIP 103467	10°	**48**	61	0	0	0	0
	Na	545		<140		<140	
	Log Na	2.74		<2.15		<2.15	
	R	**4.80**		**>5.39**		**>5.39**	
Staphylococcus aureus CIP 4.83	10°	0	0	0	0	0	0
	Na	<140		<140		<140	
	Log Na	<2.15		<2.15		<2.15	
	R	**>5.35**		**>5.35**		**>5.35**	
Enterococcus hirae CIP 58.55	10°	0	0	0	0	0	0
	Na	<140		<140		<140	
	Log Na	<2.15		<2.15		<2.15	
	R	**>5.30**		**>5.30**		**>5.30**	

R = log N_0 - log Na

A concentration is bactericidal if R 5

VI. Specific remarks

1. Ali the contrais and the mixture of validation method give values included within the fundamental limits.
2. At least one concentration of the product has a logarithmic reduction of less than S.
3. No formation of a precipitate was observed during the realization of the assay (Tested mixtures were homogeneous).

VII. Conclusion

According to the NF EN 13727+A1 standard, the batch **2013.03.01.70** of the product **F2563** (ready to use product) presents a bactericidal activity in **5 minutes contact time** at 20°C, in dirty conditions, against the strains *Pseudomonas aeruginosa, Staphylococcus aureus* and *Enterococcus hirae.*

Romainville, on August the 31st, 2015

Corinne BENOLIEL
Doctor of pharmacy
Scientific Director

TEST REPORT

BACTERICIDAL ACTIVITY OF THE SEPTALKAN PRODUCT ACCORDING TO THE EN 14561 STANDARD

For: **ALKAPHARM**
PARC BIOTECH
102 AVENUE GASTON ROUSSEL
93230 ROMAINVILLE

Study number: n°061F02-2012-01

BACTERICIDAL TESTS:

According to the European standard NF EN 14561 (March 2007) – Chemical disinfectants and antiseptics - Quantitative carrier test for the evaluation of bactericidal activity of chemical disinfectants used for instruments in the medical area -
Test method and requirements (phase 2, step 2)

Tests performed against three reference strains: *Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus hirae*.

This test report included 17 pages.

Study completion date: 07/13/2012

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

Professor Georges HERBEIN
Professor of Medicine – MD-PhD in virology
Scientific consultant

SUMMARY

- ✓ PERFORMING LABORATORY
- ✓ PRODUCT IDENTITY
- ✓ EXPERIMENTAL CONDITIONS
- ✓ VALIDATIONS
- ✓ ASSAYS
- ✓ CONCLUSIONS
- ✓ TECHNICAL APPENDIX

18, rue Alain SAVARY • 25000 Besançon • Tel: 03.81.25.09.04 • Fax: 03.81.25.53.51• SARL • RCS BESANÇON • N° SIRET 51786053200012 • N° TVA intra FR 23517860532 • info@apexlabo.com

1. PERFORMING LABORATORY

APEX BIOSOLUTIONS
18, rue Alain SAVARY
25000 BESANÇON
FRANCE

2. PRODUCT IDENTITY

SEPTALKAN

Batch N°: 2163F2563A

Expiration date: non communicated

Manufacturer: ALKAPHARM

Manufacturing date: 06/11/2012

Storage conditions: as recommended by the manufacturer

Active substances: didecyl dimethylammonium chloride and alkylbenzyl dimethyl ammonium chloride

Appearance: clear

Product diluent recommended by the manufacturer for use: none – ready-to-use product

Date of delivery of the product: 06/26/2012.

Date of tests: from 06/29/2012 to 07/11/2012

3. EXPERIMENTAL CONDITIONS

- Concentrations of the product: 100%, 80% and 10%.
- Method: EN 14561
- Exposure time: 5 minutes
- Temperature used during the assays: 20°C
- Organic soil load: dirty conditions, bovine albumin sera (bovine albumin 3 g/l finaux and sheep erythrocytes 3 ml/l).
- Diluent used for the product: trypton salt sterile solution.

- Strains: *Staphylococcus aureus* subsp. *aureus* DSMZ 799 batch march 2011 (ATCC 6538), *Pseudomonas aeruginosa* CIP 103467 batch 10807 (ATCC 15442) and *Enterococcus hirae* CIP 58.55 batch 7804 (ATCC 10541) - Institut Pasteur.

- Culture conditions: TSA (Trypton Soy Agar), at 37°C ± 1°C.

- Stop solution: 10 ml of neutralizer (egg yolk (5 %) and polysorbate 80 (3 %)).

4. RESULTS

See sheet of results.

The SEPTALKAN product demonstrated a bactericidal activity (5 log reduction is mandatory):

- S. *aureus*, R ≥ 5,56 from the 100% concentration
- *P. aeruginosa*, R ≥ 5,17 from the 100% concentration
- *E. hirae*, R ≥ 5,25 from the 100% concentration

5. CONCLUSION

Acording to the EN 14561 (March 2007), the SEPTALKAN product, batch n°2163F2563A:

- **Demonstrated a bactericidal activity against the three reference strains when used as ready-to-use product, for 5 min of time exposure, at 20°C, in dirty conditions (3 g/l bovine albumin and 3 ml/l sheep erythrocytes).**

SHEET 1 OF RESULTS
***Staphylococcus aureus* (trial)**

Standard: EN 14561, phase 2, step 2

Product: SEPTALKAN

Batch n°: 2163F2563A

Diluent used for the product: distilled water

Number of petri dishes: 1 petri dish / ml

Stop solution: Polysorbate 80 (30g/L) + egg yolk (5%)

Temperature of trials: 20°C

Organic soil load: bovine albumin sera 3 g/l with sheep erythrocytes 3 ml/l

Strain: *Staphylococcus aureus* subsp. *aureus* DSMZ 799 batch march 2011 (ATCC 6538)

Incubation temperature: 37°C ± 1°C

Study N°: 061F02-2012-01

Date of tests: 07/05/2012

Study director: Stephanie MOROT-BIZOT

Signature:

Diluent used: distilled water

Appearance of the product: clear

Validations

Validation control (Nv0)			Experimental conditions control (A)			Control of neutralization (B)			Validation of dilution-neutralization method (C) product concentration 100%		
Vc1	51	$\bar{x}$ = 48,5	Vc1	46	$\bar{x}$ = 38	Vc1	31	$\bar{x}$ = 41	Vc1	37	$\bar{x}$ = 34
Vc2	46		Vc2	30		Vc2	51		Vc2	31	
30 ≤ $\bar{x}$ of Nv0 ≤160 ☒ yes ☐ no			$\bar{x}$ of A is ≥0,5 × $\bar{x}$ of Nv0 ☒ yes ☐ no			$\bar{x}$ of B ≥ 0,5× $\bar{x}$ of Nv0 ☒ yes ☐ no			$\bar{x}$ of C ≥ 0,5 × $\bar{x}$ of Nv0 ☒ yes ☐ no		

18, rue Alain SAVARY • 25000 Besançon • Tel: 03.81.25.09.04 • Fax: 03.81.25.53.51• SARL • RCS BESANÇON • N° SIRET 51786053200012 • N° TVA intra FR 23517860532 • info@apexlabo.com

Suspension test and trials

	N	Vc1	Vc2	
Suspension test N	10^{-7}	187	207	$N = 2,00 \times 10^9$ lg N = 9,30 $9,17 \leq N \leq 9,70$? ×yes □no
	10^{-8}	25	18	

Methodology controls:
- $1,5 \times 10^9$ UFC/ml$\leq N \leq 5,0 \times 10^9$ UFC/ml
- $1,4 \times 10^7$ UFC/ml$\leq$ Nwt $\leq$ lg N-1,3
- 30$\leq$ Nv0 $\leq$160 UFC/ml
- A, B and C $\geq$0,5 x Nv0

	N	Vc1	Vc2	
Water control Nw	10^{-4}	>330	>330	$Nw = 5,80 \times 10^7$ lg Nw = 7,76 7,15 $\leq$ Nw $\leq$ lg N-1,3? ×yes □no
	10^{-5}	55	60	

Product	10^0		10^{-1}		10^{-2}		10^{-3}		lg Na	lg R = lg Nw – lg Na	Exposure time
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2			
100 %	0	0 0	0	0	0	0	0	0	2,15	**5,61**	5 min
80 %	0	0	0	0	0	0	0	0	2,15	**5,61**	5 min
10%	>330	>330	>330	>330	>330	>330	299	314	2,15	1,27	5 min

Vc: value is the number of cfu/ml
x̄: average (Vc1 and Vc2)
N : number of cfu/ml of the bacterial suspension
Nw: number of cfu/ml of the control bacterial test suspension
Na: number of survivors per ml in the test mixture at the end of the time exposure
Nv0: number of cfu/ml of the bacterial test suspension in the mixture A, B and C at the beginning of the time exposure

SHEET 2 OF RESULTS
***Staphylococcus aureus* (repetition)**

Standard: EN 14561, phase 2, step 2

Product: SEPTALKAN

Batch n°: 2163F2563A

Diluent used for the product: distilled water

Number of petri dishes: 1 petri dish / ml

Stop solution: Polysorbate 80 (30g/L) + egg yolk (5%)

Temperature of trials: 20°C

Organic soil load: bovine albumin sera 3 g/l with sheep erythrocytes 3 ml/l

Strain: *Staphylococcus aureus* subsp. *aureus* DSMZ 799 batch march 2011 (ATCC 6538)

Incubation temperature: 37°C ± 1°C

Study N°: 061F02-2012-01

Date of tests: 07/11/2012

Study director: Stephanie MOROT-BIZOT

Signature:

Diluent used: distilled water

Appearance of the product: clear

Validations

Validation control (Nv0)			Experimental conditions control (A)			Control of neutralization (B)			Validation of dilution-neutralization method (C) product concentration 100%		
Vc1	48	$\bar{x}$ = 46,5	Vc1	82	$\bar{x}$ = 77,5	Vc1	71	$\bar{x}$ = 59,5	Vc1	80	$\bar{x}$ = 73
Vc2	45		Vc2	73		Vc2	48		Vc2	66	
30 ≤ $\bar{x}$ of Nv0 ≤160 ☒ yes ☐ no			$\bar{x}$ of A is ≥0,5 × $\bar{x}$ of Nv0 ☒ yes ☐ no			$\bar{x}$ of B ≥ 0,5× $\bar{x}$ of Nv0 ☒ yes ☐ no			$\bar{x}$ of C ≥ 0,5 × $\bar{x}$ of Nv0 ☒ yes ☐ no		

18, rue Alain SAVARY • 25000 Besançon • Tel: 03.81.25.09.04 • Fax: 03.81.25.53.51• SARL • RCS BESANÇON • N° SIRET 51786053200012 • N° TVA intra FR 23517860532 • info@apexlabo.com

Suspension test and trials

	N	Vc1	Vc2	
Test suspension N	10^{-7}	230	188	$N = 2,10 \times 10^9$ lg N = 9,33 $9,17 \leq N \leq 9,70$? ×yes □no
	10^{-8}	18	30	

Methodology controls:
- $1,5 \times 10^9$ UFC/ml$\leq N \leq 5,0 \times 10^9$ UFC/ml
- $1,4 \times 10^7$ UFC/ml$\leq$ Nwt $\leq$ lg N-1,3
- 30$\leq$ Nv0 $\leq$160 UFC/ml
- A, B and C $\geq$0,5 x Nv0

	N	Vc1	Vc2	
Water control Nw	10^{-4}			$Nw = 4,60 \times 10^7$ lg Nw = 7,66 7,15 $\leq$ Nw $\leq$ lg N-1,3? ×yes □no
	10^{-5}	45	46	

Product	10^0		10^{-1}		10^{-2}		10^{-3}		lg Na	lg R = lg Nw – lg Na	Exposure time
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2			
100 %	0	0	0	0	0	0	0	0	2,15	**5,51**	5 min
80 %	0	0	0	0	0	0	0	0	2,15	**5,51**	5 min
10%	>330	>330	63	85	23	24	2	2	3,95	3,71	5 min

Vc: value is the number of cfu/ml
x̄: average (Vc1 and Vc2)
N : number of cfu/ml of the bacterial suspension
Nw: number of cfu/ml of the control bacterial test suspension
Na: number of survivors per ml in the test mixture at the end of the time exposure
Nv0: number of cfu/ml of the bacterial test suspension in the mixture A, B and C at the beginning of the time exposure

SHEET 3 OF RESULTS
***Enterococcus hirae* (trial)**

Standard: EN 14561, phase 2, step 2

Product: SEPTALKAN

Batch n°: 2163F2563A

Diluent used for the product: distilled water

Number of petri dishes: 1 petri dish / ml

Stop solution: Polysorbate 80 (30g/L) + egg yolk (5%)

Temperature of trials: 20°C

Organic soil load: bovine albumin sera 3 g/l with sheep erythrocytes 3 ml/l

Strain: *Enterococcus hirae* CIP 58.55 batch 7804 (ATCC 10541)

Incubation temperature: 37°C ± 1°C

Study N° 061F02-2012-01

Date of tests: 07/10/2012

Study director: Stephanie MOROT-BIZOT

Signature:

Diluent used: distilled water

Appearance of the product: clear

Validations

Validation control (Nv0)			Experimental conditions control (A)			Control of neutralization (B)			Validation of dilution-neutralization method (C) product concentration 100%		
Vc1	67	$\bar{x}$ = 58,5	Vc1	58	$\bar{x}$ = 65	Vc1	67	$\bar{x}$ = 56,5	Vc1	61	$\bar{x}$ = 61
Vc2	50		Vc2	72		Vc2	46		Vc2	61	
30 ≤ $\bar{x}$ of Nv0 ≤160 ☒ yes ☐ no			$\bar{x}$ of A is ≥0,5 × $\bar{x}$ of Nv0 ☒ yes ☐ no			$\bar{x}$ of B ≥ 0,5× $\bar{x}$ of Nv0 ☒ yes ☐ no			$\bar{x}$ of C ≥ 0,5 × $\bar{x}$ of Nv0 ☒ yes ☐ no		

18, rue Alain SAVARY • 25000 Besançon • Tel: 03.81.25.09.04 • Fax: 03.81.25.53.51• SARL • RCS BESANÇON • N° SIRET 51786053200012 • N° TVA intra FR 23517860532 • info@apexlabo.com

Suspension test and trials

	N	Vc1	Vc2	
Test suspension N	10^{-7}	234	255	N = 2,40 x 10^9 ufc/mL lg N =9,38 9,17 ≤ N ≤ 9,70? ×yes □no
	10^{-8}	26	14	

Methodology controls:
- 1,5 x 10^9 UFC/ml≤ N ≤ 5,0 x 10^9 UFC/ml
- 1,4 x 10^7 UFC/ml≤ Nwt ≤ lg N-1,3
- 30≤ Nv0 ≤160 UFC/ml
- A, B and C ≥0,5 x Nv0

	N	Vc1	Vc2	
Water control Nw	10^{-4}	284	269	Nw = 2,40 x 10^7 lg Nw = 7,37 7,15 ≤ Nw ≤ lg N-1,3? ×yes □no
	10^{-5}	25	22	

Product	10^0		10^{-1}		10^{-2}		10^{-3}		lg Na	lg R = lg Nw – lg Na	Exposure time
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2			
100 %	0	0	0	0	0	0	0	0	<2,15	**>5,22**	5 min
80 %	0	0	0	0	0	0	0	0	<2,15	**>5,22**	5 min
10%	>330	>330	>330	>330	>330	>330	38	31	5,54	1,83	5 min

Vc: value is the number of cfu/ml
x̄: average (Vc1 and Vc2)
N : number of cfu/ml of the bacterial suspension
Nw: number of cfu/ml of the control bacterial test suspension
Na: number of survivors per ml in the test mixture at the end of the time exposure
Nv0: number of cfu/ml of the bacterial test suspension in the mixture A, B and C at the beginning of the time exposure

SHEET 4 OF RESULTS
***Enterococcus hirae* (repetition)**

Standard: EN 14561, phase 2, step 2

Product: SEPTALKAN

Batch n°: 2163F2563A

Diluent used for the product: distilled water

Number of petri dishes: 1 petri dish / ml

Stop solution: Polysorbate 80 (30g/L) + egg yolk (5%)

Temperature of trials: 20°C

Organic soil load: bovine albumin sera 3 g/l with sheep erythrocytes 3 ml/l

Strain: *Enterococcus hirae* CIP 58.55 batch 7804 (ATCC 10541)

Incubation temperature: 37°C ± 1°C

Study N° 061F02-2012-01

Date of tests: 07/12/2012

Study director: Stephanie MOROT-BIZOT

Signature:

Diluent used: distilled water

Appearance of the product: clear

Validations

Validation control (Nv0)			Experimental conditions control (A)			Control of neutralization (B)			Validation of dilution-neutralization method (C) product concentration 100%		
Vc1	57	$\bar{x}$ = 50,5	Vc1	53	$\bar{x}$ = 62	Vc1	63	$\bar{x}$ = 66,5	Vc1	52	$\bar{x}$ = 53
Vc2	44		Vc2	71		Vc2	70		Vc2	54	
30 ≤ $\bar{x}$ of Nv0 ≤160 ☒ yes ☐ no			$\bar{x}$ of A is ≥0,5 × $\bar{x}$ of Nv0 ☒ yes ☐ no			$\bar{x}$ of B ≥ 0,5× $\bar{x}$ of Nv0 ☒ yes ☐ no			$\bar{x}$ of C ≥ 0,5 × $\bar{x}$ of Nv0 ☒ yes ☐ no		

18, rue Alain SAVARY • 25000 Besançon • Tel: 03.81.25.09.04 • Fax: 03.81.25.53.51• SARL • RCS BESANÇON • N° SIRET 51786053200012 • N° TVA intra FR 23517860532 • info@apexlabo.com

Suspension test and trials

	N	Vc1	Vc2	
Test suspension N	10^{-7}	239	218	$N = 2,30 \times 10^9$ lg N = 9,36 $9,17 \leq N \leq 9,70$? ×yes □no
	10^{-8}	27	21	

Methodology controls:
- $1,5 \times 10^9$ UFC/ml$\leq N \leq 5,0 \times 10^9$ UFC/ml
- $1,4 \times 10^7$ UFC/ml$\leq$ Nwt $\leq$ lg N-1,3
- 30$\leq$ Nv0 $\leq$160 UFC/ml
- A, B and C $\geq$0,5 x Nv0

	N	Vc1	Vc2	
Water control Nw	10^{-4}	301	275	$Nw = 2,70 \times 10^7$ lg Nw = 7,43 7,15 $\leq$ Nw $\leq$ lg N-1,3? ×yes □no
	10^{-5}	29	25	

Product	10^0		10^{-1}		10^{-2}		10^{-3}		lg Na	lg R = lg Nw – lg Na	Exposure time
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2			
100 %	0	0	0	0	0	0	0	0	2,15	**5,29**	5 min
80 %	0	0	0	0	0	0	0	0	2,15	**5,29**	5 min
10%	>330	>330	>330	>330	>330	>330	25	20	5,35	2,08	5 min

Vc: value is the number of cfu/ml
x̄: average (Vc1 and Vc2)
N : number of cfu/ml of the bacterial suspension
Nw: number of cfu/ml of the control bacterial test suspension
Na: number of survivors per ml in the test mixture at the end of the time exposure
Nv0: number of cfu/ml of the bacterial test suspension in the mixture A, B and C at the beginning of the time exposure)

SHEET 5 OF RESULTS
***Pseudomonas aeruginosa* (trial)**

Standard: EN 14561, phase 2, step 2

Product: SEPTALKAN

Batch n°: 2163F2563A

Diluent used for the product: distilled water

Number of petri dishes: 1 petri dish / ml

Stop solution: Polysorbate 80 (30g/L) + egg yolk (5%)

Temperature of trials: 20°C

Organic soil load: bovine albumin sera 3 g/l with sheep erythrocytes 3 ml/l
Strain: *Pseudomonas aeruginosa* CIP 103467 batch 10807 (ATCC 15442)

Incubation temperature: 37°C ± 1°C

Study N° 061F02-2012-01

Date of tests: 07/10/2012

Study director: Stephanie MOROT-BIZOT

Signature:

Diluent used: distilled water

Appearance of the product: clear

Validations

Validation control (Nv0)			Experimental conditions control (A)			Control of neutralization (B)			Validation of dilution-neutralization method (C) product concentration 100%		
Vc1	41	$\bar{x}$ = 43,5	Vc1	28	$\bar{x}$ = 29	Vc1	57	$\bar{x}$ = 52	Vc1	48	$\bar{x}$ = 38,5
Vc2	46		Vc2	30		Vc2	47		Vc2	29	
30 ≤ $\bar{x}$ of Nv0 ≤160 ☒ yes ☐ no			$\bar{x}$ of A is ≥0,5 × $\bar{x}$ of Nv0 ☒ yes ☐ no			$\bar{x}$ of B ≥ 0,5× $\bar{x}$ of Nv0 ☒ yes ☐ no			$\bar{x}$ of C ≥ 0,5 × $\bar{x}$ of Nv0 ☒ yes ☐ no		

18, rue Alain SAVARY • 25000 Besançon • Tel: 03.81.25.09.04 • Fax: 03.81.25.53.51• SARL • RCS BESANÇON • N° SIRET 51786053200012 • N° TVA intra FR 23517860532 • info@apexlabo.com

Suspension test and trials

	N	Vc1	Vc2	
Test suspension N	10^{-7}	191	154	$N = 1,70 \times 10^9$ lg N = 9,24 9,17 ≤ N ≤ 9,70? ×yes □no
	10^{-8}	16	22	

Methodology controls:
- $1,5 \times 10^9$ UFC/ml≤ N ≤ $5,0 \times 10^9$ UFC/ml
- $1,4 \times 10^7$ UFC/ml≤ Nwt ≤ lg N-1,3
- 30≤ Nv0 ≤160 UFC/ml
- A, B and C ≥0,5 x Nv0

	N	Vc1	Vc2	
Water control Nw	10^{-4}	148	162	$Nw = 1,50 \times 10^7$ lg Nw = 7,16 7,15 ≤ Nw ≤ lg N-1,3? ×yes □no
	10^{-5}	14	15	

Product	10^0		10^{-1}		10^{-2}		10^{-3}		lg Na	lg R = lg Nw – lg Na	Exposure time
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2			
100 %	0	0 0	0	0	0	0	0	0	2,15	**5,02**	5 min
80 %	0	0	0	0	0	0	0	0	2,15	**5,02**	5 min
10%	>330	>330	>330	>330	>330	>330	>330	>330	>6,52	<0,64	5 min

Vc: value is the number of cfu/ml
x̄: average (Vc1 and Vc2)
N : number of cfu/ml of the bacterial suspension
Nw: number of cfu/ml of the control bacterial test suspension
Na: number of survivors per ml in the test mixture at the end of the time exposure
Nv0: number of cfu/ml of the bacterial test suspension in the mixture A, B and C at the beginning of the time exposure

SHEET 6 OF RESULTS
***Pseudomonas aeruginosa* (repetition)**

Standard: EN 14561, phase 2, step 2

Product: SEPTALKAN

Batch n°: 2163F2563A

Diluent used for the product: distilled water

Number of petri dishes: 1 petri dish / ml

Stop solution: Polysorbate 80 (30g/L) + egg yolk (5%)

Temperature of trials: 20°C

Organic soil load: bovine albumin sera 3 g/l with sheep erythrocytes 3 ml/l

Strain: *Pseudomonas aeruginosa* ATCC 15442 (CIP 103467)

Incubation temperature: 37°C ± 1°C

Study N° 061F02-2012-01

Date of tests: 07/11/2012

Study director: Stephanie MOROT-BIZOT

Signature:

Diluent used: distilled water

Appearance of the product: clear

Validations

Validation control (Nv0)			Experimental conditions control (A)			Control of neutralization (B)			Validation of dilution-neutralization method (C) product concentration 100%		
Vc1	75	$\bar{x}$ = 73,5	Vc1	62	$\bar{x}$ = 66	Vc1	67	$\bar{x}$ = 71,5	Vc1	71	$\bar{x}$ = 77,5
Vc2	72		Vc2	70		Vc2	76		Vc2	84	
30 ≤ $\bar{x}$ of Nv0 ≤160 ☒ yes ☐ no			$\bar{x}$ of A is ≥0,5 × $\bar{x}$ of Nv0 ☒ yes ☐ no			$\bar{x}$ of B ≥ 0,5× $\bar{x}$ of Nv0 ☒ yes ☐ no			$\bar{x}$ of C ≥ 0,5 × $\bar{x}$ of Nv0 ☒ yes ☐ no		

18, rue Alain SAVARY • 25000 Besançon • Tel: 03.81.25.09.04 • Fax: 03.81.25.53.51• SARL • RCS BESANÇON • N° SIRET 51786053200012 • N° TVA intra FR 23517860532 • info@apexlabo.com

Suspension test and trials

	N	Vc1	Vc2	
Test suspension N	10^{-7}	232	264	$N = 2,50 \times 10^9$ lg N = 9,39 $9,17 \leq N \leq 9,70$? ×yes □no
	10^{-8}	24	23	

Methodology controls:
- $1,5 \times 10^9$ UFC/ml$\leq N \leq 5,0 \times 10^9$ UFC/ml
- $1,4 \times 10^7$ UFC/ml$\leq$ Nwt $\leq$ lg N-1,3
- $30 \leq Nv0 \leq 160$ UFC/ml
- A, B and C $\geq 0,5 \times Nv0$

	N	Vc1	Vc2	
Water control Nw	10^{-4}	294	>330	$Nw = 3,0 \times 10^7$ lg Nw = 7,47 7,15 ≤ Nw ≤ lg N-1,3? ×yes □no
	10^{-5}	28	31	

Product	10^0		10^{-1}		10^{-2}		10^{-3}		lg Na	lg R = lg Nw – lg Na	Exposure time
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2			
100 %	0	0	0	0	0	0	0	0	2,15	**5,32**	5 min
80 %	0	0	0	0	0	0	0	0	2,15	**5,32**	5 min
10%	>330	>330	>330	>330	>330	>330	>330	>330	>6,52	<0,95	5 min

Vc: value is the number of cfu/ml
x̄: average (Vc1 and Vc2)
N : number of cfu/ml of the bacterial suspension
Nw: number of cfu/ml of the control bacterial test suspension
Na: number of survivors per ml in the test mixture at the end of the time exposure
Nv0: number of cfu/ml of the bacterial test suspension in the mixture A, B and C at the beginning of the time exposure

TECHNICAL APPENDIX

Media:

TSA (Trypton Soya Agar), BD, ref. 236950, batch n°1326359

Reagents:

ORGANIC SOIL LOAD:

Bovine Albumin Sera, powder, Fraction V, SIGMA ALDRICH, ref. 05479, batch STBB7838V

DILUENT

Trypton-Salt Solution (TS)

- Trypton, Laboratories CONDA, ref. 1612, batch n° 091229 ----------1,00 g/l
- Sodium chloride, MOLECULAR BIOLOGY, ref n° 19032391, batch n° 808211----8,50 g/l

pH at 25°C : 7,0 ± 0,2

NEUTRALIZER

- Egg yolk ---------- 5 %
- Polysorbate 80, SIGMA ALDRICH, ref. 59924, batch n°1451069 ---------- 30 g/l
- Trypton-Soya Broth (TSB), CONDA, ref. n°1224, batch n° 810081 --------- 30 g/l

GLASS CARRIERS – glass carriers 15 x 60 mm, 1 mm thick – Thermo scientific/ Menzel-Gläser – ref. 100 OTM, batch n°01 1794389.

18, rue Alain SAVARY • 25000 Besançon • Tel: 03.81.25.09.04 • Fax: 03.81.25.53.51• SARL • RCS BESANÇON • N° SIRET 51786053200012 • N° TVA intra FR 23517860532 • info@apexlabo.com

Laboratoire MIDAC
Your expert in microbiology

Test report n° RE 14359-3

cofrac
ESSAIS
Acuidll:atlon n•1-0019
Ponée disponible
sur www.cofrac.fr

Bactericidal activity according to the EN 1276: 2010 standard

Product: Septalkan batch 2013.03.01.70 (exp: 2016/03)

On request of:

ALKAPHARM
Parc Biocitech
102 avenue Gaston Roussel
FR 93230
ROMAINVILLE

Date: 04/12/2014

Visa:

Technical manager
Mélinda MAUX, Ph.D

The test report includes: 5 pages

The COFRAC accreditation attests laboratories are competent for the on/y tests covered by the program.
Copy of this test report is on/y authorized from its unabridged version.
This report concerns on/y the tested product.

1 PRINCIPLE :

The bactericidal activity was evaluated according to the **EN 1276** European standard: **Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1) - March 2010."**

Test organisms(s): **see table(s) on next page(s).**
Preservation and stock cultures of test organisms following the requirements of the EN 12353 standard.

2 SAMPLE(S) IDENTIFICATION

Name(s): **Septalkan batch 2013.03.01.70 (exp: 2016/03).**

Society: **ALKAPHARM.**

Product appearance: **Liquid.**

Receipt at the laboratory: 02/10/2014.

Storage conditions at the laboratory: Room temperature, in the darkness.

3 TEST METHOD AND ITS VALIDATION:

- **Dilution neutralization:** pour plating method (number of plates : 2 i.e. 1 ml/ plate)
- **Diluent-neutralizer:** TSB Capitol IV

Addition of 10% diluent-neutralizer in the agar (v/v).

4 EXPERIMENTAL CONDITIONS:

Period of analysis: from 07/10/2014 to 25/10/2014.

Diluent used for product test solution: **distilled water.**

Product test concentration(s): **see table(s) on next page(s).**

Appearance of product dilutions: **limpid.**

Test temperature: **20°C** +/- **1°c.**

Contact time: **5 minutes+/- 10 secondes.**

Interfering substance: **3g/l bovine albumin (dirty conditions).** Prepared according to the requirements of the EN 1276 standard.

Stability of the test mixture interfering substance/ test product(s): **trouble.**

Incubation temperature: **37°C** +/- **1°C.**

5 TEST RESULTS:

Control and validations of the dilution-neutralization method in the test conditions (carried out in parallel with tests)

Strain(s)	Enumeration of bacteria {CFU/ml) :			
	Validation of the suspension **Nvo**	Experimental conditions control **A**	Neutralizer non-toxicity control **B**	Method validation **C** Product concentration: **80%**
Pseudomonos aeruginosa CIP 103467	Vcl: 36 Vc2: 45 Nvo: 40,5	Vcl: 37 Vc2: 38 A: 37,5	Vcl: 45 Vc2: 46 B: 45,5	Vcl: 40 Vc2: 36 C: 38,0
Escherichia co/i CIP 54127	Vcl: 96 Vc2: 96 Nvo: 96,0	Vcl: 91 Vc2: 94 A: 92,5	Vcl: 87 Vc2: 95 B: 91,0	Vcl: 90 Vc2: 97 C: 93,5
Staphylococcus aureus CIP 4 83	Vcl: 70 Vc2: 64 Nvo: 67,0	Vcl: 61 Vc2: 68 A: 64,5	Vcl: 64 Vc2: 67 B: 65,5	Vcl: 67 Vc2: 68 C: 67,5
Enterococcus hirae CIP 5855	Vcl: 81 Vc2: 90 Nvo: 85,5	Vcl: 89 Vc2: 91 A: 90,0	Vcl: 88 Vc2: 95 B: 91,5	Vcl: 99 Vc2: 89 C: 94,0
Validation criterio: *Nvobetween 30 and 160 CFU* *A, Band C equol or equivalent tra 0.5 x NvO*				

Conclusion:

The neutralization is validated with the dilution-neutralization method with the test organisms in the test conditions.

Evaluation of bactericidal activity (neutralization by dilution-neutralization method)

Strain(s)	Test suspension **N** and **No**	**Enumeration of bacteria at the tested concentrations(% v/v): Na**		
		0,1	10,0	80,0
Pseudomonas aeruginosa CIP 103467	10^{-6} v c1 : 158 Vc2: 172 10^{-1} v c1 : 21 Vc2: 25 N= 1,71 x 10^8 No= 1,71 x 10^7 log No= 7,23	Vcl: > 330 Vc2: > 330 Na : > 3,3 x 10^3 log Na : > 3,52	**Vcl: < 14** **Vc2: < 14** **Na: < 140** **log Na : < 2,15**	**Vcl: < 14** **Vc2: < 14** **Na: < 140** **log Na: < 2,15**
Escherichia co/i CIP 54 127	10^{-6} v c1 : 280 Vc2: 268 10^{-1} v c1 : 28 Vc2: 29 N= 2,75 x 10^8 No= 2,75 x 10^7 log No= 7,44	Vcl: > 330 Vc2: > 330 Na : > 3,3 x 10^3 log Na : > 3,52	**Vcl: < 14** **Vc2: < 14** **Na: < 140** **log Na : < 2,15**	**Vcl: < 14** **Vc2: < 14** **Na: < 140** **log Na : < 2,15**
Staphy/ococcus aureus CIP 4 83	10^{-6} v c1 : 286 Vc2: 290 10^{-1} v c1 : 27 Vc2: 29 N= 2,87 x 10^8 No= 2,87 x 10^7 log No= 7,46	Vcl: > 330 Vc2: > 330 Na : > 3,3 x 10^3 log Na : > 3,52	**Vcl: < 14** **Vc2: < 14** **Na: < 140** log Na : < **2,15**	**Vcl: < 14** **Vc2: < 14** **Na: < 140** log Na : < 2,15
Enterococcus hirae CIP 5855	10^{-6} v c1 : 288 Vc2: 294 10^{-1} v c1 : 31 Vc2: 27 N= 2,91 x 10^8 No= 2,91 x 10^7 log No= 7,46	Vcl: > 330 Vc2: > 330 Na : > 3,3 x 10^3 log Na : > 3,52	Vcl: < 14 Vc2: < 14 **Na: < 140** **log Na : < 2,15**	Vcl: < 14 Vc2: < 14 **Na: < 140** **log Na : < 2,15**

Validation criteria:
N between 1.5 x 1(11 and 5.0 x 1(11
No between 1.5 x 10^7 and 5.0 x 10^7
log No between 7.17 and 7.70

Reduction (R) of the number of viable cells at the tested concentration(% v/v) :

	0,1	10,0	80,0
Pseudomonas aeruginosa CIP 103467	log R: < 3,71	**log R: > 5,08**	**log R: > 5,08**
Escherichia coti CIP 54 127	log R: < 3,92	**log R: > 5,29**	**log R: > 5,29**
Staphylococcus aureus CIP 4 83	log R: < 3,94	**log R: > 5,31**	**log R: > 5,31**
Enterococcus hirae CIP 5855	log R: < 3,94	**log R: > 5,31**	**log R: > 5,31**

Interpretation criteria:
active concentration if log R è 5
non active concentration if log R 5'5

6 CONCLUSION :

According to the EN 1276 standard (March 2010), the product **Septalkan batch 2013.03.01.70 (exp: 2016/03) possesses a bactericidal activity** in the test conditions: dirty conditions - 5 minutes of contact at 20°C +/- 1°C - at **10% (v/v)** against the four tested strains.

Laboratoire MIDAC
Your expert in microbiology

Test report n° RE 14361-2

cofrac
ESSAIS
Accréditation n•t-0019
Pon:h di1ponibte:
sv, www.cofrac.fr

Bactericidal activity according to the EN 13697: 2001 standard

Product: Septalkan batch 2013.03.01.70 (exp 03/2016)

On request of:

ALKAPHARM
Parc Biocitech
102 avenue Gaston Roussel
FR 93230
ROMAINVILLE

Date: 04/12/2014

Visa:

Technical manager
Mélinda MAUX, Ph.D

The test report includes: 4 pages

The COFRAC accreditation attests loboratories are competent for the on/y tests covered by the program.
Copy of this test report is on/y authorized from its unabridged version.
This report concerns on/y the tested product.

1 PRINCIPLE:

The bactericidal activity on surface has been evaluated in accordance to the **EN 13697** European Standard: " **Chemical disinfectants and antiseptics -Quantitative non porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements without mechanic action - (Phase 2/ Step 2) - November 2001."**

Test organisms(s): **see table(s) on next page(s).**
Preservation and stock cultures of test organisms following the requirements of the EN 12353 standard.

2 SAMPLE(S) IDENTIFICATION:

Name(s): **Septalkan batch 2013.03.01.70 (exp 03/2016)**

Society: **ALKAPHARM.**

Receipt at the laboratory: **02/10/2014.**

Storage conditions at the laboratory: Room temperature, in the darkness.

3 TEST METHOD AND ITS VALIDATION:

- Dilution neutralization:
- Diluent-neutralizer: TSB Capitol IV
Addition of 10% diluent-neutralizer in the agar (v/v).

4 EXPERIMENTAL CONDITIONS:

Period of analysis: from 04/11/2014 to 06/11/2014.

Diluent used for product test solution: **distilled water.**

Product test concentration(s): **see table(s) on next page(s).**

Interfering substance: **3g/l bovine albumin (dirty conditions).** Prepared according to the requirements of the EN 13697 standard.

Appearance of product dilutions: **colourless, homogeneous.**

Stability of the test mixture interfering substance/ test product(s): **none precipitate.**

Test temperature: **20°C** +/- **1°C.**

Contact time: **5 minutes+/- 10 secondes.**

Incubation temperature: **37°C** +/- **1°C.**

Tested surface: **Stainless steel dises 304 with grade 2B,** 0 **2 cm, finish on both sides according to the requirements of the EN 10 088-2 standard.**

Drying conditions: **30 minutes at 37°C** +/- **1°c in an incubator with a fan.**

5 TEST RESULTS:

Test organisms	Validation test			Distilled water contrai	Test procedure at concentrations in% (v/v)		
	Bacterial suspension test N	Neutralization contrai (distilled water) NC	Neutralization test (product) NT	Ne	0,1	10,0	100,0
Pseudomonas aeruginosa CIP 103467	10-6: 287; 281 10-7: 28; 24 N: 6,85	10-3: >300 ;>300 10-4: 58; 60 10-5: 7; 8 NC: 6,77	10-3: >300 ;>300 10-4: 60; 64 10-5: 5; 2 NT: 6,79	10-3: >300 ;>300 10-4: 59; 53 10-5: 6; 7 10-6: 1; 0 Nc:6,75 Nts: > 300	10° : >300; >300 10-l: >300; >300 10-2: >300 ;>300 Nd : > 5,48 Nts: > 300 ME:< 1,27	**10° : 51; 50** **10-l: 8; 9** **10-2: 1; 1** **Nd: 2,70** **Nts: 30** **ME: 4,05**	**10° : 0; 0** **10-l: 0; 0** **10-2: 0; 0** **Nd:< 0,1** **Nts: 0** **ME:> 6,65**
Escherichia coli CIP 54127	10-6: 259 ; 264 10-7: 30; 25 N: 6,82	10-3: >300 ;>300 10-4: 51; 52 10-5: 4; 3 NC:6,72	10-3: >300 ;>300 10-4: 53; 50 10-5: 6; 3 NT: 6,72	10-3: >300 ;>300 10-4: 50; 54 10-5: 5; 7 10-6: 0; 0 Nc:6,72 Nts: > 300	10° : >300 ; >300 10-l: 42; 56 10-2: 13; 7 Nd: 3,69 Nts: > 300 ME: 3,03	10° : 0; 0 **10-l: 0; 0** **10-2: 0; 0** **Nd:< 0,1** **Nts: 0** **ME:>6,62**	**10° : 0; 0** 10-l: 0; 0 10-2: 0; 0 **Nd:< 0,1** **Nts: 0** **ME:> 6,62**
Staphylococcus aureus CIP 4. 83	10-6: 269; 274 10-7: 24; 29 N: 6,83	10-3: 132 ; 167 10-4: 12; 10 10-5: 1; 0 NC: 6,14	10-3: 140; 138 10-4: 13; 16 10-5: 1; 1 NT: 6,14	10-3: 127; 140 10-4: 13; 17 10-5: 1; 2 10-6: 0; 0 Ne: 6,13 Nts: > 300	10° : >300; >300 10-l : >300; >300 10-2 : 200; 189 Nd: 5,29 Nts: > 300 ME: 0,84	10° : 168; 172 10-l: 20; 22 10-2: 4; 1 Nd: 3,23 Nts: 124 ME: 2,90	10° : o ; o **10-l: 0; 0** **10-2: 0; 0** **Nd:< 0,1** **Nts: 0** **ME:> 6,03**
Enterococcus hirae CIP 58 55	10-6: 267; 281 10-7: 25; 29 N: 6,84	10-3: >300 ;>300 10-4: 68; 51 10-5: 4; 7 NC: 6,77	10-3: >300 ;>300 10-4: 69; 54 10-5 : 5; 6 NT: 6,79	10-3: >300 ;>300 10-4: 56; 62 10-5: 2; 2 10-6: 0; 0 Ne: 6,77 Nts: > 300	10° : >300; >300 10-l : >300; >300 10-2 : >300; >300 Nd:> 5,48 Nts : > 300 ME: 1,29	**10° : 30; 27** **10-l: 2; 5** **10-2: 0; 0** **Nd: 2,45** **Nts: 3** **ME: 4,32**	**10° : 0; 0** **10-l: 0; 0** 10-2: 0; 0 **Nd:<** 0,1 **Nts: 0** **ME:>6,67**

10-x : number of CFU for the dilution x.
N: Log number of CFU for 0.05 ml of test suspension
NC: Log number of CFU per test surface of neutralisation contrai: log (CFU/test surface)
NT: Log number of CFU per test surface of neutralisation contrai.
Ne: Log number of CFU per test surface of water contrai.
Nd: Log number of CFU per test surface of desinfectant test: log (CFU/test surface)
Nts : Number of CFU remoining on the surface test
ME: Bactericidol effect: ME= Ne-Nd. Effective concentration if ME<? 4

6 CONCLUSION :

According to the **EN 13697** standard (November 2001), the product **Septalkan batch 2013.03.01.70 (exp 03/2016) possesses a bactericidal activity** on surfaces in the test conditions: dirty conditions - 5 minutes of contact at 20°C +/- 1•c - at **100% (v/v)** against the four tested strains.

Laboratoire MIDAC
Your expert in microbiology

Test report n° RE 16088-4
Cancel and replace RE 16088-2

Bactericidal and yeasticidal activity
according to the NF EN 16615: 2015 standard

Product : Septalkan wipes - Flow pack
batch 2015 06 03 5201

On request of:

Alkapharm
Parc Biocitech
102 avenue Gaston Roussel
93230 ROMAINVILLE

Loos, the 15 July 2016

Mélinda MAUX, Ph.D
Technical manager

The test report includes: 6 pages

Copy of this test report is only authorized from its unobridged version.
This report concerns only the tested product.

1. PRINCIPLE

The bactericidal and yeasticidal activity has been evaluated according to the NF EN 16615 standard: "Chemical disinfectants and antiseptics - Quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes in the medical area (4- field test) - Test method and requirements (phase 2, step 2)." Mai 2015.

2. SAMPLE(S) IDENTIFICATION

Name(s): **Septalkan wipes - flow pack** - lot **2015 06 03-5201**

Society: **Alkapharm**

Receipt at the laboratory : 15 March 2016
Storage conditions at the laboratory: **Room temperature,** in the **darkness**
Appearance of the product: pre-impregnated wipes

3. METHODE D'ESSAI ET VALIDATION

- Neutralization method: **Dilution-neutralization method** (pour plating method)

- Diluent-neutralizer: **neutralizing buffered diluent at 2X concentration**
Addition of 10% diluent-neutralizer (v/v) in the agar

4. EXPERIMENTAL CONDITIONS

Period of analysis: from 13 April 2016 to 20 May 2016

Test organisms(s): see table(s) on next page(s)
Preservation and stock cultures of test organisms following the requirements of the EN 12353 standard

Product test concentration(s): see table(s) on next page(s)
Diluent used for product test solution:/
Appearance of product dilutions:/

Interfering substance(s): 3g/l bovine albumin with 3 ml/L sheep erythrocytes (dirty conditions)
Stability of the test mixture interfering substance/ test product(s): **none precipitate**

Test temperature(s): **20°c ± 2.5 •c**
Contact time(s): **5 minutes± 15 seconds**

Incubation temperature: **37°C ± 1•c**

Test surface identification: **PVC with PUR surface coating** (dimension 20 cm x 50 cm)
Unitary weight identification: granite block (dimension 12 cm x 8.6 cm x 8.6 cm)
Identification of the wipe used for mechanical action: see Sample(s) Identification part

Drying conditions: 45 minutes at the test temperature

5. TEST RESULTS

Contrais and validations of the dilution-neutralization method in the test conditions
(carried out in parallel with tests)

Test organism{s)	Number of survivor cells { CFU /ml)		
	Validation suspension Nvo	Neutralizer control B	Method validation C
Pseudomonos aeruginosa DSM 939	VCl: 85 VC2: 84 Nvo: 84.5	VCl: 74 VC2: 71 **B:** 72.5	VCl: 98 VC2: 92 **C:** 95.0
Enterococcus hirae DSM 3320	VCl: 113 VC2: 118 Nvo: 115.5	VCl: 97 VC2: 91 **B:** 94.0	VCl: 114 VC2: 117 **C:** 115.5
Stophylococcus aureus DSM 799	VCl: 71 VC2: 70 **Nvo:** 70.5	VCl: 85 VC2: 91 **B:** 88.0	VCl: 74 VC2: 81 **C:** 77.5
Candida a/bicans DSM 1386	VCl: 131 VC2: 140 **Nvo:** 135.5	VCl: 111 VC2: 110 **B:** 110.5	VCl: 113 VC2: 120 **C:** 116.5

10-x : number of CFU for the dilution x

Validation criteria:
NvO between 30 UFC/ml and 160 UFC/ml
8 Z 0.5 Nvo
C 2 0.5 Nv_0

Actua/ test contrais *(neutralization by dilution-neutra/ization)*

Test organism{s)	Numeration for test **T** on the test fields 2 to 4 {CFU/25 cm^2)	Numeration for the water control **NW** on the test fields 2 to 4 (CFU/25 cm^2)
Pseudomonas aeruginosa DSM 939	VC T2: 0;0 VC n: 0;0 VC T4: 0;0 Vr2to4: 0	VC r2: 41; 45 VC n: 24; 24 VC r,: 12; 16 VNwT2to4: 135.0
Enterococcus hirae DSM 3320	VC T2: 0;0 VC n: 0;0 V(T4: 0;0 Vnto4: 0	VC r2: 24; 26 VC n: 14; 16 VC T4: 9 ; 9 VNwT2 to4: 81.6
Staphylococcus aureus DSM 799	VC T2: 0;0 VC T3: 0;0 V(T4: 0;0 Vr2,04: 0	VC r2: 10; 15 VCn: 20; 20 V(T4: 21; 20 VNwT2 to4: 88.3
Candida a/bicans DSM 1386	VC T2: 0;0 VC n: 0;0 VC T4: 0;0 Vr2to4: 0	VC r2: 14; 17 VC n: 21; 21 VC T4: 9; 11 VNwT2 to4: 77.5

Validation criteria:
$V_{12 to 4}$ > 50 UFC /25cm'
$V_{aw 1 to 4}$ > 10 UFC /25cm²

***Actua/ test results** against bacteria :*

Test organism(s)	Test suspension **N** and Nb	Drying control *O,o*	Contact time control **Oct**	Numeration on the test field 1 **Na** (CFU/ml)
Pseudomonos aeruginosa DSM 939	10•7; 190; 191 10-s: 24; 26 Log N: 9.29 **Log No:** 7.99	10..: 195; 182 10·5: 18; 20 **log Oco:** 6.97	10⁴: 181; 175 10·5: 24; 24 **log Oct:** 6.96	10°: 0;0 10-1: 0;0 **Na:** <70 **Log Na:** <1.85
Enteracoccus hirae DSM 3320	10-7: 193; 201 10-8: 23; 21 **Log N:** 9.30 **Log No:** 8.00	10..: 191; 201 10·5: 23; 21 **log Oco:** 7.00	10-4: 151; 156 IQ·S: 16; 17 **log Oct:** 6.89	10°: 0;0 10-1: 0;0 **Na:** <70 **Log Na:** <1.85
Staphylocaccus aureus DSM 799	*10-1:* 164; 181 10·8: 19; 21 **Log N:** 9.24 **Log No:** 7.94	10⁴: 141; 153 10·5: 17; 19 **log Oco:** 6.88	10..: 190; 181 10·5: 19; 20 **log Oct:** 6.97	100: 0;0 IQ·1: 0;0 **Na:** <70 **Log Na:** <1.85

10• : number of CFU for the dilution x

Volidotion criteria:
Log N between 9.17 ond 9 70
Log Oco and Log Oct: between 6 88 and 8.40

Reduction (R) of microorganisms number:

Test organism(s)	Reduction
Pseudomonas aeruginosa DSM 939	Log R: >5.11
Enterococcus hirae DSM 3320	Log R: >5.04
Staphylococcus aureus DSM 799	Log R: >5.12

Reduction R: log R= log Oct• log Na

Interpretation criteria:
active concentration if R i s
non active concentration if R 55

Actua/testresu/ts*against Candida a/bicans*

Test organism(s)	Test suspension **N** and N_0	Drying control **Dco**	Contact time control **Dct**	Numeration on the test field 1 **Na** (CFU/ml)
Candida a/bicans DSM 1386	10-6: 151; 160 10-1: 25; 20 **log N:** 8.21 **Log No:** 6.91	10-3: 181; 190 10-4: 22; 22 **log Dco:** 5.97	10-3: 221; 225 10-4: 25; 26 **log Dct:** 6.05	10°: 21; 16 10-1: 2 ; 2 **Na:** 92.5 **log Na:** 1.97

10-•: *number of CFU for the dilution x*

Validation criteria:
log N between 8.17 and 8 70
log Dca and log Oct: between 5.88 and 7.40

Reduction (R) of microorganisms number:

Test organism(s)	Reduction
Candida albicans DSM 1386	Log R: 4.08

Reduction R: log R = log Oct• log Na

Interpretatian criteria:
active concentration if R 4
non octive concentration ,f R ≤ 4

6. CONCLUSION

According to the NF EN 16615 standard (mai 2015), the product

Septalkan wipes - flow pack- lot 2015 06 03 5201

possesses a bactericidal and yeasticidal activity on surfaces after **5 minutes** of contact at **20°c,** in **dirty** conditions (presence of Bovine albumin 3 g/L with 3 ml/L sheep erythrocytes).

sc1ent.1s

RAPPORT D'ESSAI
037-IREA 15 CI AN

Seule la version française fait foi / Only the French version is valid

Test according to the NF EN 13624 (November 2013) standard

Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area
Test method and requirements (phase2, step 1)

Test* on *one referenced strains: Candida albicans
This report includes 4 pages and relates on/y the sample tested.

1 Sample identification

Name of the product:	**F2563**
Batch number:	2013.03.01.70
Manufacturer:	**ALKAPHARM** Parc Biocitech - 102 avenue Gaston Roussel **93230 ROMAINVILLE**
Date of delivery :	**28/07/2015**
Storage conditions:	Ambient temperature
Product diluent recommended by the manufacturer :	None
Active substance(s):	Didecyldimethylammonium chloride Alkyl dimethyl benzyl ammonium chloride
Appearance of the product:	Clear liquid

II. Completion of the test

111. Subcontractor laboratory identification

SCIENTIS
Parc Biocitech - 102 avenue Gaston Roussel
93230 ROMAINVILLE

Scientis is one of the commission members T72Q - Antiseptics and chemical disinfectants and is involved in a quality approach in accordance with the NF EN ISO/CE/ 17025 v2005 standard.

112. Reference of test report

N° 037-IREA 15 Cl AN delivered on August 31st 2015

III. Principle of the test

The purpose of the test according to the NF EN 13624 (2013) standard is to draw attention to a reduction in the number of microorganisms, when these corne into contact with the test product under conditions specified below.

This test conducted under suspension conditions is used to ascertain a fungicidal or yeasticidal activity if the reduction rate is 10^4 or more.

IV. Experimental conditions

NF EN 13624 (2013)	**F2563**	Batch: 2013.03.01.70

Test period:	from July 31st, 2015 to August 3rd, 2015
Test temperature:	20°C +/-1°C
Contact time:	**5 minutes**
Test microorganisms:	*Candida albicans* DSM 1386
Product diluent:	Sterile water for injection

Diluent used for product test solutions: tryptone sodium chloride sterile solution

Appearance of product test solutions: clear liquid

Dilution neutralization method: technical stop the bactericidal activity

Neutralizer:	Lécithin (3g/l); Polysorbate 80 (30g/l); Sodium Thiosulfate (5g/l); L-histidin (lg/l); Saponin (30g/l); Tryptone sodium chloride (qsp 11)
Pour plate	
Counting agar:	Malt agar
Incubation:	30°C +/-1°C for 48-72h
Interfering substances:	**Dirty conditions** (Bovine albumin 3g/l and sheep erythrocytes 3ml/l)

Product stability with sterile water for injection: stable

Concentrations of the tested product: 10%; 50%; 100%; (80% as final concentration)

V. Results of the test

a. Test suspension

Strain (Collection and number)	*Candida albicans* DSM 1386	
Dilution 105	255	224
Dilution 10^{-6}	21	17
Test suspension (N)	2.35×10^7	
Log N	7.37	
No=N/10	2.35×10^6	
Log No	**6.37**	

N is between 1.5×10^7 and 5.0×10^7 CFU /ml (log **N** is between 7.17 and 7.70)
N_0 is between 1.5×10^6 and 5.0×10^6 CFU /ml (log No is between 6.17 and 6.70)

b. Validation and controls

Strain (Collection and umber)	Validation suspension (N_{v0})		Validation suspension for neutralization (N_{va}/1000)		Control of experimental conditions (A)		Validation of the neutralising agent (B)		Validation of the method (C)	
Candida albicans DSM 1386	59	69	93	90	**64**	67	64	**64**	73	**48**
	64		**91.5**		**65.5**		**64**		**60.5**	

N_{v0} is between 30 and 160 CFU /ml, Nve/1000 is between 30 and 160 CFU/ml
A, Band C are greater than or equal to 0,5 x N_{v0}, B is greater than or equal to 0.0005 x N_{v8}
The method is validated in the test conditions

c. Test

Strain (Collection and number)		Number of viable cells (Na) and reduction rate (R) for the concentrations of product tested					
		10% (v/v)		50% (v/v)		100% (v/v)	
Candida albicans DSM 1386	100	>330	>330	6	3	9	0
	Na	>3300		<140		<140	
	Log Na	>3.52		<2.15		<2.15	
	R	**<2.85**		**>4.22**		**>4.22**	

R = log N_0 - log Na
A concentration is fungicidal or yeasticidal if R .?.4

VI. Specific remarks

1. Ali the controls and the mixture of validation method give values included within the fundamental limits.
2. At least one concentration of the product has a logarithmic reduction of less than 4.
3. No formation of a precipitate was observed during the realization of the assay (Tested mixtures were homogeneous).

VII. Conclusion

According to the NF EN 13624 standard, the batch **2013.03.01.70** of the product **F2563** (ready to use product) presents a yeasticidal activity in **5 minutes contact time** at 20°c, in dirty conditions, against the strain *Candida albicans.*

Romainville, on August 31st 2015

Corinne BENOLIEL
Doctor of pharmacy
Scientific Director

TEST REPORT

YEASTICIDAL ACTIVITY OF THE SEPTALKAN PRODUCT ACCORDING TO THE EN 14562 STANDARD

For: **ALKAPHARM**
PARC BIOTECH
102 AVENUE GASTON ROUSSEL
93230 ROMAINVILLE

Study number: n°061F02-2012-02

YEASTICIDAL TESTS:

According to the European standard NF EN 14562 (September 2006) – Chemical disinfectants and antiseptics - Quantitative carrier test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants used for instruments in the medical area -
Test method and requirements (phase 2, step 2)

Tests performed against one reference strain: *Candida albicans.*

This test report included 9 pages.

Study completion date: 07/13/2012

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

Professor Georges HERBEIN
Professor of Medicine – MD-PhD in virology
Scientific consultant

SUMMARY

- ✓ PERFORMING LABORATORY
- ✓ PRODUCT IDENTITY
- ✓ EXPERIMENTAL CONDITIONS
- ✓ VALIDATIONS
- ✓ ASSAYS
- ✓ CONCLUSIONS
- ✓ TECHNICAL APPENDIX

18, rue Alain SAVARY • 25000 Besançon • Tel: 03.81.25.09.04 • Fax: 03.81.25.53.51• SARL • RCS BESANÇON • N° SIRET 51786053200012 • N° TVA intra FR 23517860532 • info@apexlabo.com

1. PERFORMING LABORATORY

APEX BIOSOLUTIONS
18, rue Alain SAVARY
25000 BESANÇON
FRANCE

2. PRODUCT IDENTITY

SEPTALKAN

Batch N°: 2163F2563A

Expiration date: non communicated

Manufacturer: ALKAPHARM

Manufacturing date: 06/11/2012

Storage conditions: as recommended by the manufacturer

Active substances: didecyl dimethylammonium chloride and alkylbenzyl dimethyl ammonium chloride

Appearance: clear

Product diluent recommended by the manufacturer for use: none – ready-to-use product

Date of delivery of the product: 06/26/2012.

Date of tests: from 07/05/2012 to 07/13/2012

3. EXPERIMENTAL CONDITIONS

- Concentrations of the product: 100%, 80% and 10%.
- Method: EN 14562
- Exposure time: 5 minutes
- Temperature used during the assays: 20°C
- Organic soil load: dirty conditions, bovine albumin sera (bovine albumin 3 g/l finaux and sheep erythrocytes 3 ml/l).
- Diluent used for the product: trypton salt sterile solution.

- Strain: *Candida albicans* CIP 48.72 batch 265.09.
- Culture conditions: MEA (Malt Extract Agar), at 30°C ± 1°C.
- Stop solution: 10 ml of neutralizer (egg yolk (5 %) and polysorbate 80 (3 %)).

4. RESULTS

See sheet of results.

The SEPTALKAN product demonstrated a yeasticidal activity (4 log reduction is mandatory):

- *C.albicans*, R ≥ 4,19 from the concentration 100%

5. CONCLUSION

According to the EN 14562 (September 2006), the SEPTALKAN product, batch n°2163F2563A:

- **Demonstrated a yeasticidal activity against the reference strain *Candida albicans* when used as ready-to-use product, for 5 min of time exposure, at 20°C, in dirty conditions (3 g/l bovine albumin and 3 ml/l sheep erythrocytes).**

SHEET 1 OF RESULTS
***Candida albicans* (trial)**

Standard: EN 14562, phase 2, step 2

Product: SEPTALKAN

Batch n°: 2163F2563A

Diluent used for the product: distilled water

Number of petri dishes: 1 petri dish / ml

Stop solution: Polysorbate 80 (30g/L) + egg yolk (5%)

Temperature of trials: 20°C

Organic soil load: bovine albumin sera 3 g/l with sheep erythrocytes 3 ml/l

Strain: *Candida albicans* CIP 48.72 batch 265.09

Incubation temperature: 30°C ± 1°C

Study N°: 061F02-2012-02

Date of tests: 07/11/2012

Study director: Stephanie MOROT-BIZOT

Signature:

Diluent used: distilled water

Appearance of the product: clear

Validations

Validation control (Nv0)			Experimental conditions control (A)			Control of neutralization (B)			Validation of dilution-neutralization method (C) product concentration 100%		
Vc1	86	$\bar{x}$ = 72	Vc1	54	$\bar{x}$ = 52	Vc1	58	$\bar{x}$ = 61	Vc1	68	$\bar{x}$ = 66
Vc2	58		Vc2	50		Vc2	64		Vc2	64	
30 ≤ $\bar{x}$ of Nv0 ≤160 ☒ yes ☐ no			$\bar{x}$ of A is ≥0,5 × $\bar{x}$ of Nv0 ☒ yes ☐ no			$\bar{x}$ of B ≥ 0,5× $\bar{x}$ of Nv0 ☒ yes ☐ no			$\bar{x}$ of C ≥ 0,5 × $\bar{x}$ of Nv0 ☒ yes ☐ no		

Suspension test and trials

	N	Vc1	Vc2	
Suspension test N	10^{-6}	273	251	N = 2,70x 10^8 lg N = 8,43 8,17 ≤ N ≤ 8,70? ×yes □no
	10^{-7}	36	35	

Methodology controls:
- 1,5 x 10^8 UFC/ml≤ N ≤ 5,0 x 10^8 UFC/ml
- 1,4 x 10^6 UFC/ml≤ Nwt ≤ lg N-1,3
- 30≤ Nv0 ≤160 UFC/ml
- A, B and C ≥0,5 x Nv0

	N	Vc1	Vc2	
Water control Nw	10^{-3}	>330	>330	Nw = 2,80 x 10^6 lg Nw = 6,44 6,15 ≤ Nw ≤ lg N-1,3? ×yes □no
	10^{-4}	27	28	

Product	10^0		10^{-1}		10^{-2}		10^{-3}		lg Na	lg R = lg Nw – lg Na	Exposure time
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2			
100 %	0	0	0	0	0	0	0	0	<2,15	**>4,29**	**5 min**
80 %	0	0	0	0	0	0	0	0	<2,15	**>4,29**	**5 min**
10%	>330	>330	50	98	0	0	0	0	3,87	2,57	5 min

Vc: value is the number of cfu/ml
x̄: average (Vc1 and Vc2)
N : number of cfu/ml of the yeastidal suspension
Nw: number of cfu/ml of the control yeastidal test suspension
Na: number of survivors per ml in the test mixture at the end of the time exposure
Nv0: number of cfu/ml of the yeastidal test suspension in the mixture A, B and C at the beginning of the time exposure

SHEET 2 OF RESULTS
***Candida albicans* (repetition)**

Standard: EN 14562, phase 2, step 2

Product: SEPTALKAN

Batch n°: 2163F2563A

Diluent used for the product: distilled water

Number of petri dishes: 1 petri dish / ml

Stop solution: Polysorbate 80 (30g/L) + egg yolk (5%)

Temperature of trials: 20°C

Organic soil load: bovine albumin sera 3 g/l with sheep erythrocytes 3 ml/l

Strain: *Candida albicans* CIP 48.72 batch 265.09

Incubation temperature: 30°C ± 1°C

Study N°: 061F02-2012-02

Date of tests: 07/11/2012

Study director: Stephanie MOROT-BIZOT

Signature:

Diluent used: distilled water

Appearance of the product: clear

Validations

Validation control (Nv0)			Experimental conditions control (A)			Control of neutralization (B)			Validation of dilution-neutralization method (C) product concentration 100%		
Vc1	53	$\bar{x}$ = 51	Vc1	82	$\bar{x}$ = 62,5	Vc1	35	$\bar{x}$ = 36	Vc1	55	$\bar{x}$ = 57,5
Vc2	49		Vc2	43		Vc2	37		Vc2	60	
30 ≤ $\bar{x}$ of Nv0 ≤160 ☒ yes ☐ no			$\bar{x}$ of A is ≥0,5 × $\bar{x}$ of Nv0 ☒ yes ☐ no			$\bar{x}$ of B ≥ 0,5× $\bar{x}$ of Nv0 ☒ yes ☐ no			$\bar{x}$ of C ≥ 0,5 × $\bar{x}$ of Nv0 ☒ yes ☐ no		

18, rue Alain SAVARY • 25000 Besançon • Tel: 03.81.25.09.04 • Fax: 03.81.25.53.51• SARL • RCS BESANÇON • N° SIRET 51786053200012 • N° TVA intra FR 23517860532 • info@apexlabo.com

Suspension test and trials

	N	Vc1	Vc2	
Suspension test N	10^{-6}	262	204	$N = 2,4 \times 10^{8}$ lg N = 8,38 8,17 ≤ N ≤ 8,70? ×yes □no
	10^{-7}	34	23	

Methodology controls:
- $1,5 \times 10^{8}$ UFC/ml≤ N ≤ $5,0 \times 10^{8}$ UFC/ml
- $1,4 \times 10^{6}$ UFC/ml≤ Nwt ≤ lg N-1,3
- 30≤ Nv0 ≤160 UFC/ml
- A, B and C ≥0,5 x Nv0

	N	Vc1	Vc2	
Water control Nw	10^{-3}	189	175	$Nw = 1,70 \times 10^{6}$ lg Nw = 6,23 6,15 ≤ Nw ≤ lg N-1,3? ×yes □no
	10^{-4}	16	18	

Produit	10^{0}		10^{-1}		10^{-2}		10^{-3}		lg Na	lg R = lg Nw – lg Na	Temps de contact
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2			
100 %	0	0	0	0	0	0	0	0	<2,15	**>4,08**	**5 min**
80 %	0	0	0	0	0	0	0	0	<2,15	**>4,08**	**5 min**
10%	>330	>330	22	23	0	0	0	0	3,35	2,88	5 min

Vc: value is the number of cfu/ml
x̄: average (Vc1 and Vc2)
N : number of cfu/ml of the yeastidal suspension
Nw: number of cfu/ml of the control yeastidal test suspension
Na: number of survivors per ml in the test mixture at the end of the time exposure
Nv0: number of cfu/ml of the yeastidal test suspension in the mixture A, B and C at the beginning of the time exposure

TECHNICAL APPENDIX

Media:

MEA (Malt Extract Agar), Laboratories CONDA, ref. n° 1038, batch n° 111151

Reagents:

ORGANIC SOIL LOAD:

Bovine Albumin Sera, powder, Fraction V, SIGMA ALDRICH, ref. 05479, batch STBB7838V

DILUENT

Trypton-Salt Solution (TS)

- Trypton, Laboratories CONDA, ref. 1612, batch n° 091229 ----------1,00 g/l
- Sodium chloride, MOLECULAR BIOLOGY, ref n° 19032391, batch n° 808211----8,50 g/l

pH at 25°C : 7,0 ± 0,2

NEUTRALIZER

- Egg yolk ---------- 5 %
- Polysorbate 80, SIGMA ALDRICH, ref. 59924, batch n°1451069 ---------- 30 g/l
- Trypton-Soya Broth (TSB), CONDA, ref. n°1224, batch n° 810081 --------- 30 g/l

GLASS CARRIERS – glass carriers 15 x 60 mm, 1 mm thick – Thermo scientific/ Menzel-Gläser – ref. 100 OTM, batch n°01 1794389.

Laboratoire MIDAC
Your expert in microbiology

Test report n° RE 14357-5

Quantitative suspension test for the evaluation of yeasticidal activity according to EN 1650: 2013 standard

11

Product : Septalkan batch 2013.03.01.70 (exp: 2016/03}

On request of:

ALKAPHARM
Parc Biocitech
102 avenue Gaston Roussel
FR 93230
ROMAINVILLE

Date: 04/12/2014

Visa:

Technical Manager
Mélinda MAUX, Ph.D

The test report includes: 5 pages

Copy of this test report is only authorized from its unabridged version.
This report concerns only the tested product.

1 PRINCIPLE:

The yeasticidal activity has been determined in accordance to the **EN 1650** European standard:
" Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements - (Phase 2, step 1} - July 2013."

Test organisms(s): **see table(s) on next page(s).**

Preservation and stock cultures of test organisms following the requirements of the EN 12353 standard.

2 SAMPLE(S) IDENTIFICATION:

Name(s): **Septalkan batch 2013.03.01.70 (exp: 2016/03}.**

Society: **ALKAPHARM.**

Product appearance: **Liquid.**

Receipt at the laboratory: **02/10/2014.**

Storage conditions at the laboratory: Room temperature, in the darkness.

3 TEST METHOD AND ITS VALIDATION:

- Dilution neutralization: pour plating method (number of plates : 2 i.e. 1 ml/ dish)
- Diluent-neutralizer: TSB Capital IV
Addition of 10% diluent-neutralizer in the agar (v/v).

4 EXPERIMENTAL CONDITIONS:

Period of analysis: from 13/10/2014 to 06/11/2014.

Diluent used for product test solution: **distilled water**

Product test concentration(s): **see table(s) on next page(s).**

Appearance of product dilutions: **whitening.**

Test temperature: **20°c** +/- **1°c.**

Additional contact temperature: **5 minutes+/- 10 secondes.**

Interfering substance: **3g/l bovine albumin (dirty conditions).** Prepared according to the requirements of the EN 1650 standard.

Stability of the test mixture interfering substance/ test product(s): **trouble.**

Incubation temperature: **30°C** +/- **1°C.**

5 TEST RESULTS:

Control and validations of the dilution-neutralization method in the test conditions

Strain(s)	Enumeration of viable cells (CFU/ml) :			
	Validation of the yeasticidal suspension **Nvo**	Experimental conditions control **A**	Neutralizer non-toxicity control **B**	Method validation **C** Product concentration : *BO%*
***Candida albicans* CIP4872**	Vcl: 48 Vc2: 42 Nvo: 45,0	Vcl: 35 Vc2: 39 A: 37,0	Vcl: 43 Vc2: 36 B: 39,5	Vcl: 41 Vc2: 37 C: 39,0
Validation criteria: *Nvabetween 30 and 160 CFU* *A, Band C equa/ or equivalent to 0.5 x NvO* *spiny spores ≥ 75%*				

Conclusion:

The neutralization is validated with the dilution-neutralization method with the test organisms in the test conditions.

Actual test results (neutralization by dilution-neutralization method)

Strain(s)	Test suspension **N** and **No**	**Enumeration of viable cells (CFU/ml) : Na in accordance to the concentrations in% (v/v):**		
		0,1	10,0	80,0
***Candida albicans* CIP4872**	10^{-5} Vcl : 185 Vc2: 173 10^{-6} vc1 : 22 Vc2: 19 N= 1,81 x 10^{7} No= 1,81 x 10^{6} log No= 6,26	Vcl: > 330 Vc2: > 330 Na:> 3 300 log Na : > 3,52	Vcl: 18 Vc2: 20 Na: 190 log Na: 2,28	**Vcl: < 14** **Vc2: < 14** **Na:< 140** log Na : < **2,15**
Validation criteria: *N between 1.5 x 10^{7} and 5.0 x 10^{7}* *No between 1.5 x 1a^{5} and 5.0 x 10^{6}* *log Nobetween 6.17 and 6.70*				

Reduction (R) of the number of viable cells at the tested concentration:

	0,1	10,0	80,0
***Candida albicans* CIP4872**	logR:<2,74	log R: 3,98	**log R: > 4,11**

6 CONCLUSION :

According to the **EN 1650 standard (July 2013),** the product

Septalkan batch 2013.03.01.70 (exp: 2016/03)

possesses a yeasticidal activity diluted at the test concentration of **80% (v/v)** in the following conditions:

- In 5 minutes of contact at 2o·c
- In presence of Bovine albumin 3 g/1 (Dirty conditions)
- Against the strain *Candida albicans* CIP 4872

Laboratoire MIDAC
Your expert in microbiology

Test report n° RE 14363-5

Yeasticidal activity according to the EN 13697: 2001 standard

Product: Septalkan batch 2013.03.01.70 (exp 03/2016}

On request of:

ALKAPHARM
Parc Biocitech
102 avenue Gaston Roussel
FR 93230
ROMAINVILLE

Date: 04/12/2014

Visa:

Technical manager
Mélinda MAUX, Ph.D

The test report includes: 4 pages

Copy of this test report is only authorized from its unabridged version.
This report concerns only the tested product.

1 PRINCIPLE :

The yeasticidal activity on surface has been evaluated in accordance to the **EN 13697** European Standard:" **Chemical disinfectants and antiseptics - Quantitative non porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements without mechanic action - (Phase 2/ Step 2) - November 2001."**

Test organisms(s): **see table{s) on next page(s).**
Preservation and stock cultures of test organisms following the requirements of the EN 12353 standard.

2 SAMPLE(S) IDENTIFICATION:

Name(s): **Septalkan batch 2013.03.01.70 (exp 03/2016)**

Society: **ALKAPHARM.**

Receipt at the laboratory: **02/10/2014.**

Storage conditions at the laboratory: Room temperature, in the darkness.

3 TEST METHOD AND ITS VALIDATION:

- **Dilution neutralization** :
- **Diluent-neutralizer:** TSB Capitol IV

Addition of 10% diluent-neutralizer in the agar (v/v).

4 EXPERIMENTAL CONDITIONS:

Period of analysis: from 05/11/2014 to 08/11/2014.

Diluent used for product test solution: **distilled water.**

Product test concentration(s): **see table{s) on next page(s).**

Interfering substance: **3g/l bovine albumin {dirty conditions).** Prepared according to the requirements of the EN 13697 standard.

Appearance of product dilutions: **whitening.**

Stability of the test mixture interfering substance/ test product(s): **none precipitate.**

Test temperature: **21°C** +/- **1°C.**

Additional contact time: **5 minutes+/- 10 secondes.**

Incubation temperature: **30°C** +/- **1°C.**

Tested surface: **Stainless steel dises 304 with grade 2B,** 0 **2 cm, finish on both sides according to the requirements of the EN 10 088-2 standard.**

Drying conditions: **30 minutes at 37°C** +/- **1°C in an incubator with a fan.**

5 TEST RESULTS:

Test organisms	Validation test			Distilled water contrai	Test procedure at concentrations in% (v/v)		
	Yeasticidal suspension test N	Neutralization contrai (distilled water) NC	Neutralization test (product) NT	Ne	0,1	10,0	100,0
Candida albicans CIP 4872.	10-5: 267; 276 10-6: 29; 24 N: 5,83	10-3: 79; 73 10-4: 8; 6 NC: 5,88	10-3: 70; 64 10-4: 9; 8 NT: 5,82	10-3: 79; 69 10-4: 8; 10 10-5: 0; 1 Ne: 5,87 Nts: > 300	10˚ : 189; 197 10-l: 26; 22 10-2 : 3; 5 Nd: 3,29 Nts: 121 ME: 2,58	**10˚ : 17; 23** **10-l: 2; 2** **10-2: 0; 0** **Nd: 2,30** **Nts: 14** **ME: 3,57**	**10˚ : 0; 0** **10-l: 0; 0** **10-2: 0; 0** **Nd:< 0,1** **Nts: 0** **ME:>5,77**

10-x: number of CFU for the dilution x.
N: Log number of CFU for 0.05 ml of test suspension
NC: Log number of CFU per test surface of neutralisation contrai: log {CFU/test surface)
NT: Log number of CFU per test surface of neutralisation contrai.
Ne : Log number of CFU per test surface of water contrai.
Nd: Log number of CFU per test surface of desinfectant test: log (CFU/test surface)
Nts : Number of CFU remaining on the surface test
ME: Fungicidal ejfect: ME= Ne-Nd. Effective concentration if ME 3

6 CONCLUSION :

According to the **EN 13697** standard (November 2001), the product

Septalkan batch 2013.03.01.70 (exp 03/2016)

possesses a yeasticidal on surfaces at **10,0%** (v/v) in the following test conditions:

- In 5 minutes of contact at 21 °C
- In presence of 3 g/l bovine albumin (dirty conditions)
- Against *Candida albicans* CIP 4872

TEST REPORT

BACTERICIDAL AND YEASTICIDAL ACTIVITY OF THE SEPTALKAN PRODUCT BY AERIAL DISINFECTION, ACCORDING TO THE NF T72-281 STANDARD

For : ALKAPHARM
PARC BIOCITECH
102 AVENUE GASTON ROUSSEL
93230 ROMAINVILLE

Date of request: 08.06.2012

Study number: n° 156B11-2012-00

According to the NF T 72-281 (May 2009) standard – aerial desinfection. Determination of the bactericidal, fungicidal, yeasticidal and sporicidal activity.

Trials performed on 4 reference strains: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus hirae*, and one yeastidal strain: *Candida albicans*.

This test report included 10 pages.

Study completion date: 10/05/2012

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

Professor Georges HERBEIN
Professor of Medicine – MD-PhD in virology
Scientific consultant

SUMMARY

- ✓ PERFORMING LABORATORY
- ✓ PRODUCT IDENTITY
- ✓ EXPERIMENTAL CONDITIONS
- ✓ VALIDATIONS
- ✓ ASSAYS
- ✓ CONCLUSIONS
- ✓ TECHNICAL APPENDIX

18, rue Alain SAVARY • 25000 Besançon • Tel: 03.81.25.09.04 • Fax: 03.81.25.53.51• SARL • RCS BESANÇON • N° SIRET 51786053200012 • N° TVA intra FR 23517860532 • info@apexlabo.com

1 PERFORMING LABORATORY

APEX BIOSOLUTIONS
18, rue Alain SAVARY
25000 BESANÇON
FRANCE

2 PRODUCT IDENTIFICATION

SEPTALKAN:

Batch n°: 2163F2563A

Expiration date: non communicated

Manufacturer: ALKAPHARM

Date of manufacturing: 06/11/2012

Storage conditions: as recommended by the manufacturer

Active substances: didecyl dimethylammonium chloride and alkylbenzyl dimethyl ammonium chloride.

Appearance of the product: clear

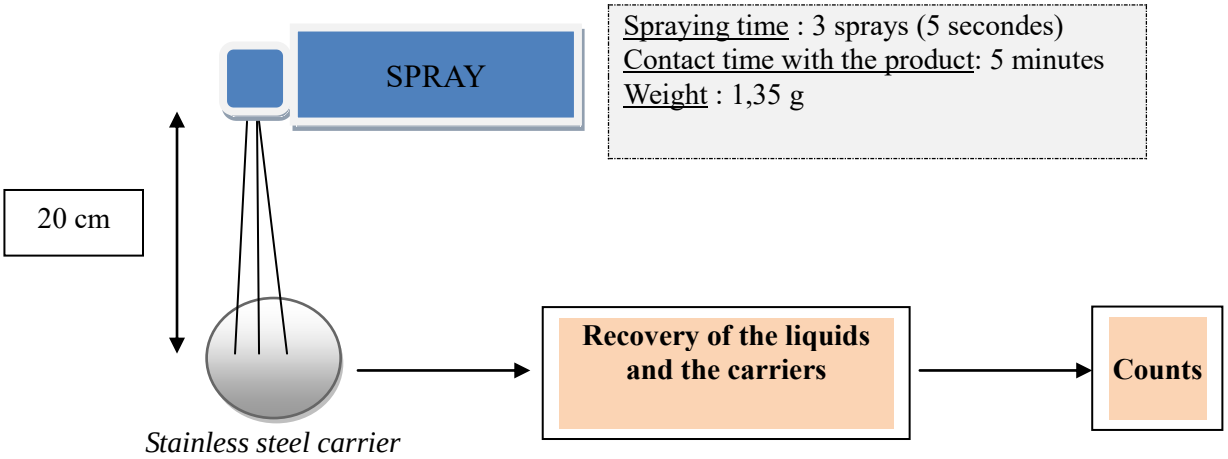
Diluent of the product: none, ready-to-use product

Date of delivery of the product: 06/26/2012

Date of tests: from 08/29/2012 to 09/25/2012

3 EXPERIMENTAL CONDITIONS

- Disinfection method : by manual spraying
- Description of the study protocol :



- Volume of the product: 3 sprays at 20 cm above the carrier
- 5 min of contact time between the contaminated carrier and the sprayed product
- Time of manual spraying : 5 secondes
- Culture conditions: TSA agar (Trypton Soya Agar), at 37°C ± 1°C (bacteria), MEA agar (Malt Extract Agar) (yeast) at 30°C.
- Bacterial strains: *Staphylococcus aureus* subsp. *aureus* DSMZ 799 batch march 2011 (ATCC 6538), *Escherichia coli* CIP 54.127 batch 7207 (ATCC 10536), *Pseudomonas aeruginosa* CIP 103467 batch 10807 (ATCC 15442) and *Enterococcus hirae* CIP 58.55 batch 7804 (ATCC 10541) - Institut Pasteur.
- Yeastidal strain: *Candida albicans* CIP 48.72 batch 265.09 (ATCC 10231) - Institut Pasteur.
- Microbial suspensions: contamination of the carriers with 50 µl of microbial suspensions in skimmed milk.

4 VALIDATIONS AND RESULTS

(1) VALIDATIONS

Experimental conditions: 21°0 C; 62% relative humidity

Stainless steel carriers: 2 cm diameter, 0,15 cm thick

Staphylococcus aureus

Counts (CFU/ml)				
		VC1	VC2	
N	10^{-7}	215	221	2,18E+09
	10^{-8}	20	24	
N2	10^{-7}	>165	>165	2,00E+01
	10^{-8}	20	20	

validations							
T	4,63E+06	**n1**	2,39E+02	**n2**	1,80E+01	**n3**	2,85E+02
T>10^6 x yes □ no		n1 > 0,5*N1 x yes □ no		n2 > 0,5 N2 x yes □ no		n3 > 0,5*N1 x yes □ no	

Enterococcus hirae

Counts (CFU/ml)				
		VC1	VC2	
N	10^{-7}	201	197	1,97E+09
	10^{-8}	19	16	
N2	10^{-7}	>165	>165	1,55E+01
	10^{-8}	15	16	

validations							
T	2,58E+06	**n1**	1,92E+02	**n2**	1,70E+01	**n3**	2,68E+02
T>10^6 x yes □ no		n1 > 0,5*N1 x yes □ no		n2 > 0,5 N2 x yes □ no		n3 > 0,5*N1 x yes □ no	

Pseudomonas aeruginosa

Counts (CFU/ml)				
		VC1	VC2	
N	10^{-7}	230	212	2,15E+09
	10^{-8}	16	14	
N2	10^{-7}	>165	>165	3,35E+01
	10^{-8}	34	33	

validations							
T	3,22E+07	**n1**	2,52E+02	**n2**	2,35E+01	**n3**	2,67E+02
T>10^6 x yes □ no		n1 > 0,5*N1 x yes □ no		n2 > 0,5 N2 x yes □ no		n3 > 0,5*N1 x yes □ no	

Escherichia coli

Counts (CFU/ml)				
		VC1	VC2	
N	10^{-7}	191	214	2,03E+09
	10^{-8}	23	18	
N2	10^{-7}	>165	>165	2,90E+01
	10^{-8}	28	30	

validations							
T	4,90E+06	**n1**	2,34E+02	**n2**	2,40E+01	**n3**	3,00E+02
T>10^6 x yes □ no		n1 > 0,5*N1 x yes □ no		n2 > 0,5 N2 x yes □ no		n3 > 0,5*N1 x yes □ no	

Candida albicans

Counts (CFU/ml)				
		VC1	VC2	
N	10^{-7}	123	119	1,27E+09
	10^{-8}	19	19	
N2	10^{-7}	166	166	9,90E+01
	10^{-8}	20	20	

validations							
T	1,81E+06	**n1**	9,65E+01	**n2**	9,75E+01	**n3**	8,30E+01
T>10^6 x yes □ no		n1 > 0,5*N1 x yes □ no		n2 > 0,5 N2 x yes □ no		n3 > 0,5*N1 x yes □ no	

(2) RESULTS

Experimental conditions: 20°7 C; 56% relative humidity
Stainless steel carriers: 2 cm diameter, 0,15 cm thick

Staphylococcus aureus

	Trials							
	Trial 1		**Trial 2**		**Trial 3**		**carrier**	
Mb (87mL)	10	/	0	/	3	/	**carrier 1**	5
Mb (10mL)	2	/	0	/	0	/	**carrier 2**	0
10^{0}	0	0	0	0	0	0	**carrier 3**	4
10^{-1}	0	0	0	0	0	0		
10^{-2}	0	0	0	0	0	0		
10^{-3}	0	0	0	0	0	0		
n'1	0	**d**	**6,67E+00**					
n'2	3	**d>5** **x yes □ no**						

Enterococcus hirae

	Trials							
	Trial 1		Trial 1		Trial 1		Trial 1	
Mb (87mL)	0	/	0	/	0	/	**carrier 1**	0
Mb (10mL)	0	/	0	/	0	/	**carrier 2**	1
10^0	0	0	0	0	0	0	**carrier 3**	0
10^{-1}	0	0	0	0	0	0		
10^{-2}	0	0	0	0	0	0		
10^{-3}	0	0	0	0	0	0		
n'1	0	**d**	**6,41E+00**					
n'2	0,33	**d>5** x yes □ no						

Pseudomonas aeruginosa

	Trials							
	Trial 1		Trial 2		Trial 3		carrier	
Mb (87mL)	7	/	0	/	0	/	**carrier 1**	0
Mb (10mL)	1	/	0	/	0	/	**carrier 2**	0
10^0	0	0	0	0	0	0	**carrier 3**	0
10^{-1}	0	0	0	0	0	0		
10^{-2}	0	0	0	0	0	0		
10^{-3}	0	0	0	0	0	0		
n'1	0	**d**	**7,51E+00**					
n'2	0	**d>5** x yes □ no						

Escherichia coli

	Trials							
	Trial 1		Trial 2		Trial 3		Carrier	
Mb (87mL)	0	/	0	/	0	/	carrier 1	0
Mb (10mL)	0	/	0	/	0	/	carrier 2	0
10^0	0	0	0	0	0	0	carrier 3	0
10^{-1}	0	0	0	0	0	0		
10^{-2}	0	0	0	0	0	0		
10^{-3}	0	0	0	0	0	0		
n'1	0,00E+00	d	6,69E+00					
n'2	0,00E+00	d>5 x yes □ no						

Candida albicans

	Trials							
	Trial 1		Trial 2		Trial 3		Carrier	
Mb (87mL)	0	/	0	/	0	/	carrier 1	0
Mb (10mL)	0	/	0	/	0	/	carrier 2	0
10^0	0	0	0	0	0	0	carrier 3	0
10^{-1}	0	0	0	0	0	0		
10^{-2}	0	0	0	0	0	0		
10^{-3}	0	0	0	0	0	0		
n'1	0,00E+00	d	6,26E+00					
n'2	0	d>4 x yes □ no						

5 CONCLUSION

The SEPTALKAN product, batch n°2163F2563A, demonstrated a bactericidal and yeasticidal activity by aerial disinfection method, according to the NF T 72-281 standard (may 2009), with three sprays (manual spraying) and for a 5 minutes of time exposure on the carriers.

TECHNICAL APPENDIX

MEDIA:

TSA (Trypto casein Soya Agar), CONDA Laboratories, ref. n° 1068, batch n° 102231

MEA (Malt Extract Agar), Thermofisher, ref. n°1202-7606, batch n°2311C

RECOVERY LIQUID

Trypton-Salt Solution (TS)

- Trypton, Laboratories CONDA, ref. 1612, batch n° 091229 ----------1,00 g/l
- Sodium chloride, PANREAC, ref n° 141659, batch n° 204679 ----------8,50 g/l

pH at 25°C : 7,0 ± 0,2

SKIM MILK

Skim milk, Régilait, batch n° L M F N 15:27.

STAINLESS STEEL CARRIERS – stainless steel disks**,** 2 cm diameter, 1.4301, 2B grade (MECAPOL).

TEST REPORT

BACTERICIDAL AND YEASTICIDAL ACTIVITY OF THE SEPTALKAN PRODUCT BY AERIAL DISINFECTION, ACCORDING TO THE NF T72-281 STANDARD

For : ALKAPHARM
PARC BIOCITECH
102 AVENUE GASTON ROUSSEL
93230 ROMAINVILLE

Date of request: 08.06.2012

Study number: n° 156B11-2012-01

According to the NF T 72-281 (May 2009) standard – aerial desinfection. Determination of the bactericidal, fungicidal, yeasticidal and sporicidal activity.

Trials performed on 4 reference strains: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus hirae*, and one yeastidal strain: *Candida albicans*.

The study protocol is based on the NF T 72-281 standard, with a wiping step of the surfaces disinfected with the product.

This test report included 12 pages.

Study completion date: 10/05/2012

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

Professor Georges HERBEIN
Professor of Medicine – MD-PhD in virology
Scientific consultant

SUMMARY

– PERFORMING LABORATORY

– PRODUCT IDENTITY

– EXPERIMENTAL CONDITIONS

– VALIDATIONS

– ASSAYS

– CONCLUSIONS

– TECHNICAL APPENDIX

18, rue Alain SAVARY • 25000 Besançon • Tel: 03.81.25.09.04 • Fax: 03.81.25.53.51• SARL • RCS BESANÇON • N° SIRET 51786053200012 • N° TVA intra FR 23517860532 • info@apexlabo.com

1 PERFORMING LABORATORY

APEX BIOSOLUTIONS
18, rue Alain SAVARY
25000 BESANÇON
FRANCE

2 PRODUCT IDENTIFICATION

SEPTALKAN:

Batch n°: 2163F2563A

Expiration date: non communicated

Manufacturer: ALKAPHARM

Date of manufacturing: 06/11/2012

Storage conditions: as recommended by the manufacturer

Active substances: didecyl dimethylammonium chloride and alkylbenzyl dimethyl ammonium chloride.

Appearance of the product: clear

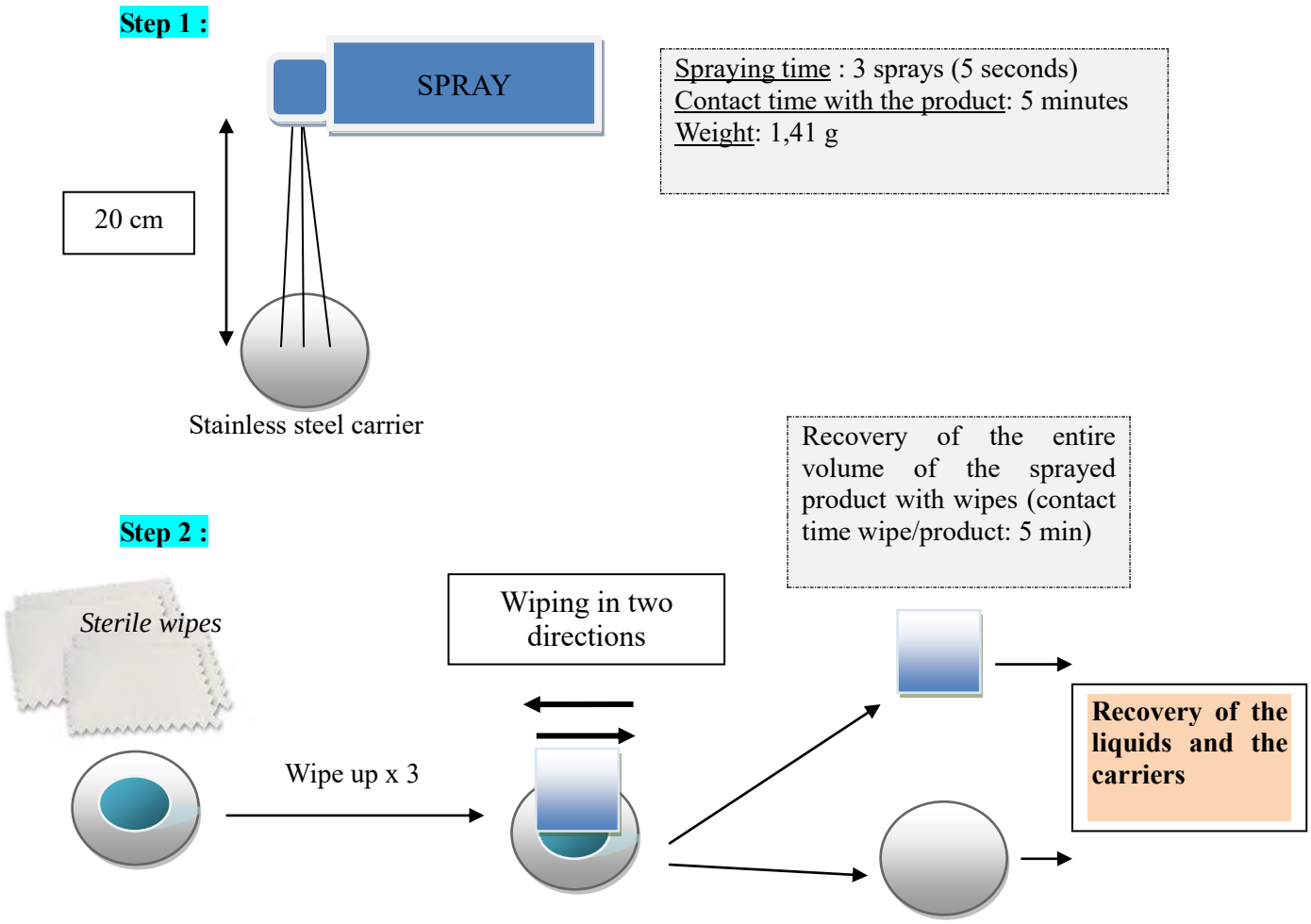
Diluent of the product: none, ready-to-use product

Date of delivery of the product: 06/26/2012

Date of tests: from 08/29/2012 to 09/25/2012

3 EXPERIMENTAL CONDITIONS

- Disinfection method : by manual spraying and wiping step of the product sprayed
- Description of the study protocol :



- Volume of the product: 3 sprays at 20 cm above the carrier
- 5 min of contact time between the contaminated carrier and the sprayed product, then, 5 min of contact time between the wipe and the recovered product (after the wiping step of the carriers)
- With or without wiping step of the contaminated carriers disinfected by the sprayed product
- Time of manual spraying : 5 secondes
- Culture conditions: TSA agar (Trypton Soya Agar), at 37°C ± 1°C (bacteria), MEA agar (Malt Extract Agar) (yeast) at 30°C.
- Bacterial strains: *Staphylococcus aureus* subsp. *aureus* DSMZ 799 batch march 2011 (ATCC 6538), *Escherichia coli* CIP 54.127 batch 7207 (ATCC 10536), *Pseudomonas aeruginosa* CIP 103467 batch 10807 (ATCC 15442) and *Enterococcus hirae* CIP 58.55 batch 7804 (ATCC 10541) - Institut Pasteur.
- Yeastidal strain: *Candida albicans* CIP 48.72 batch 265.09 (ATCC 10231) - Institut Pasteur.
- Microbial suspensions: contamination of the carriers with 50 µl of microbial suspensions in skimmed milk.

4 VALIDATIONS AND RESULTS

(1) VALIDATIONS

Experimental conditions: 21°1 C; 63% relative humidity

Stainless steel carriers: 2 cm diameter, 0,15 cm thick

Staphylococcus aureus

Counts (CFU/ml)				
		VC1	VC2	
N	10^{-7}	230	228	2,36E+09
	10^{-8}	33	28	**N1 =** 2,29E+02
N2	10^{-7}	>165	>165	3,20E+01
	10^{-8}	30	34	

validations							
T	5,18E+06	**n1**	2,49E+02	**n2**	2,80E+01	**n3**	2,95E+02
T>10^6 x yes □ no		n1 > 0,5*N1 x yes □ no		n2 > 0,5 N2 x yes □ no		n3 > 0,5*N1 x yes □ no	

Enterococcus hirae

Counts (CFU/ml)				
		VC1	VC2	
N	10^{-7}	212	210	2,10E+09
	10^{-8}	19	21	**N1 =** 2,11E+02
N2	10^{-7}	>165	>165	2,15E+01
	10^{-8}	20	23	

validations							
T	4,05E+06	**n1**	2,02E+02	**n2**	3,20E+01	**n3**	2,74E+02
T>10^6 x yes □ no		n1 > 0,5*N1 x yes □ no		n2 > 0,5 N2 x yes □ no		n3 > 0,5*N1 x yes □ no	

Pseudomonas aeruginosa

Counts (CFU/ml)				
		VC1	VC2	
N	10^{-7}	241	245	2,44E+09
	10^{-8}	26	25	**N1 =** 2,43E+02
N2	10^{-7}	>165	>165	3,13E+07
	10^{-8}	40	45	

validations							
T	3,13E+07	**n1**	2,40E+02	**n2**	2,90E+01	**n3**	2,74E+02
T>10^6 x yes □ no		n1 > 0,5*N1 x yes □ no		n2 > 0,5 N2 x yes □ no		n3 > 0,5*N1 x yes □ no	

Escherichia coli

Counts (CFU/ml)				
		VC1	VC2	
N	10^{-7}	200	208	2,03E+09
	10^{-8}	18	21	**N1 =** 2,04E+02
N2	10^{-7}	>165	>165	2,85E+01
	10^{-8}	30	27	

validations							
T	5,50E+06	**n1**	2,37E+02	**n2**	2,95E+01	**n3**	3,03E+02
T>10^6 x yes □ no		n1 > 0,5*N1 x yes □ no		n2 > 0,5 N2 x yes □ no		n3 > 0,5*N1 x yes □ no	

Candida albicans

Counts (CFU/ml)					
		VC1	VC2		
N	10^{-7}	130	128	**1,36E+09**	
	10^{-8}	21	20	**N1**	**1,29E+02**
N2	10^{-7}	110	105	**1,08E+02**	
	10^{-8}	10	9		

validations							
T	1,87E+06	**n1**	1,09E+02	**n2**	9,85E+01	**n3**	9,05E+01
T>10^6 x yes □ no		n1 > 0,5*N1 x yes □ no		n2 > 0,5 N2 x yes □ no		n3 > 0,5*N1 x yes □ no	

(1) RESULTS

Experimental conditions: 21°6 C; 65% relative humidity
Stainless steel carriers: 2 cm diameter, 0,15 cm thick

Staphylococcus aureus

	Trials							
	Trial 1		**Trial 2**		**Trial 3**		**Carrier**	
Mb (87mL)	0	/	0	/	0	/	**carrier 1**	0
Mb (10mL)	0	/	0	/	0	/	**carrier 2**	0
10^0	0	0	0	0	0	0	**carrier 3**	0
10^{-1}	0	0	0	0	0	0		
10^{-2}	0	0	0	0	0	0		
10^{-3}	0	0	0	0	0	0		
n'1	0,00E+00	**d**	**6,71E+00**					
n'2	0	**d>5** **x yes □ no**						

	lingettes					
	Trial 1		**Trial 2**		**Trial 3**	
Mb (87mL)	2	/	0	/	0	/
Mb (10mL)	0	/	0	/	0	/
10^0	0	0	0	0	0	0
10^{-1}	0	0	0	0	0	0
10^{-2}	0	0	0	0	0	0
10^{-3}	0	0	0	0	0	0
n'1	2,30E+00	**d**	**6,35E+00**			
n'2	0	**d>5** **x oui □ non**				

Enterococcus hirae

	Trials							
	Trial 1		Trial 2		Trial 3		carrier	
Mb (87mL)	0	/	0	/	0	/	**carrier 1**	0
Mb (10mL)	0	/	0	/	0	/	**carrier 2**	0
10^0	0	0	0	0	0	0	**carrier 3**	0
10^{-1}	0	0	0	0	0	0		
10^{-2}	0	0	0	0	0	0		
10^{-3}	0	0	0	0	0	0		
n'1	0,00E+00	**d**	**6,61E+00**					
n'2	0	**d>5** x yes □ no						

	wipes					
	Trial 1		Trial 2		Trial 3	
Mb (87mL)	0	/	0	/	0	/
Mb (10mL)	0	/	0	/	0	/
10^0	0	0	0	0	0	0
10^{-1}	0	0	0	0	0	0
10^{-2}	0	0	0	0	0	0
10^{-3}	0	0	0	0	0	0
n'1	0,00E+00	**d**	**6,61E+00**			
n'2	0	**d>5** x yes □ no				

Pseudomonas aeruginosa

	Trials							
	Trial 1		Trial 2		Trial 3		carrier	
Mb (87mL)	0	/	0	/	0	/	**carrier 1**	0
Mb (10mL)	0	/	0	/	0	/	**carrier 2**	2
10^0	0	0	0	0	0	0	**carrier 3**	1
10^{-1}	0	0	0	0	0	0		
10^{-2}	0	0	0	0	0	0		
10^{-3}	0	0	0	0	0	0		
n'1	0,00E+00	**d**	**7,50E+00**					
n'2	1	**d>5** **x yes □ no**						

	wipes					
	Trial 1		Trial 2		Trial 3	
Mb (87mL)	0	/	0	/	0	/
Mb (10mL)	0	/	0	/	0	/
10^0	0	0	0	0	0	0
10^{-1}	0	0	0	0	0	0
10^{-2}	0	0	0	0	0	0
10^{-3}	0	0	0	0	0	0
n'1	0,00E+00	**d**	**7,02E+00**			
n'2	3	**d>5** **x yes □ no**				

Escherichia coli

	Trials							
	Trial 1		Trial 2		Trial 3		Carrier	
Mb (87mL)	0	/	0	/	0	/	**carrier 1**	0
Mb (10mL)	0	/	0	/	0	/	**carrier 2**	0
10^0	0	0	0	0	0	0	**carrier 3**	0
10^{-1}	0	0	0	0	0	0		
10^{-2}	0	0	0	0	0	0		
10^{-3}	0	0	0	0	0	0		
n'1	0,00E+00	**d**	**6,74E+00**					
n'2	0,00E+00	**d>5** **x yes □ no**						

	wipes					
	Trial 1		Trial 2		Trial 3	
Mb (87mL)	0	/	0	/	0	/
Mb (10mL)	0	/	0	/	0	/
10^0	0	0	0	0	0	0
10^{-1}	0	0	0	0	0	0
10^{-2}	0	0	0	0	0	0
10^{-3}	0	0	0	0	0	0
n'1	0,00E+00	**d**	**6,74E+00**			
n'2	0	**d>5** **x yes □ no**				

Candida albicans

	Trials							
	Trial 1		Trial 2		Trial 3		carrier	
Mb (87mL)	0	/	0	/	0	/	**carrier 1**	0
Mb (10mL)	0	/	0	/	0	/	**carrier 2**	0
10^0	0	0	0	0	0	0	**carrier 3**	0
10^{-1}	0	0	0	0	0	0		
10^{-2}	0	0	0	0	0	0		
10^{-3}	0	0	0	0	0	0		
n'1	0,00E+00	**d**	**6,27E+00**					
n'2	0	**d>4**						
		x yes □ no						

	wipes					
	Trial 1		Trial 2		Trial 3	
Mb (87mL)	1	/	0	/	0	/
Mb (10mL)	0	/	0	/	0	/
10^0	0	0	0	0	0	0
10^{-1}	0	0	0	0	0	0
10^{-2}	0	0	0	0	0	0
10^{-3}	0	0	0	0	0	0
n'1	1,15E+00	**d**	**6,27E+00**			
n'2	0	**d>4**				
		x yes □ no				

5 CONCLUSION

The SEPTALKAN product, batch n° 2163F2563A, demonstrated a bactericidal and yeasticidal activity by aerial disinfection method, according to the NF T 72-281 standard (may 2009), with three sprays (manual spraying) and for a 5 minutes of time exposure on the carriers, with a wiping step after the contact time with the sprayed product.

TECHNICAL APPENDIX

Media:

TSA (Trypto casein Soya Agar), CONDA Laboratories, ref. n° 1068, batch n° 102231

MEA (Malt Extract Agar), Thermofisher, ref. n°1202-7606, batch n°2311C

Recovery liquid

Trypton-Salt Solution (TS)

- Trypton, Laboratories CONDA, ref. 1612, batch n° 091229 ----------1,00 g/l
- Sodium chloride, PANREAC, ref n° 141659, batch n° 204679 ----------8,50 g/l

pH at 25°C : 7,0 ± 0,2

SKIM MILK

Skim milk, Régilait, batch n° L M F N 15:27.

WIPES

Cellulose and polyester wipes: ADS laminaire, Lymotech, ref.C-WIPES, 7 cm x 7 cm, steriles

STAINLESS STEEL CARRIERS – stainless steel disks**,** 2 cm diameter, 1.4301, 2B grade (MECAPOL).

TEST REPORT

VIRUCIDAL QUANTITATIVE SUSPENSION TEST USING BOVINE ROTAVIRUS ON SEPTALKAN PRODUCT

For : **ALKAPHARM**
PARC BIOCITECH
102 AVENUE GASTON ROUSSEL
93230 ROMAINVILLE
FRANCE

Study number: 23/11/2010

References: n°326V40-2010

VIRUCIDAL TESTS:

According the European standard EN 14476+A1 (January 2007) – chemical antiseptics and disinfectants – virucidal quantitative suspension tests for chemical disinfectants and antiseptics used in human medicine.

Tests using the ready-to-use product SEPTALKAN against the bovine rotavirus.

This test report included 13 pages.

Study completion date: 01-28-2011
Date of republication : 11-07-2013

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

Professeur Georges HERBEIN
Professor of Medicine – MD-PhD in virology
Scientific consultant

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

SUMMARY

1. PERFORMING LABORATORY
2. PRODUCT IDENTITY
3. EXPERIMENTAL CONDITIONS
4. VALIDATIONS
5. VIRUCIDAL ASSAYS
6. CONCLUSIONS
7. TECHNICAL APPENDIX

1 PERFORMING LABORATORY

APEX BIOSOLUTIONS
18, rue Alain SAVARY
25000 BESANÇON
FRANCE

2 PRODUCT IDENTITY

SEPTALKAN product

Lot # 830.11.10
Storage conditions: as recommended by the manufacturer
Active substances: alkylbenzyl dimethyl ammonium chloride; propan-2-ol ; ethylic alcohol
Appearance of the product: liquid, clear.
Product diluent recommended by the manufacturer for use: none (ready-to-use)
Date of delivery of the product: 12-01-2010
Date of tests: 12-13-2010 to 01-21-2011

3 EXPERIMENTAL CONDITIONS

Temperature used during the assays: 20°C ± 1°C
Titration units: log $TCID_{50}$
Exposure time: 5 minutes
Diluent used for the product: distilled sterile water
Viral strain: bovine rotavirus ATCC VR-1290, B223 strain, grown on VERO cells, at 37°C, under 5% CO2 atmosphere
Organic soil load: 3 g/l of albumin bovine serum + 3 ml sheep erythrocytes (dirty conditions)
Product stability: clear solution
Stop solution: cold shock

Viral titer:

Viral titers are expressed in log $TCID_{50}$ per ml, according to the SPEARMAN-KÄRBER method. Titer of bovine rotavirus suspension assay = 8 log $TCID_{50}$.

4 VALIDATIONS

a) Methodology

The VERO cells were exposed to the SEPTALKAN product and a weak cytotoxic effect was observed at the dilution 10^{-2}

b) Sensibility assays

The bovine rotavirus was titrated on cell cultures untreated with the SEPTALKAN product (indicator cell line), and titrated on cell cultures treated with the SEPTALKAN product.

	Viral titer (log $TCID_{50}$)		
Product dilution	Untreated cell cultures	Treated cell cultures	Log reduction
SEPTALKAN 10^{-3}	8	7,125	**0,875**

According to the European standard EN 14476 + A1, the SEPTALKAN product used at the dilution of 10^{-3} does not have an effect on the bovine rotavirus titration method (the difference between viral titers must be < 1,0 log).

c) Validations of the cold shock method on the virucidal effect of the SEPTALKAN product:

Product concentration	**Organic soil load**	**Viral titer (log $TCID_{50}$)**	**Difference with the viral suspension assay**
SEPTALKAN 100%	3 g/l BSA + 3 ml sheep erythrocytes	Trial 1 : 8	0
		Trial 2 : 8,125	-0,125
	3 g/l BSA	Trial 1 : 8,25	-0,25
		Trial 2 : 8,25	-0,25

The method is validated for a difference in viral titers ≤ |0,5| log.

d) Inactivation assays of the virus with a control solution (1,4 % formaldehyde)

	Viral titer (log $TCID_{50}$)	**Viral titer reduction (log $TCID_{50}$)**
Viral suspension control	8,125	
With 1,4%formaldehyde		
Inactivation exposure 5 min	6,375	1,75
Inactivation exposure 15 min	6,25	1,875
Inactivation exposure 30 min	5,75	**2,375**

The viral titer reduction (difference between the titers of the viral suspension treated with 1,4 % formaldehyde and the viral suspension control) must be between -0,5 and -2,5 log after 30 min of exposure. In the assays, the viral titer reduction is 2,375 log, indicated that the bovine rotavirus is inactivated after 30 min of exposure with the 1,4 % formaldehyde solution.

5 VIRUCIDAL ASSAYS

Trial 1

The viral test suspension was titrated at 8 log prior the assays for the SEPTALKAN product at concentration 100 % and 90 %, and 8,25 log $TCID_{50}$ for the concentration 50 %.

PRODUCT	Concentration (v/v)	Time exposure	Temperature	Viral titer (log $TCID_{50}$)	Viral titer reduction
SEPTALKAN	100%	5 min	20°C	3,75	**4,25**
	90%	5 min	20°C	5,375	**2,625**
	50%	5 min	20°C	8,125	**0,125**

Trial 2

The viral test suspension was titrated at 8,125 log $TCID_{50}$ prior the assays for the SEPTALKAN product 100 %.

PRODUCT	Concentration (v/v)	Time exposure	Temperature	Viral titer (log $TCID_{50}$)	Viral titer reduction
SEPTALKAN	100%	5 min	20°C	4	**4,125**

The concentrations of the product demonstrated a virucidal activity on the virus tested if the viral titer reduction is ≥ 4,0 log.

The assays were validated as required by the European standard EN 14476+A1:

- The viral titers of the suspension tests were sufficient in order to observe a reduction of 4 log after time exposure with the product. The viral titer of the bovine rotavirus was of 8 log $TCID_{50}$.

- The virus was inactivated with the control solution of 1,4 % formaldehyde after 30 min of exposure : the reduction observed was of 2,375 log for the bovine rotavirus.

- The SEPTALKAN product does not have a cytotoxic effect on the VERO cells.

- The dirty conditions (sheep erythrocytes + BSA) do not affect the infectious capacity of the bovine rotavirus

- The SEPTALKAN product does not affect the infectious capacity of the virus: the differences in viral titers between the virus inoculated on VERO cells and the virus inoculated on the VERO cells treated with the SEPTALKAN was ≤ 1,0 log (0,875 log).

- The difference between the viral suspension assay titer and the viral suspension titer in the cold shock validation was ≤ $|0,5|$ log (0 log $TCID_{50}$ trial 1, and $|0,125|$ log $TCID_{50}$ trial 2)

6 CONCLUSIONS

The assays performed with SEPTALKAN product demonstrated that:

- **the SEPTALKAN product demonstrated a virucidal activity on the bovine rotavirus from the concentration 100%,** as required by the European standard EN 14476+A1, **following a 5 minutes exposure period, at 20°C, in dirty conditions.**

7 TECHNICAL APPENDIX

Cell line: VERO cells (R&D BIOTECH, lot n°110118-110V)

Viral strain: bovine rotavirus, strain B223, ATCC VR-1290 (lot n° 4/200409/220409)

Buffer and reagents:

- Buffer PBS: sodium chloride, Panreac, ref. 141659.1211, lot n° 0000204679; sodium phosphate dibasic, Sigma Aldrich, ref. S5136, lot n° BCBC7067V; sodium phosphate monobasic, Sigma Aldrich, ref. S5011, lot n° 1019K01021V
- MEM media, Sigma Aldrich, ref. M0268, lot n° 040M8301
- DMEM media, Sigma Aldrich, ref. D6546, lot n° RNBB3646
- Fetal calf serum, Sigma Aldrich, ref. F7524, lot n° 078K3396

Organic soil load:

- Bovine albumin serum (powder), Sigma Aldrich, réf. A7906, lot n° 078K0729
- Sheep erythrocytes, Oxoïd, réf. n° SR0051C, lot n° 25987000

Inactivation solution:

- Formaldehyde, Sigma Aldrich, réf. F-1635, lot n° BCBB3510

Table A1 - Titration of bovine rotavirus by cytopathic effect, with the Spaerman-Kärber method :

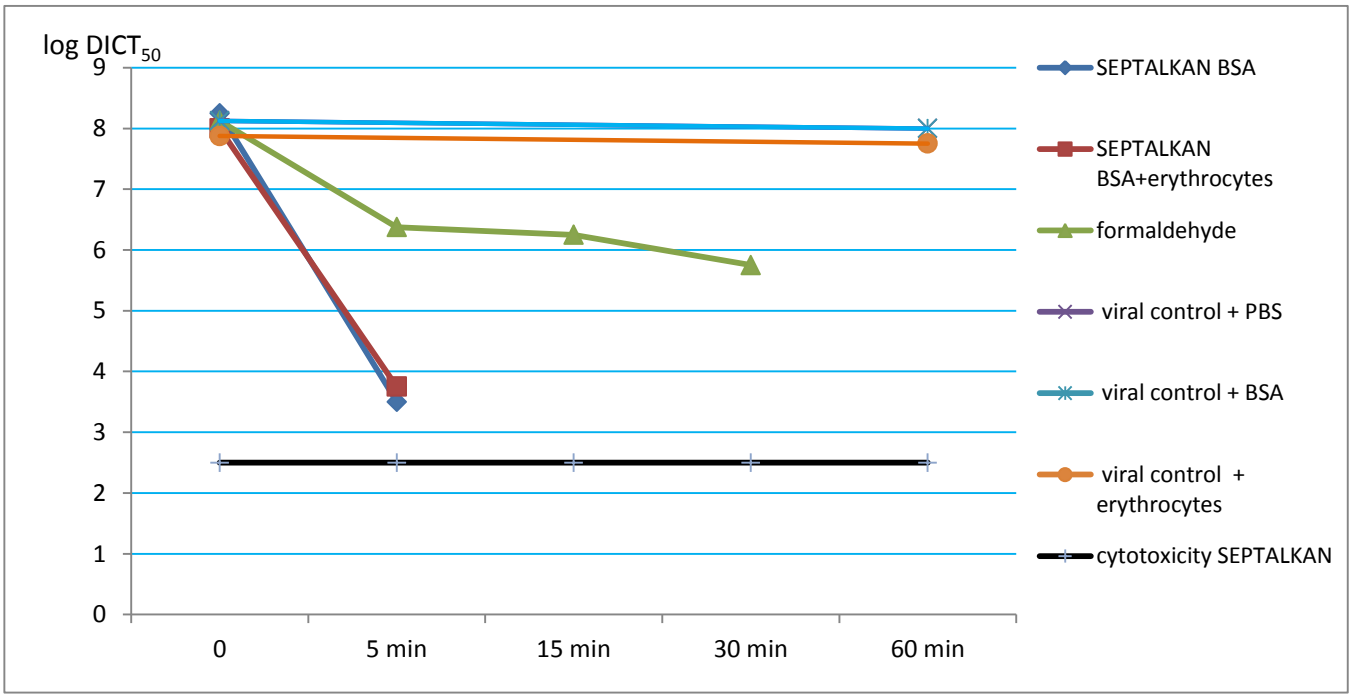
log $TCID_{50}$ = 8

Dilution (- log)	Results	% positive results
-3	44444444	100
-4	44444444	100
-5	44444444	100
-6	44444444	100
-7	33443444	100
-8	12200200	50
-9	00000000	0
-10	00000000	0
Sum of the positives results		550

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Fig. 1 – Chart of the results:

Trial 1



Trial 2

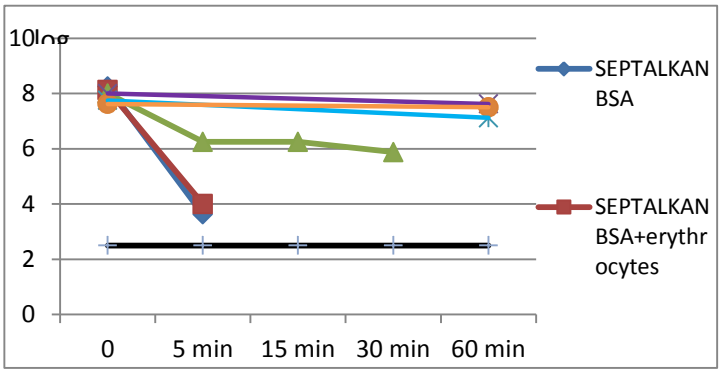


Table A2 — results for the SEPTALKAN product and the bovine rotavirus in dirty conditions (3 g/l BSA + 3 ml sheep erythrocytes)

Product	Concentration	Organic soil load	Cytotoxicity level	Log $TCID_{50}$					Reduction > 4 lg after
				0	5 min	15 min	30 min	60 min	
SEPTALKAN trial 1	100,00%	3 g/l BSA + 3 ml erythrocytes	2,5	2,5	8	3,75	N.T.	N.T.	N.T. R = 4,125
	100,00%	3 g/l BSA	2,5	2,5	8,25	3,5	N.T.	N.T.	N.T. R = 4,5
SEPTALKAN trial 2	100,00%	3 g/l BSA + 3 ml erythrocytes	2,5	2,5	8,125	4	N.T.	N.T.	N.T. R = 4,375
		3 g/l BSA	2,5	2,5	8,25	3,625	N.T.	N.T.	N.T. R = 4,5
Formaldehyde trial 1	1,40%	PBS	1,5	1,5	8,125	6,375	6,25	5,75	N.T.
Formaldehyde trial 2	1,40%	PBS	1,5	1,5	8	6,25	6,25	5,875	N.T.
Infectivity control trial 1	N.A.	PBS	N.A.	N.A.	8,125	N.T.	N.T.	N.T.	8
Infectivity control trial 1	N.A.	3 g/l BSA	N.A	N.A	8,125	N.T.	N.T.	N.T.	8
Infectivity control trial 1	N.A.	3 g/l BSA + 3 ml erythrocytes	N.A	N.A	7,875	N.T.	N.T.	N.T.	7,75
Infectivity control trial 2	N.A.	PBS	N.A	N.A	8	N.T.	N.T.	N.T.	7,625
Infectivity control trial 2	N.A.	3 g/l BSA	N.A	N.A	7,625	N.T.	N.T.	N.T.	7,375
Infectivity control trial 2	N.A.	3 g/l BSA + 3 ml erythrocytes	N.A	N.A	7,625	N.T.	N.T.	N.T.	7,5
Cells sensitivity to the virus	SEPTALKAN	N.A.	Untreated cells	N.T.	N.T.	N.T.	N.T.	8	
	10^{-3}	N.A.	Treated cells	N.T.	N.T.	N.T.	N.T.	7,125	

N.A. : Non Applicable N.T. : Non tested

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Table A3 — Raw data for SEPTALKAN product (3 g/l BSA + 3 ml sheep erythrocytes) tested against the bovine rotavirus (titration by cytopathic effect; 8 wells)

Trial 1

Product	Concen -tration	Organic soil load	Time exposure	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	100,00%	3 g/l BSA + 3 ml erythrocytes	5 min	4444	4444	4444	0040	0000	0000	0000	0000	0000	0000
				4444	4444	4444	0004	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	4444	4444	4444	2200	0000	0000
				4444	4444	4444	4444	4444	4444	4444	2400	0000	0000
		3 g/l BSA	5 min	4444	4444	4444	0000	0000	0000	0000	0000	0000	0000
				4444	4444	4444	0000	0000	0000	0000	0000	0000	0000
			viral control	4444	4444	4444	4444	4444	4444	4444	2420	0000	0000
				4444	4444	4444	4444	4444	4444	4444	2202	0000	0000
SEPTALKAN cytotoxicity	100,00%	3 g/l BSA + 3 ml erythrocytes	N.A.	4444	4444	0000	0000	0000	0000	0000	0000	0000	N.T.
				4444	4444	0000	0000	0000	0000	0000	0000	0000	N.T.
Formaldehyde	1,40%	PBS	5	4444	4444	4444	4444	2222	1001	0000	0000	0000	N.T.
				4444	4444	4444	4444	2222	1111	1000	0000	0000	
			15	4444	4444	4444	4444	3333	1211	0000	0000	0000	N.T.
				4444	4444	4444	4444	3333	2002	0000	0000	0000	
			30	4444	4444	4444	3333	0211	0100	0000	0000	0000	N.T.
				4444	4444	4444	3333	1111	0110	0000	0000	0000	
Formaldehyde (cytotoxicity)	1,40%	PBS	N.A.	4444	0000	0000	0000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
				4444	0000	0000	0000						
Viral control of infectivity	N.A.	PBS	0	4444	4444	4444	4444	4444	4444	3333	0004	0000	0000
				4444	4444	4444	4444	4444	4444	4444	2222	0000	0000
			60	4444	4444	4444	4444	4444	4444	2303	0033	0000	0000
				4444	4444	4444	4444	4444	3444	3333	3033	0000	0000
Viral control of infectivity	N.A.	3 g/l BSA	0	4444	4444	4444	4444	4444	4444	4044	3030	0000	0000
				4444	4444	4444	4444	4444	4444	4444	3333	0000	0000
			60	4444	4444	4444	4444	4444	4444	4022	0222	0000	0000
				4444	4444	4444	4444	4444	4444	2024	2210	0000	0000
Viral control of infectivity	N.A.	3 g/l BSA + 3 ml erythrocytes	0	4444	4444	4444	4444	4444	4444	2002	3022	0000	0000
				4444	4444	4444	4444	4444	4444	2222	3020	0000	0000
			60	4444	4444	4444	4444	4444	4444	2022	0022	0000	0000
				4444	4444	4444	4444	4444	4444	4022	2002	0000	0000

Trial 2

Product	Concen -tration	Organic soil load	Time exposure	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	100,00%	3 g/l BSA + 3 ml erythrocytes	5 min	4444	4444	4444	0020	0000	0000	0000	0000	0000	0000
				4444	4444	3433	0222	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	4444	4444	3344	2203	0000	0000
				4444	4444	4444	4444	4444	4444	2233	2030	0000	0000
		3 g/l BSA	5 min	4444	4444	3344	2000	0000	0000	0000	0000	0000	0000
				4444	4444	4444	0000	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	4444	4444	4333	4003	0000	0000
				4444	4444	4444	4444	4444	4444	3344	3333	0000	0000
SEPTALKAN cytotoxicity	100,00%	3 g/l BSA + 3 ml erythrocytes	N.A.	4444	4444	0000	0000	0000	0000	0000	0000	0000	N.T.
				4444	4444	0000	0000	0000	0000	0000	0000	0000	
Formaldehyde	1,40%	PBS	5	4444	4444	4444	4444	3444	3033	0030	0000	0000	N.T.
				4444	4444	4444	4444	3333	0033	0000	0000	0000	
			15	4444	4444	4444	4444	3333	2210	0000	0000	0000	N.T.
				4444	4444	4444	4444	4222	1101	0000	0000	0000	
			30	4444	4444	4444	4444	1222	0100	0000	0000	0000	N.T.
				4444	4444	4444	4444	2112	0101	0000	0000	0000	
Formaldehyde (cytotoxicity)	1,40%	PBS	N.A.	4444	0000	0000	0000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
				4444	0000	0000	0000						
Viral control of infectivity	N.A.	PBS	0	4444	4444	4444	4444	4444	3443	3032	0222	0000	0000
				4444	4444	4444	4444	4444	3333	2222	2200	0000	0000
			60	4444	4444	4444	4444	4444	4444	3403	0300	0000	0000
				4444	4444	4444	4444	4444	4444	3344	0300	0000	0000
Viral control of infectivity	N.A.	3 g/l BSA	0	4444	4444	4444	4444	4444	3333	3033	0200	0000	0000
				4444	4444	4444	4444	4444	3333	3230	2002	0000	0000
			60	4444	4444	4444	4444	4444	2322	2200	0000	0000	0000
				4444	4444	4444	4444	4444	2333	2220	1020	0000	0000
Viral control of infectivity	N.A.	3 g/l BSA + 3 ml erythrocytes	0	4444	4444	4444	4444	4444	2221	1101	0001	0000	0000
				4444	4444	4444	4444	4444	2222	1011	1010	0000	0000
			60	4444	4444	4444	4444	4444	1112	1010	0011	0000	0000
				4444	4444	4444	4444	4444	1111	1111	0000	0000	0000

Validations :

Product	Concen -tration	Organic soil load	Time exposure	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	90,00%	3 g/l BSA + 3 ml erythrocytes	5 min	4444	4444	4444	4444	2200	0000	0000	0000	0000	0000
				4444	4444	4444	4444	2220	1100	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	4444	4444	4333	0010	0000	0000
				4444	4444	4444	4444	4444	4433	2333	4044	0000	0000
SEPTALKAN	50,00%	3 g/l BSA + 3 ml erythrocytes	5 min	4444	4444	4444	4444	4444	4444	4433	1101	0000	0000
				4444	4444	4444	4444	4444	4444	3333	1100	0000	0000
			Viral control	4444	4444	4444	4444	4444	4444	4444	2210	0000	0000
				4444	4444	4444	4444	4444	4444	4444	1101	0000	0000

Product	Concentration	Organic soil load	Cytotoxic level	Log $TCID_{50}$		Reduction
				0	5 min	
SEPTALKAN	90,00%	3 g/l BSA + 3 ml erythrocytes	2,5	8	5,375	5 min R = 2,625
SEPTALKAN	50,00%	3 g/l BSA + 3 ml erythrocytes	2,5	8,25	8,125	5 min R = 0,125

Sensitivity of cells to the bovine rotavirus:

Product	dilution	Organic soil load		Dilutions								
				-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	10^{-3}	3 g/l BSA + 3 ml erythrocytes	Untreated cells	4444	4444	4444	4444	4444	4444	0023	0000	0000
				4444	4444	4444	4444	4444	4443	3300	0000	0000
			Treated cells	4444	4444	4444	4444	4444	4040	0000	0000	0000
				4444	4444	4444	4444	4444	4404	0000	0000	0000

TEST REPORT

VIRUCIDAL QUANTITATIVE SUSPENSION TEST USING MURINE NOROVIRUS ON SEPTALKAN PRODUCT

For : **ALKAPHARM**
PARC BIOCITECH
102 AVENUE GASTON ROUSSEL
93230 ROMAINVILLE
FRANCE

Study number: 11/23/2010

References: n°306V36-2010

VIRUCIDAL TESTS:

According the European standard EN 14476+A1 (January 2007) – chemical antiseptics and disinfectants – virucidal quantitative suspension tests for chemical disinfectants and antiseptics used in human medicine.

Tests using the ready-to-use product SEPTALKAN against the murine norovirus.

This test report included 13 pages.

Study completion date: 01-13-2011
Date of republication : 11-07-2013

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

Professeur Georges HERBEIN
Professor of Medicine – MD-PhD in virology
Scientific consultant

SUMMARY

1. PERFORMING LABORATORY
2. PRODUCT IDENTITY
3. EXPERIMENTAL CONDITIONS
4. VALIDATIONS
5. VIRUCIDAL ASSAYS
6. CONCLUSIONS
7. TECHNICAL APPENDIX

1 PERFORMING LABORATORY

APEX BIOSOLUTIONS
18, rue Alain SAVARY
25000 BESANÇON
FRANCE

2 PRODUCT IDENTITY

SEPTALKAN product

Lot # 830.11.10
Storage conditions: as recommended by the manufacturer
Active substances: alkylbenzyl dimethyl ammonium chloride; propan-2-ol; ethylic alcohol
Appearance of the product: liquid, clear.
Product diluent recommended by the manufacturer for use: none (ready-to-use)
Date of delivery of the product: 12-01-2010
Date of tests: 12-06-2010 to 01-07-2011

3 EXPERIMENTAL CONDITIONS

Temperature used during the assays: 20°C ± 1°C
Titration units: log $TCID_{50}$
Exposure time: 5 minutes
Diluent used for the product: distilled sterile water
Viral strain: murine norovirus MNV-1, strain S99, grown on RAW 264.7 cells, at 37°C, under 5% CO2 atmosphere
Organic soil load: 3 g/l of albumin bovine serum + 3 ml sheep erythrocytes (dirty conditions)
Product stability: clear solution
Stop solution: cold shock

Viral titer:

Viral titers are expressed in log $TCID_{50}$ per ml, according to the SPEARMAN-KÄRBER method. Titer of murine norovirus suspension assay = 8,375 log $TCID_{50}$.

4 VALIDATIONS

a) Methodology

The RAW 264.7 cells were exposed to the SEPTALKAN product and a weak cytotoxic effect was observed at the dilution 10^{-2}

b) Sensibility assays

The murine norovirus was titrated on cell cultures untreated with the SEPTALKAN product (indicator cell line), and titrated on cell cultures treated with the SEPTALKAN product.

	Viral titer (log $TCID_{50}$)		
Product dilution	Untreated cell cultures	Product dilution	Untreated cell cultures
SEPTALKAN 10^{-3}	8,25	7,625	**0,625**

According to the European standard EN 14476 + A1, the SEPTALKAN product used at the dilution of 10^{-3} does not have an effect on the murine norovirus titration method (the difference between viral titers must be < 1,0 log).

c) Validations of the cold shock method on the virucidal effect of the SEPTALKAN product:

Product concentration	**Organic soil load**	**Viral titer (log $TCID_{50}$)**	**Difference with the viral suspension assay**
SEPTALKAN 100%	3 g/l BSA + 3 ml sheep erythrocytes	Trial 1 : 8,125	0,25
		Trial 2 : 8,25	0,125
	3 g/l BSA	Trial 1 : 8,25	0,125
		Trial 2 : 8	0,375

The method is validated for a difference in viral titers ≤ 0,5 log.

c) Inactivation assays of the virus with a control solution (1,4 % formaldehyde)

	Viral titer (log $TCID_{50}$)	**Viral titer reduction (log $TCID_{50}$)**
Viral suspension control	8,375	
With 1,4%formaldehyde		
Inactivation exposure 5 min	7	1,375
Inactivation exposure 15 min	6,125	2,25
Inactivation exposure 30 min	6	**2,375**

The viral titer reduction (difference between the titers of the viral suspension treated with 1,4 % formaldehyde and the viral suspension control) must be between -0,5 and -2,5 log after 30 min of exposure. In the assays, the viral titer reduction is 2,375 log, indicated that the murine norovirus is inactivated after 30 min of exposure with the 1,4 % formaldehyde solution.

5 VIRUCIDAL ASSAYS

Trial 1

The viral test suspension was titrated at 8,125 log prior the assays for the SEPTALKAN product at concentration 100 %, 8,375 log $TCID_{50}$ for the concentration 90 % and 8,5 log $TCID_{50}$ for the concentration 50 %.

PRODUCT	Concentration (v/v)	Time exposure	Temperature	Viral titer (log $TCID_{50}$)	Viral titer reduction
SEPTALKAN	100%	5 min	20°C	4	**4,125**
	90%	5 min	20°C	5,125	**3,25**
	50%	5 min	20°C	8	**0,5**

Trial 2

The viral test suspension was titrated at 8,25 log $TCID_{50}$ prior the assays for the SEPTALKAN product 100 %.

PRODUCT	Concentration (v/v)	Time exposure	Temperature	Viral titer (log $TCID_{50}$)	Viral titer reduction
SEPTALKAN	100%	5 min	20°C	3,875	**4,375**

The concentrations of the product demonstrated a virucidal activity on the virus tested if the viral titer reduction is ≥ 4,0 log.

The assays were validated as required by the European standard EN 14476+A1:

- The viral titers of the suspension tests were sufficient in order to observe a reduction of 4 log after time exposure with the product. The viral titer of the murine norovirus was of 8,375 log $TCID_{50}$.

- The virus was inactivated with the control solution of 1,4 % formaldehyde after 30 min of exposure : the reduction observed was of 2,375 log for the murine norovirus.

- The SEPTALKAN product does not have a cytotoxic effect on the RAW cells.

- The dirty conditions (sheep erythrocytes + BSA) do not affect the infectious capacity of the murine norovirus

- The SEPTALKAN product does not affect the infectious capacity of the virus: the differences in viral titers between the virus inoculated on RAW cells and the virus inoculated on the RAW cells treated with the SEPTALKAN was ≤ 1,0 log (0,625 log).

- The difference between the viral suspension assay titer and the viral suspension titer in the cold shock validation was ≤ 0,5 log (0,25 log $TCID_{50}$ trial 1, and 0,125 log $TCID_{50}$ trial 2)

6 CONCLUSIONS

The assays performed with SEPTALKAN product demonstrated that:

- **the SEPTALKAN product demonstrated a virucidal activity on the murine norovirus from the concentration 100%,** as required by the European standard EN 14476+A1, **following a 5 minutes exposure period, at 20°C, in dirty conditions.**

7 TECHNICAL APPENDIX

7.1 TECHNICAL APPENDIX 1

Cell line: RAW 264.7 (ATCC TIB-71) cells

Viral strain: murine norovirus, strain S99 (lot n° 4/200409/220409- Friedrich Loeffler Institut)

Buffer and reagents:

- Buffer PBS: sodium chloride, Panreac, ref. 141659.1211, lot n° 0000204679; sodium phosphate dibasic, Sigma Aldrich, ref. S5136, lot n° BCBC7067V; sodium phosphate monobasic, Sigma Aldrich, ref. S5011, lot n° 1019K01021V
- MEM media, Sigma Aldrich, ref. M0268, lot n° 040M8301
- DMEM media, Sigma Aldrich, ref. D6546, lot n° RNBB3646
- Fetal calf serum, Sigma Aldrich, ref. F7524, lot n° 078K3396

Organic soil load:

- Bovine albumin serum (powder), Sigma Aldrich, réf. A7906, lot n° 078K0729
- Sheep erythrocytes, Oxoïd, réf. n° SR0051C, lot n° 25987000

Inactivation solution:

- Formaldehyde, Sigma Aldrich, réf. F-1635, lot n° BCBB3510

7.2 TECHNICAL APPENDIX 2

Table A1 - Titration of murine norovirus by cytopathic effect, with the Spaerman-Kärber method :

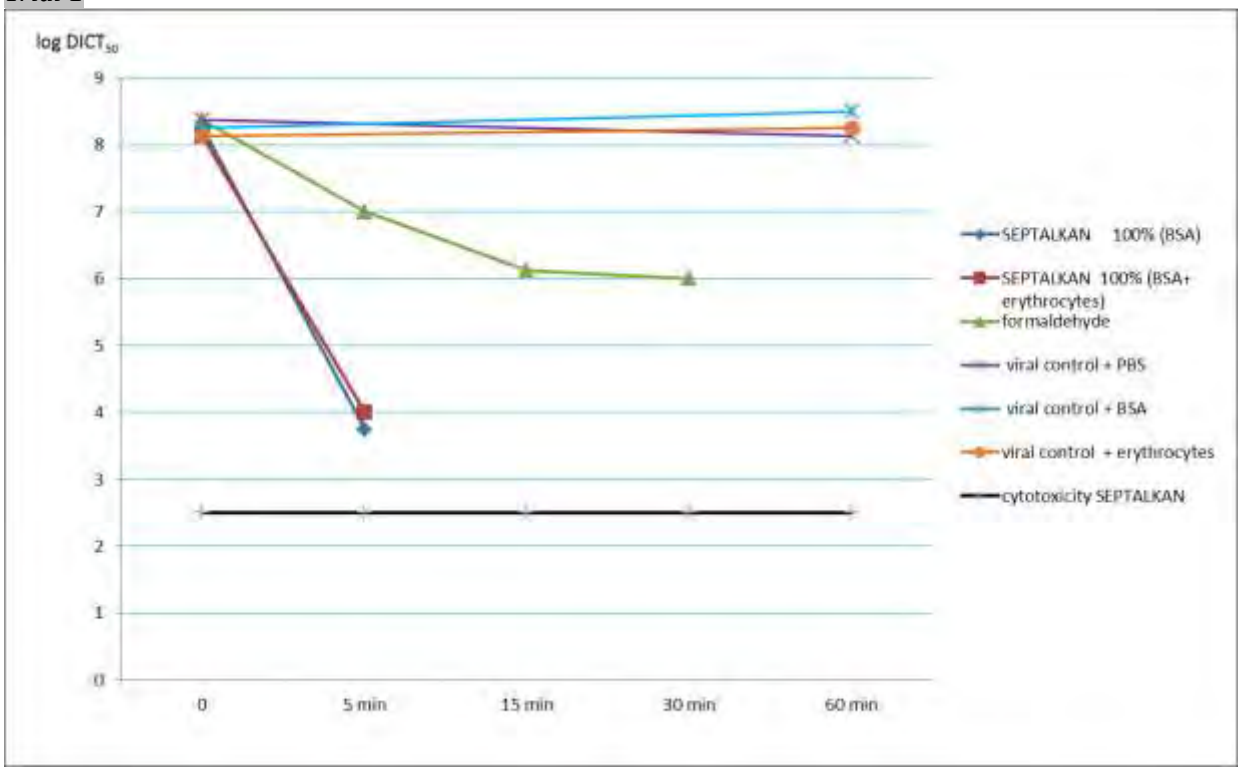
log $TCID_{50}$ = 8,375

Dilution (- log)	Results	% positive results
-3	44444444	100
-4	44444444	100
-5	44444444	100
-6	44444444	100
-7	22223220	87,5
-8	11122002	75
-9	20000002	25
-10	00000000	0
Sum of the positives results		587,5

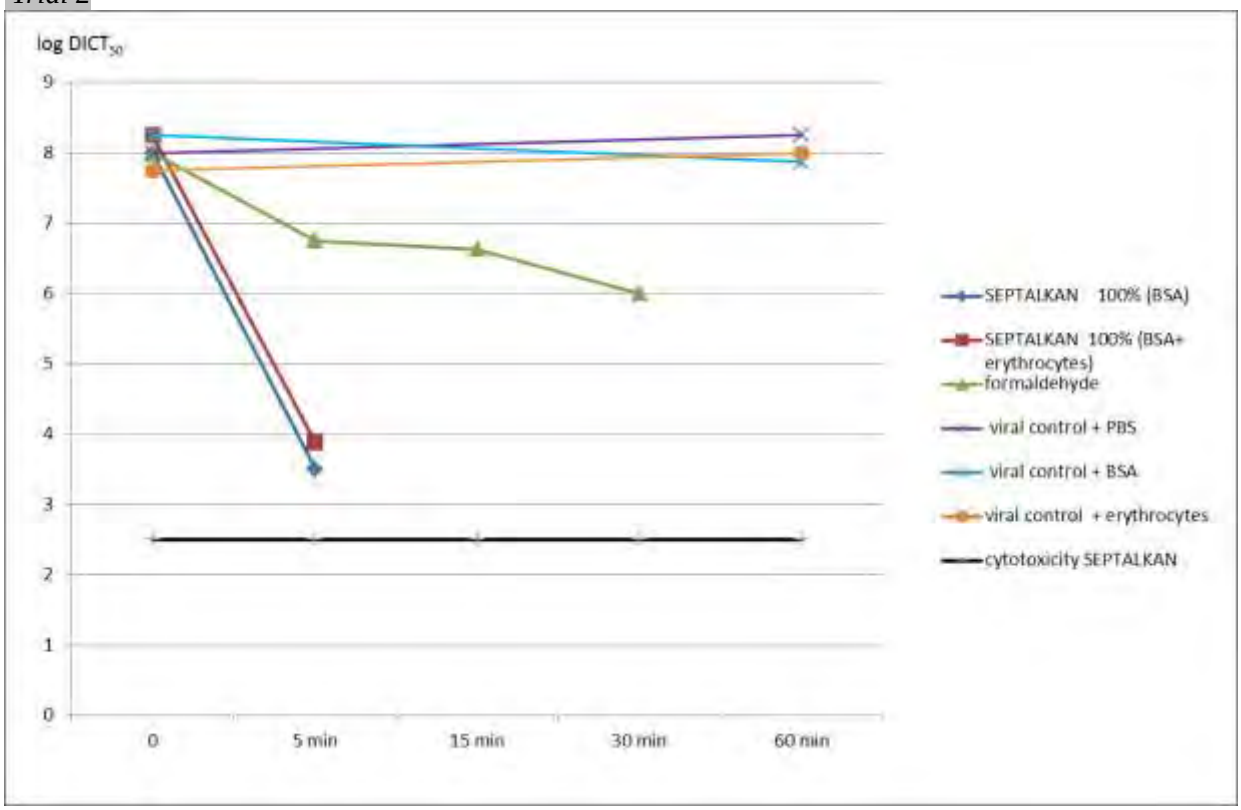
18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Fig. 1 – Chart of the results:

Trial 1



Trial 2



18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Table A2 — results for the SEPTALKAN product and the murine norovirus in dirty conditions (3 g/l BSA + 3 ml sheep erythrocytes)

Product	Concentration	Organic soil load	Cytotoxicity level	Log $TCID_{50}$					Reduction > 4 lg after
				0	5 min	15 min	30 min	60 min	
SEPTALKAN trial 1	100,00%	3 g/l BSA + 3 ml erythrocytes	2,5	8,125	4	N.T.	N.T.	N.T.	5 min R = 4,125
	100,00%	3 g/l BSA	2,5	8,25	3,75	N.T.	N.T.	N.T.	5 min R = 4,5
SEPTALKAN trial 2	100,00%	3 g/l BSA + 3 ml erythrocytes	2,5	8,25	3,875	N.T.	N.T.	N.T.	5 min R = 4,375
		3 g/l BSA	2,5	8	3,5	N.T.	N.T.	N.T.	5 min R = 4,5
Formaldehyde trial 1	1,40%	PBS	1,5	8,375	7	6,125	6	N.T.	
Formaldehyde trial 2	1,40%	PBS	1,5	8	6,75	6,63	6	N.T.	
Infectivity control trial 1	N.A.	PBS	N.A.	8,375	N.T.	N.T.	N.T.	8,125	
Infectivity control trial 1	N.A.	3 g/l BSA	N.A	8,25	N.T.	N.T.	N.T.	8,5	
Infectivity control trial 1	N.A.	3 g/l BSA + 3 ml erythrocytes	N.A	8,125	N.T.	N.T.	N.T.	8,25	
Infectivity control trial 2	N.A.	PBS	N.A	8	N.T.	N.T.	N.T.	8,25	
Infectivity control trial 2	N.A.	3 g/l BSA	N.A	8,25	N.T.	N.T.	N.T.	7,875	
Infectivity control trial 2	N.A.	3 g/l BSA + 3 ml erythrocytes	N.A	7,75	N.T.	N.T.	N.T.	8	
Cells sensitivity to the virus	SEPTALKAN	N.A.	Untreated cells	N.T.	N.T.	N.T.	N.T.	8,25	
	10^{-3}	N.A.	Treated cells	N.T.	N.T.	N.T.	N.T.	7,625	

N.A. : Non Applicable N.T. : Non tested

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Table A3 — Raw data for SEPTALKAN product (3 g/l BSA + 3 ml sheep erythrocytes) tested against the murine norovirus (titration by cytopathic effect; 8 wells)

Trial 1

Product	Concen -tration	Organic soil load	Time exposure	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	100,00%	3 g/l BSA + 3 ml erythrocytes	5 min	4444	4444	4444	4200	0000	0000	0000	0000	0000	0000
				4444	4444	4444	2040	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	4444	4444	4444	1100	0000	0000
				4444	4444	4444	4444	4444	4444	4444	0111	0000	0000
		3 g/l BSA	5 min	4444	4444	4444	0000	0000	0000	0000	0000	0000	0000
				4444	4444	4444	0044	0000	0000	0000	0000	0000	0000
			viral control	4444	4444	4444	4444	4444	4444	3333	2111	0000	0000
				4444	4444	4444	4444	4444	4444	3343	1100	0000	0000
SEPTALKAN cytotoxicity	100,00%	3 g/l BSA + 3 ml erythrocytes	N.A.	4444	4444	0000	0000	0000	0000	0000	0000	0000	N.T.
				4444	4444	0000	0000	0000	0000	0000	0000	0000	N.T.
Formaldehyde	1,40%	PBS	5	4444	4444	4444	4444	2233	1211	1100	0000	0000	N.T.
				4444	4444	4444	4444	3343	2222	0022	0000	0000	
			15	4444	4444	4444	4444	0444	4300	0003	0000	0000	N.T.
				4444	4444	4444	4444	3333	3330	0000	0000	0000	
			30	4444	4444	4444	4443	3403	0023	0000	0000	0000	N.T.
				4444	4444	4444	3343	3333	3330	0000	0000	0000	
Formaldehyde (cytotoxicity)	1,40%	PBS	N.A.	4444	0000	0000	0000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
				4444	0000	0000	0000						
Viral control of infectivity	N.A.	PBS	0	4444	4444	4444	4444	4444	4444	3434	4003	0200	0000
				4444	4444	4444	4444	4444	4444	4444	2440	0001	0000
			60	4444	4444	4444	4444	4444	4404	4444	4040	1140	0000
				4444	4444	4444	4444	4444	4044	3333	3400	0000	0000
Viral control of infectivity	N.A.	3 g/l BSA	0	4444	4444	4444	4444	4444	4444	2322	1100	0100	0000
				4444	4444	4444	4444	4444	4444	2222	0011	1000	0000
			60	4444	4444	4444	4444	4444	4444	3111	4003	0101	0000
				4444	4444	4444	4444	4444	4333	3333	3333	0000	0000
Viral control of infectivity	N.A.	3 g/l BSA + 3 ml erythrocytes	0	4444	4444	4444	4444	4444	4444	4444	4044	0100	0000
				4444	4444	4444	4444	4444	4444	4444	4000	0000	0000
			60	4444	4444	4444	4444	4444	4444	4304	3043	0000	0000
				4444	4444	4444	4444	4444	4444	4444	3233	0000	0000

Trial 2

Product	Concen -tration	Organic soil load	Time exposure	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	100,00%	3 g/l BSA + 3 ml erythrocytes	5 min	4444	4444	4444	0101	0000	0000	0000	0000	0000	0000
				4444	4444	4444	0003	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	4444	4444	1111	0101	0000	0000
				4444	4444	4444	4444	4444	4444	1111	1111	0000	0000
		3 g/l BSA	5 min	4444	4444	4444	0000	0000	0000	0000	0000	0000	0000
				4444	4444	4444	0000	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	3333	3333	1211	1100	0000	0000
				4444	4444	4444	4444	3333	2333	1111	1001	0000	0000
SEPTALKAN cytotoxicity	100,00%	3 g/l BSA + 3 ml erythrocytes	N.A.	4444	4444	0000	0000	0000	0000	0000	0000	0000	N.T.
				4444	4444	0000	0000	0000	0000	0000	0000	0000	
Formaldehyde	1,40%	PBS	5	4334	3333	3333	3333	1122	1122	0021	0000	0000	N.T.
				4444	3333	3333	3333	2222	1110	0200	0000	0000	
			15	4434	3333	3333	3222	2212	0111	0000	0000	0000	N.T.
				4344	3333	3333	2232	2222	0011	0000	0000	0000	
			30	3333	3333	3333	3333	1222	1010	0000	0000	0000	N.T.
				3333	3333	3333	3333	3222	1100	0000	0000	0000	
Formaldehyde (cytotoxicity)	1,40%	PBS	N.A.	4444	0000	0000	0000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
				4444	0000	0000	0000						
Viral control of infectivity	N.A.	PBS	0	4444	4444	4444	4444	4444	4444	3333	4300	0000	0000
				4444	4444	4444	4444	4444	4444	3444	3040	0000	0000
			60	4444	4444	4444	4444	4444	4434	3333	3003	0000	0000
				4444	4444	4444	4444	4444	4334	3031	3100	1110	0000
Viral control of infectivity	N.A.	3 g/l BSA	0	4444	4444	4444	4444	4444	3333	2222	0102	0100	0000
				4444	4444	4444	4444	4444	4343	2102	2002	0110	0000
			60	4444	4444	4444	4444	4444	4444	3202	1002	0000	0000
				4444	4444	4444	4444	4444	3334	3022	3031	0000	0000
Viral control of infectivity	N.A.	3 g/l BSA + 3 ml erythrocytes	0	4444	4444	4444	4444	4444	4444	4444	0440	0000	0000
				4444	4444	4444	4444	4444	4444	4040	4400	0000	0000
			60	4444	4444	4444	4444	4444	4444	4340	3003	0100	0000
				4444	4444	4444	4444	4444	4444	4400	2222	0000	0000

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Validations :

Product	Concen -tration	Organic soil load	Time exposure	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	90,00%	3 g/l BSA + 3 ml erythrocytes	5 min	4444	4444	4444	3443	1004	0000	0000	0000	0000	0000
				4444	4444	4444	3333	0144	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	4444	4433	4422	4402	0000	0000
				4444	4444	4444	4444	4311	1333	4224	1220	4000	0000
SEPTALKAN	50,00%	3 g/l BSA + 3 ml erythrocytes	5 min	4444	4444	4444	4444	4444	4444	4444	4100	0000	0000
				4444	4444	4444	4444	4444	4444	4444	1100	0000	0000
			Viral control	4444	4444	4444	4444	4444	4444	4444	4333	0000	0000
				4444	4444	4444	4444	4444	4444	4444	4444	0000	0000

Product	Concentration	Organic soil load	Cytotoxic level	Log $TCID_{50}$		Reduction > 4 log after
				0	5 min	
SEPTALKAN	90,00%	3 g/l BSA + 3 ml erythrocytes	2,5	8,375	5,125	5 min R = 3,25
SEPTALKAN	50,00%	3 g/l BSA + 3 ml erythrocytes	2,5	8,5	8	5 min R = 0,5

Sensitivity of cells to the murine norovirus:

Product	dilution	Organic soil load		Dilutions								
				-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	10^{-3}	3 g/l BSA + 3 ml erythrocytes	Untreated cells	4444	4444	4444	4444	4444	4444	3003	0000	0000
				4444	4444	4444	4444	4444	3333	3333	0000	0000
			Treated cells	4444	4444	4444	4444	4444	4444	4000	0000	0000
				4444	4444	4444	4444	4444	4444	0000	0000	0000

TEST REPORT

VIRUCIDAL ACTIVITY OF THE SEPTALKAN PRODUCT AGAINST ADENOVIRUS

For : **ALKAPHARM**
PARC BIOCITECH
102 AVENUE GASTON ROUSSEL
93230 ROMAINVILLE

Date of request: 07/20/2012

Study number: n°201V8-2012

VIRUCIDAL ACTIVITY:

According to the NF EN 14476+A1 (January 2007) standard – chemical antiseptics and disinfectants – virucidal quantitative suspension tests for chemical disinfectants and antiseptics used in human medicine.

Tests using the SEPTALKAN product, on the adenovirus VR-5, adenoïd 75 strain.

This test report included 14 pages.

Study completion date: 10/05/2012

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

Professeur Georges HERBEIN
Professor of Medicine – MD-PhD in virology
Scientific consultant

SUMMARY

1. PRODUCT IDENTITY
2. EXPERIMENTAL CONDITIONS
3. VALIDATIONS
4. VIRUCIDAL ASSAYS
5. CONCLUSIONS
6. TECHNICAL APPENDIX

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

1 PERFORMING LABORATORY

APEX BIOSOLUTIONS
18, rue Alain SAVARY
25000 BESANÇON
FRANCE

2 PRODUCT IDENTITY

SEPTALKAN

Batch n°: 2463F2563A

Expiration date: non communicated

Manufacturer: ALKAPHARM

Manufacturing date: non communicated

Storage conditions: as recommended by the manufacturer

Active substances: didecyl dimethyl ammonium chloride ≤2,5%, alkylbenzyl dimethyl ammonium chloride ≤9,5%

Appearance: clear.

Product diluent recommended by the manufacturer for use: none, ready-to-use product

Date of delivery of the product: 06/26/2012

Date of tests: from 08/02/2012 to 09/24/2012

3 EXPERIMENTAL CONDITIONS

Temperature used during the assays: 20°C ± 1°C

Titration units: log $TCID_{50}$

Exposure time: 5 minutes

Diluent used for the product: distilled water

Viral strain: adenovirus type 5, adenoïd 75 strain (ATCC VR5), grown on HEp-2 cells, at 37°C, under 5% CO2 atmosphere

Organic soil load: 3 g/l bovine serum albumin + sheep erythrocytes

Product stability: good

Stop solution: cold shock

Viral titer:

Viral titers are expressed in log $TCID_{50}$ per ml, according to the SPEARMAN-KÄRBER method. Titer of adenovirus suspension assay = 7,00 log $TCID_{50}$.

4 VALIDATION

a) Methodology

The HEp-2cells were exposed to the SEPTALKAN product and a weak cytotoxic effect was observed at the dilution 10^{-1}.

b) Sensibility assays

The adenovirus was titrated on cell cultures untreated with the SEPTALKAN product (indicator cell line), and titrated on cell cultures treated with SEPTALKAN product.

	Viral titer (log TCID50)		
Product dilution	Untreated cell cultures	Treated cell cultures	**Viral titer (log $TCID_{50}$)**
SEPTALKAN 10^{-1}	7,125	6,5	**0,625**

According to the European standard EN 14476, the SEPTALKAN product used at the dilution of 10^{-1}does not have an effect on the adenovirus titration method (the difference between viral titers must be < 1,0 log).

c) Validations of the cold shock method:

Product Concentration	**Organic soil load**	**Viral titer (log $TCID_{50}$)**	**Difference with the viral suspension**
SEPTALKAN 100 %	3 g/l BSA	Trial 1 : 7,125	0,125
		Trial 2 : 7,125	0,125
SEPTALKAN 100 %	3 g/l BSA + 3 ml sheep erythrocytes	Trial 1 : 7,125	0,125
		Trial 2 : 7,25	0,25

d) Inactivation assays of the virus with a control solution

	Viral titer (log $TCID_{50}$)	**Viral titer reduction (log $TCID_{50}$)**
Viral suspension control	6,75	
formaldehyde 1,4%		
inactivation 5 min	6,875	-0,125
inactivation 15 min	6,75	0,000
inactivation 30 min	5,75	**1,000**

The viral titer reduction (difference between the titers of the viral suspension treated with 1,4 % formaldehyde and the viral suspension control) must be between -0,5 and -2,5 log after 30 min of exposure. In the assays, the viral titer reduction is 1,00 log, indicated that the adenovirus is inactivated after 30 min of exposure with the 1,4 % formaldehyde solution.

5 VIRUCIDAL ASSAYS

Trial 1

The viral suspension was titrated at 7,125 log $TCID_{50}$ prior the assays.

PRODUCT	Organic soil load	Concentration (v/v)	Time of exposure	Temperature	**Viral titer (log $TCID_{50}$)**	**Viral titer reduction**
SEPTALKAN	3 g/l BSA + 3 ml sheep erythrocytes	100 %	5 min	20°C	2,500	**4,625**
	3 g/l BSA	100 %	5 min	20°C	2,250	**4,875**

Trial 2

The viral suspension was titrated at 7,25 log $TCID_{50}$ prior the assays (BSA+ erythrocytes) and 7,125 log $TCID_{50}$ (BSA).

PRODUCT	Organic soil load	Concentration (v/v)	Time of exposure	Temperature	**Viral titer (log $TCID_{50}$)**	**Viral titer reduction**
SEPTALKAN	3 g/l BSA + 3 ml sheep erythrocytes	100 %	5 min	20°C	2,750	**4,500**
	3 g/l BSA	100 %	5 min	20°C	2,500	**4,625**

The concentrations of the product demonstrated a virucidal activity on the virus tested if the viral titer reduction is ≥ 4,0 log.

6 VALIDATION OF THE METHODOLOGY

The assays were validated as required by the European standard EN 14476+A1:

- The viral titers of the suspension tests were sufficient in order to observe a reduction of 4 log after time exposure with the product. The viral titer of the adenovirus was 7,00 log TCID50.
- The virus was inactivated with the control solution of 1,4 % formaldehyde after 30 min of exposure : the reduction observed was of 1,0 log for the adenovirus.
- The SEPTALKAN product does not have a cytotoxic effect on the Hep-2 cells.
- The organic soil load (3 g/l BSA + 3 ml/l sheep erythrocytes) does not affect the infectious capacity of the adenovirus.
- The SEPTALKAN product does not affect the infectious capacity of the virus: the differences in viral titers between the virus inoculated on Hep-2 cells and the virus inoculated on the Hep-2 cells treated with the SEPTALKAN product was ≤ 1,0 log (0,625 log).

- The difference of viral titers and viral titers in the cold shock method is ≤ 0,5 log (0,125 log $TCID_{50}$ trial 1, and 0,125 log $TCID_{50}$ trial 2)

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

7 CONCLUSIONS

The assays performed with the SEPTALKAN product demonstrated that:

- the **SEPTALKAN product** **demonstrated a virucidal activity on the adenovirus from the concentration 100%,** as required by the European standard EN 14476+A1, following a **5 minutes** exposure period, at 20°C, in dirty conditions.

TECHNICAL APPENDIX 1

Cell line: HEp-2 celes (RD-Biotech ref. 84011, batch n°110315-118)

Viral strain: adenovirus type 5, adenoïd 75 strain (ATCC ref. VR-5, batch n°3679877)

Buffer and reagents:

- PBS buffer: sodium chloride, Panreac, ref. 141659.1211, batch n° 0000204679; sodium phosphate dibasic, Sigma Aldrich, ref. S5136, batch n° BCBC7067V; sodium phosphate monobasic, Sigma Aldrich, ref. S5011, batch n° 1019K01021V
- MEM media, PAN Biotech, ref. P03-2310, batch n° 1330-1/34
- DMEM media, Sigma Aldrich, ref. D5796, batch n° RNBB6646
- fœtal calf sera, Dutscher, batch n° S05491S0750

Reagents:

- Bovine albumin serum (powder), Sigma Aldrich, 05479, batch n° STBB7838V
- Sheep erythrocytes, Oxoïd, ref. SR 0051E, batch n° 4234000

Inactivation solution:

- Formaldehyde, Sigma Aldrich, ref. F-1635, batch n° BCBB3510

TECHNICAL APPENDIX 2

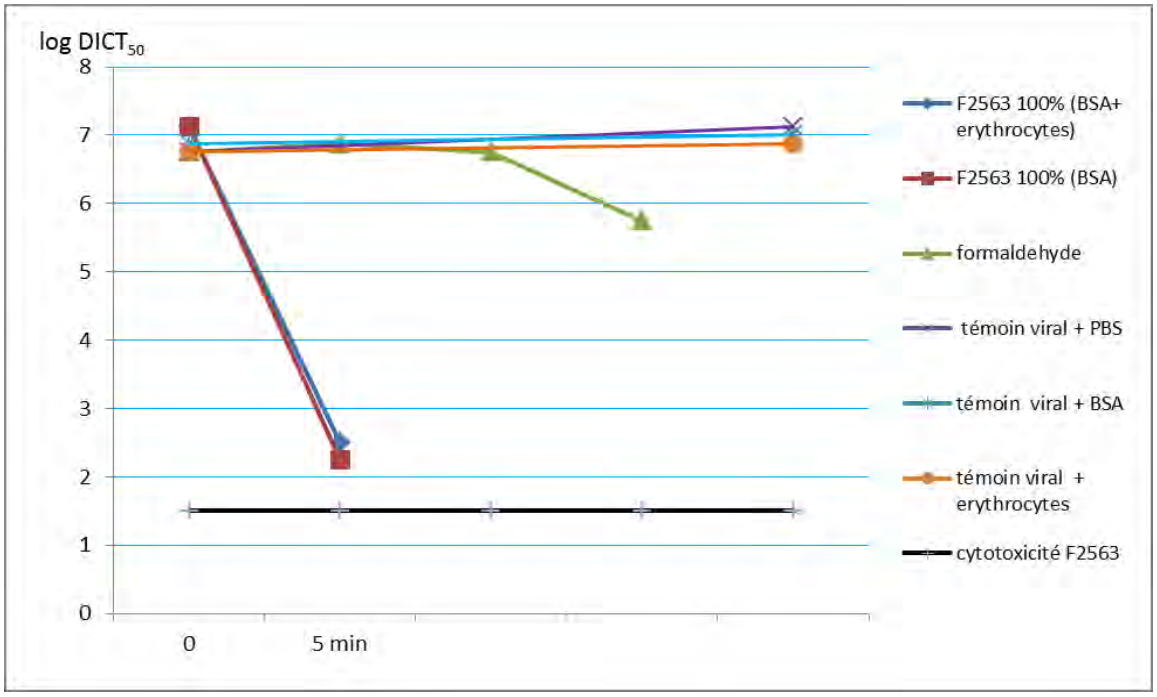
Table A1 - Titration of adenovirus virus by cytopathic effect, with the Spaerman-Kärber method:

log $TCID_{50}$ = 7,00

Dilution (- log)	Results	% positive results
-3	44444444	100
-4	44444444	100
-5	23222332	100
-6	11121111	100
-7	00111010	50
-8	00000000	0
-9	00000000	0
-10	00000000	0
Sum of positives cultures %		450

Figure 1 – – Chart of the results :

Trial 1



Trial 2

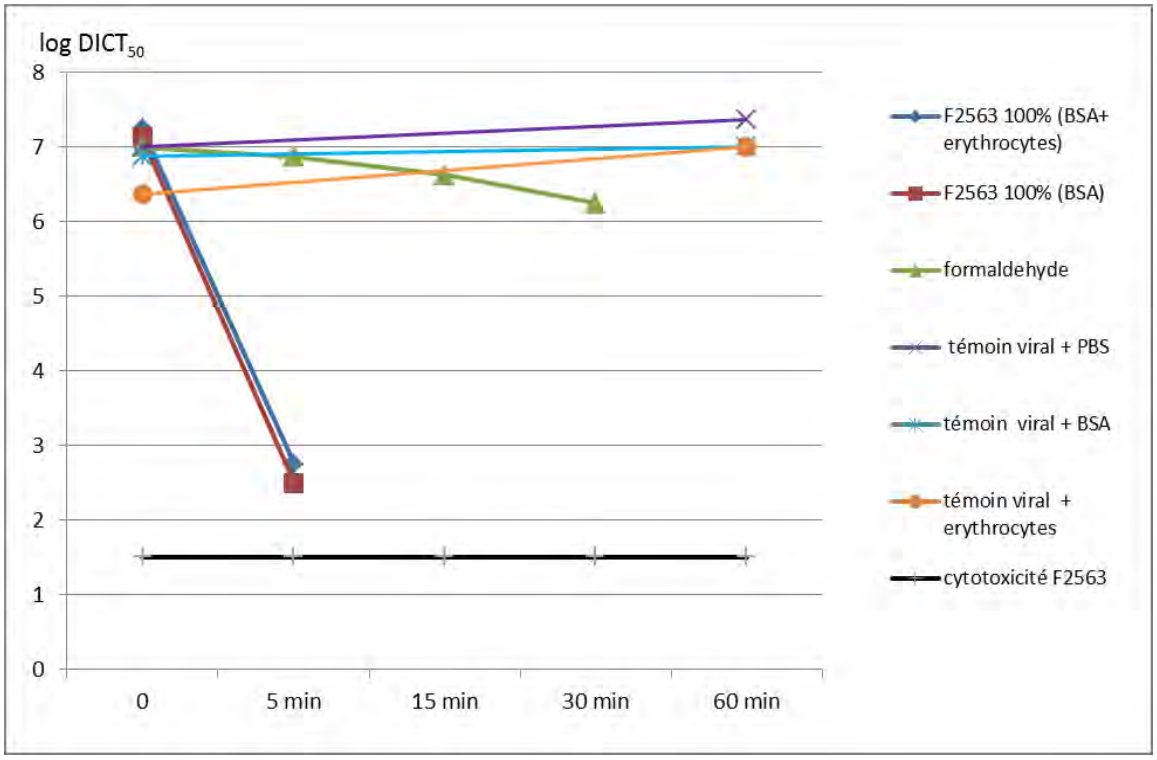


Tableau A2 — results for the SEPTALKAN product and the adenovirus in dirty conditions (3 g/l BSA + 3 ml/l sheep erythrocytes)

Product	Concentration	Organic soil load	Cytotoxicity level	Lg $TCID_{50}$					Reduction
				0	5 min	15 min	30 min	60 min	
SEPTALKAN trial 1	100,00%	3 g/l BSA + 3 ml erythrocytes	1,5	7,125	2,5	N.T.	N.T.	N.T.	5 min R = 4,625
		3 g/l BSA	1,5	7,125	2,25	N.T.	N.T.	N.T.	5 min R = 4,875
SEPTALKAN trial 2	100,00%	3 g/l BSA + 3 ml erythrocytes	1,5	7,25	2,75	N.T.	N.T.	N.T.	5 min R = 4,5
		3 g/l BSA	1,5	7,125	2,5	N.T.	N.T.	N.T.	5 min R = 4,625
Formaldehyde trial 1	1,40%	PBS	2,875	6,75	6,875	6,75	5,75	N.T.	
Formaldehyde trial 2	1,40%	PBS	2,75	7	6,875	6,625	6,25	N.T.	
Infectivity control trial 1	N.A.	PBS	N.A.	6,75	N.T.	N.T.	N.T.	7,125	
Infectivity control trial 1	N.A.	3 g/l BSA	N.A	6,875	N.T.	N.T.	N.T.	7	
Infectivity control trial 1	N.A.	3 g/l BSA + 3 ml erythrocytes	N.A	6,75	N.T.	N.T.	N.T.	6,875	
Infectivity control trial 2	N.A.	PBS	N.A	7	N.T.	N.T.	N.T.	7,375	
Infectivity control Essai 2	N.A.	3 g/l BSA	N.A	6,875	N.T.	N.T.	N.T.	7	
Infectivity control trial 2	N.A.	3 g/l BSA + 3 ml erythrocytes	N.A	6,375	N.T.	N.T.	N.T.	7	
Sensitivity of the cells	10^{-2}	N.A.	Non treated cells	N.T.	N.T.	N.T.	N.T.	7,125	
		N.A.	Treated cells	N.T.	N.T.	N.T.	N.T.	6,5	

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Tableau A3 — Raw results for the SEPTALKAN product in dirty conditions (3 g/l BSA + 3 ml/l sheep erythrocytes), against adenovirus

Trial 1

	Concentration	Organic soil load	Exposure time	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	100% en BSA+ erythrocytes	3 g/l BSA + 3 ml erythrocytes	5 min	4444	4444	0000	0000	0000	0000	0000	0000	0000	0000
				4444	4444	0000	0000	0000	0000	0000	0000	0000	0000
			viral	4444	4444	4444	4444	2223	3333	0001	0000	0000	0000
			control	4444	4444	4444	4444	3333	3333	1111	0000	0000	0000
	100% en BSA	3 g/l BSA	5 min	4444	0011	0000	0000	0000	0000	0000	0000	0000	0000
				4444	1112	0000	0000	0000	0000	0000	0000	0000	0000
			viral	4444	4444	4444	3333	3333	1111	1011	0000	0000	0000
			control	4444	4444	4444	2333	2221	1221	2010	0000	0000	0000
SEPTALKAN cytotoxicity	100,00%	3 g/l BSA + 3 ml erythrocytes	N.A.	3444	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
				4444	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
Formaldehyde	1,40%	PBS	5	4444	4444	4444	3333	1111	1111	0011	0000	0000	0000
				4444	4444	4444	3333	2213	1110	0011	0000	0000	0000
			15	4444	4444	4444	3333	2222	1111	0000	0000	0000	0000
				4444	4444	4444	3222	2221	1111	0000	0000	0000	0000
			30	4444	4444	3233	1222	1111	0011	0000	0000	0000	0000
				4444	4444	3333	2222	1111	0000	0000	0000	0000	0000
Formaldehyde (cytotoxicity)	1,40%	PBS	N.A.	4444	1111	1110	0000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
				4444	2333	0000	0000						
Infectivity control	N.A.	PBS	0	4444	4444	4444	3333	3222	1111	1000	0000	0000	0000
				4444	4444	4444	3333	2223	2112	1000	0000	0000	0000
			60	4444	4444	4444	4444	3333	1111	1110	0000	0000	0000
				4444	4444	4444	4444	1211	11111	1100	0000	0000	0000
Infectivity control	N.A.	3 g/l BSA	0	4444	4444	4444	4444	3223	3333	1000	0000	0000	0000
				4444	4444	4444	4444	2333	3333	0011	0000	0000	0000
			60	4444	4444	4444	4444	3332	2222	1110	0000	0000	0000
				4444	4444	4444	4444	3333	2222	0010	0000	0000	0000
Infectivity control	N.A.	3 g/l BSA + 3 ml erythrocytes	0	4444	4444	4444	4444	2222	1121	0000	0000	0000	0000
				4444	4444	4444	4444	2232	1111	1100	0000	0000	0000
			60	4444	4444	4444	4444	3333	1111	0011	0000	0000	0000
				4444	4444	4444	4444	3223	1111	1000	0000	0000	0000

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Trial 2

	Concentration	Organic soil load	Exposure time	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	100% en BSA+ erythrocytes	3 g/l BSA + 3 ml erythrocytes	5 min	4444	3333	0000	0000	0000	0000	0000	0000	0000	0000
				4444	3444	1100	0000	0000	0000	0000	0000	0000	0000
			viral	4444	4444	4444	4444	3334	1111	0111	0000	0000	0000
			control	4444	4444	4444	4444	3333	1211	1011	0000	0000	0000
	100% en BSA	3 g/l BSA	5 min	4444	4444	0000	0000	0000	0000	0000	0000	0000	0000
				4444	3333	0000	0000	0000	0000	0000	0000	0000	0000
			viral	4444	4444	4444	4444	3333	1111	1111	0000	0000	0000
			control	4444	4444	4444	4444	3333	2222	0001	0000	0000	0000
SEPTALKAN cytotoxicity	100,00%	3 g/l BSA + 3 ml erythrocytes	N.A.	4444	1000	0000	0000	0000	0000	0000	0000	0000	N.T.
				3334	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
Formaldehyde	1,40%	PBS	5	4444	4444	4444	4444	4444	2222	1110	0000	0000	0000
				4444	4444	4444	4444	4444	2122	0000	0000	0000	0000
			15	4444	4444	4444	3333	2222	1111	1000	0000	0000	0000
				4444	4444	4444	3333	1111	1111	0000	0000	0000	0000
			30	4444	4444	4444	3333	3333	1111	0000	0000	0000	0000
				4444	4444	4444	3333	3333	0011	0000	0000	0000	0000
Formaldehyde (cytotoxicity)	1,40%	PBS	N.A.	4444	1112	0000	0000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
				4444	2222	1100	0000						
Infectivity control	N.A.	PBS	0	4444	4444	4444	3333	2222	1111	1110	0000	0000	0000
				4444	4444	4444	3333	2223	1111	1000	0000	0000	0000
			60	4444	4444	4444	4444	3333	1111	1110	0000	0000	0000
				4444	4444	4444	4444	3333	1111	1111	0000	0000	0000
Infectivity control	N.A.	3 g/l BSA	0	4444	4444	4444	4444	3223	2222	1000	0000	0000	0000
				4444	4444	4444	4444	2333	2212	0011	0000	0000	0000
			60	4444	4444	4444	4444	3334	2222	1111	0000	0000	0000
				4444	4444	4444	4444	3333	2222	0000	0000	0000	0000
Infectivity control	N.A.	3 g/l BSA + 3 ml erythrocytes	0	4444	4444	4444	4444	3322	1111	0000	0000	0000	0000
				4444	4444	4444	4444	2232	1101	0000	0000	0000	0000
			60	4444	4444	4444	4444	3333	1111	0011	0000	0000	0000
				4444	4444	4444	4444	3333	1111	1100	0000	0000	0000

Validations:

Product	Concentration	Organic soil load	Cytotoxicity level	Lg $TCID_{50}$		Reduction > 4 lg	
				0	5 min		
SEPTALKAN	90,00%	3 g/l BSA + 3 ml erythrocytes	1,5	7	3,5	5 min R = 3,5	INACTIVE
SEPTALKAN	50,00%	3 g/l BSA + 3 ml erythrocytes	1,5	7,125	6,875	5 min R = 0,25	INACTIVE

Cells sensitivity:

Product	dilution	Organic soil load		Dilutions								
				-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	10^{-2}	3 g/l BSA + 3 ml erythrocytes	untreated cells	4444	4444	3333	2321	1111	1111	0000	0000	0000
				4444	4444	3333	2222	1122	1000	0000	0000	0000
			Treated cells	4444	4444	3333	2211	1111	0000	0000	0000	0000
				4444	4444	3333	2221	1111	0000	0000	0000	0000

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

TEST REPORT

VIRUCIDAL ACTIVITY OF THE SEPTALKAN PRODUCT AGAINST HIV

For : **ALKAPHARM**
PARC BIOCITECH
102 AVENUE GASTON ROUSSEL
93230 ROMAINVILLE

Date of request: 07/20/2012

Study number: n°201V11-2012

VIRUCIDAL ACTIVITY:

According to the NF EN 14476+A1 (January 2007) standard – chemical antiseptics and disinfectants – virucidal quantitative suspension tests for chemical disinfectants and antiseptics used in human medicine.

Tests using the SEPTALKAN product, on the HIV.

Test report included 13 pages.

Study completion date: 10/25/2012

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

Professeur Georges HERBEIN
Professor of Medicine – MD-PhD in virology
Scientific consultant

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

SUMMARY

1. PRODUCT IDENTITY
2. EXPERIMENTAL CONDITIONS
3. VALIDATIONS
4. VIRUCIDAL ASSAYS
5. CONCLUSIONS
6. TECHNICAL APPENDIX

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

1 PERFORMING LABORATORY

APEX BIOSOLUTIONS
18, rue Alain SAVARY
25000 BESANÇON
FRANCE

2 PRODUCT IDENTITY

SEPTALKAN

Batch n°: 2463F2563A

Expiration date: non communicated

Manufacturer: ALKAPHARM

Manufacturing date: non communicated

Storage conditions: as recommended by the manufacturer

Active substances: didecyl dimethyl ammonium chloride ≤2,5%, alkylbenzyl dimethyl ammonium chloride ≤9,5%

Appearance: clear.

Product diluent recommended by the manufacturer for use: none, ready-to-use product

Date of delivery of the product: 06/26/2012

Date of tests: from 08/02/2012 to 09/24/2012

3 EXPERIMENTAL CONDITIONS

Temperature used during the assays: 20°C ± 1°C

Titration units: log $TCID_{50}$

Exposure time: 5 minute

Test concentration: 100 % - 70% - 30%

Diluent used for the product: distilled sterile water

Viral strain: HIV-1 pNL4-3Luc strain, grown on 1G5 cells, at 37°C, under 5% CO2 atmosphere

Organic soil load: 3 g/l albumin bovine serum + 3 ml sheep erythrocytes (dirty conditions)

Product stability: good

Stop solution: cold shock

Viral titer:

Viral titers are expressed in log $TCID_{50}$ per ml, according to the SPEARMAN-KÄRBER method. Titer of HIV suspension assay = 7,00 log $TCID_{50}$.

4 VALIDATION

a) Methodology

The 1G5 cells were exposed to the SEPTALKAN product and a weak cytotoxic effect was observed at the dilution 10^{-1}.

b) Sensibility assays

The HIV was titrated on cell cultures untreated with the SEPTALKAN product (indicator cell line), and titrated on cell cultures treated with the SEPTALKAN product.

	Viral titer (log $TCID_{50}$)		
Product dilution	Untreated cell cultures	Treated cell cultures	**Difference of viral titer (log $TCID_{50}$)**
SEPTALKAN 10^{-1}	7,000	6,25	**0,75**

According to the European standard EN 14476 + A1, the SEPTALKAN product used at the dilution of 10^{-1} does not have an effect on the HIV titration method (the difference between viral titers must be < 1,0 log).

c) Validations of the cold shock method on the virucidal effect of the SEPTALKAN product:

Product concentration	**Organic soil load**	**Viral titer (log $TCID_{50}$)**	**Difference with the viral suspension assay**
SEPTALKAN 100%	3 g/l BSA	7,000	0,0
	3 g/l BSA + 3 ml sheep erythrocytes	6,500	0,5

The method is validated for a difference in viral titers ≤ 0,5 log.

c) Inactivation assays of the virus with a control solution (0,7 % formaldehyde)

	Viral titer (log $TCID_{50}$)	**Viral titer reduction (log $TCID_{50}$)**
Viral suspension control	7,000	
With 0,7%formaldehyde		
Inactivation exposure 5 min	5,125	1,875
Inactivation exposure 15 min	5,000	2,000
Inactivation exposure 30 min	4,625	**2,375**

The viral titer reduction (difference between the titers of the viral suspension treated with 0,7% formaldehyde and the viral suspension control) must be between -0,5 and -2,5 log after 30 min of exposure. In the assays, the viral titer reduction is 2,375 log, indicated that the HIV is inactivated after 30 min of exposure with the 0,7% formaldehyde solution.

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

5 VIRUCIDAL ASSAYS

Trial 1

The viral test suspension was titrated at **6,5 log $TCID_{50}$** prior the assays (3 g/l BSA) and **6,75 log $TCID_{50}$** prior the assays (3 g/l BSA + 3 ml sheep erythrocytes).

PRODUCT	ORGANIC SOIL LOAD	Concentration (v/v)	Time exposure	Temperature	Viral titer (log $TCID_{50}$)	Viral titer reduction
SEPTALKAN	3 g/l BSA + 3 ml sheep erythrocytes	100 %	5 min	20°C	2,375	**4,125**
	3 g/l BSA	100 %	5 min	20°C	2,125	**4,625**

Trial 2

The viral test suspension was titrated at **6,5 log $TCID_{50}$** prior the assays (3 g/l BSA) and **6,75 log $TCID_{50}$** prior the assays (3 g/l BSA + 3 ml sheep erythrocytes).

PRODUCT	ORGANIC SOIL LOAD	Concentration (v/v)	Time exposure	Temperature	Viral titer (log $TCID_{50}$)	Viral titer reduction
SEPTALKAN	3 g/l BSA + 3 ml sheep erythrocytes	100%	5 min	20°C	2,500	**4,000**
	3 g/l BSA	100%	5 min	20°C	2,500	**4,250**

The concentrations of the product demonstrated a virucidal activity on the virus tested if the viral titer reduction is ≥ 4,0 log.

The assays were validated as required by the European standard EN 14476+A1:

- The viral titers of the suspension tests were sufficient in order to observe a reduction of 4 log after time exposure with the product. The viral titer of the HIV was 7,00 log $TCID_{50}$.
- The virus was inactivated with the control solution of 0,7% formaldehyde after 30 min of exposure : the reduction observed was of 2,375 log for the HIV.

- The product does not have a cytotoxic effect on the cells. The dirty conditions do not affect the infectious capacity of the HIV.

- The SEPTALKAN product does not affect the infectious capacity of the virus: the differences in viral titers between the virus inoculated on cells and the virus inoculated on the cells treated with the SEPTALKAN product was ≤ 1,0 log (0,75 log).

- The difference between the viral suspension assay titer and the viral suspension titer in the cold shock validation was ≤ 0,5 log.

6 CONCLUSIONS

The assays performed with the SEPTALKAN product (batch#n°2463F2563A) demonstrated that:

- the **SEPTALKAN product** **demonstrated a virucidal activity on the HIV from the concentration 100%,** as required by the European standard EN 14476+A1, following a **5 minutes** exposure period, at 20°C, in dirty conditions

TECHNICAL APPENDIX 1

Cell line : 1G5 cells, ATCC, USA

Viral strain: VIH-1 (pNL4-3Luc), NIH, Bethesda, MA, USA

Buffers and media:

- Buffer PBS: sodium chloride, Dominique DUTSCHER, ref. 19032391, batch # n°836751; sodium phosphate dibasic, Sigma Aldrich, ref. S5136, batch # n° BCBC7067V; sodium phosphate monobasic, Sigma Aldrich, ref. S5011, batch # n° 1019K01021V
- DMEM media, Sigma Aldrich, ref. D5796, batch n° RNBB9336
- Fetal calf sera, Sigma Aldrich, ref. F7524, batch n° 098K3397
- MEM media, Sigma Aldrich, ref. 0268, batch n° 040M8301

Reagents:

- Bovine albumin serum (powder), Sigma Aldrich, ref. 05479, batch STBB7838V
- Sheep erythrocytes, Oxoïd, ref. SR 0051C, batch n° 48972000

Inactivation solution:

- formaldehyde, Sigma Aldrich, ref. F-1635, batch n° BCBB3510

TECHNICAL APPENDIX 2

Table A1 - Titration of VIH by cytopathic effect, with the Spaerman-Kärber method :

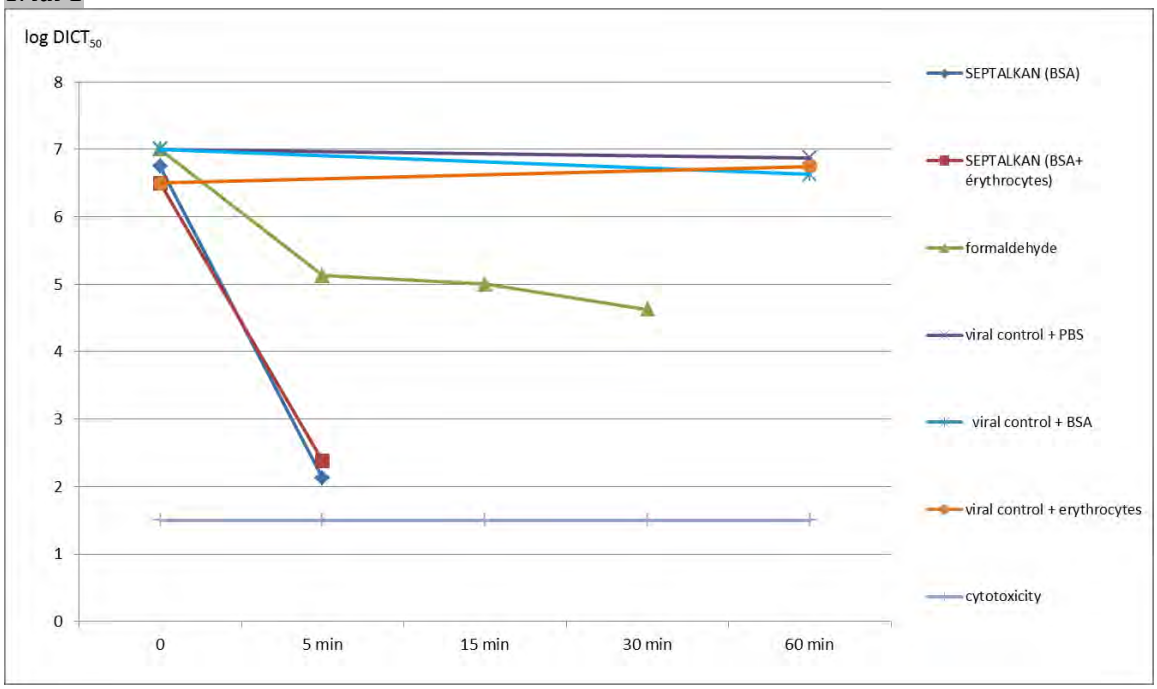
log $TCID_{50}$ = 7,00

Dilution (- log)	Results	% of positive results
-3	44444444	100
-4	44444444	100
-5	33334444	100
-6	22233112	100
-7	11110000	50
-8	00000000	0
-9	00000000	0
-10	00000000	0
Sum of the % of the positive wells		450

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Figure 1 – chart of the results :

Trial 1



Trial 2

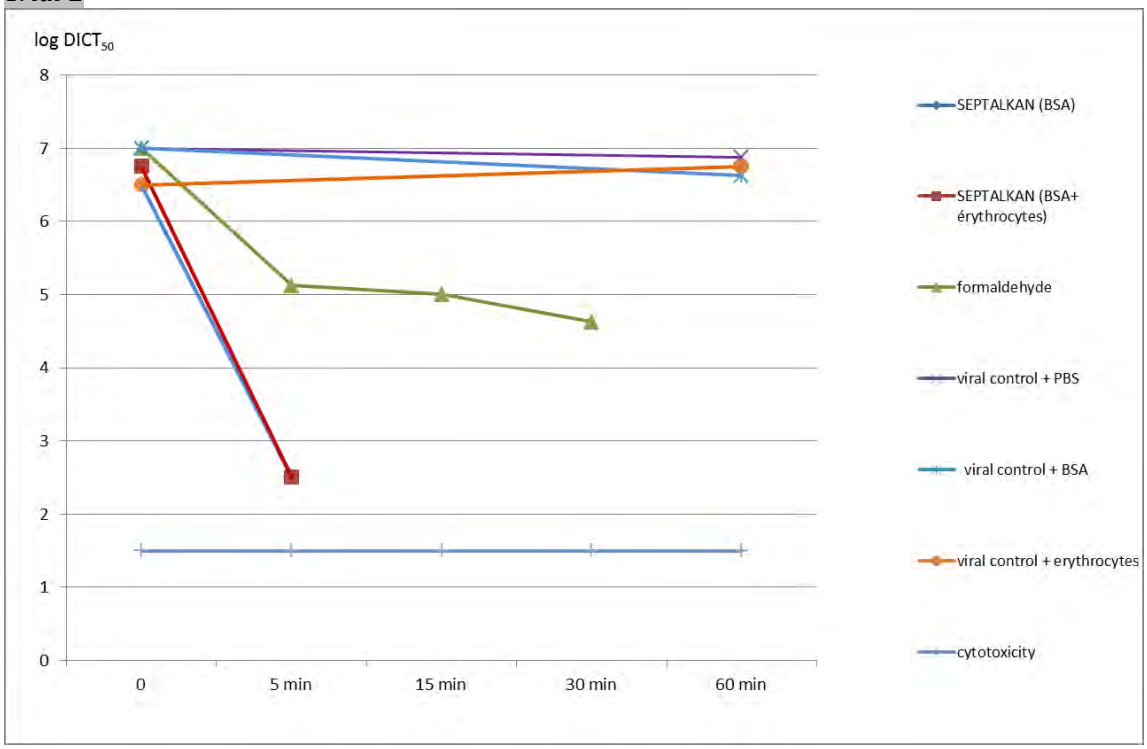


Table A2 —Results for the SEPTALKAN product against the HIV in dirty conditions (3 g/l BSA + 3 ml erythrocytes)

Product	Concentration	Organic soil load	Cytotoxicity level	Lg $TCID_{50}$					Reduction
				0	5 min	15 min	30 min	60 min	
SEPTALKAN trial 1	100,00%	3 g/l BSA + 3 ml erythrocytes	1,5	6,5	2,375	N.T.	N.T.	N.T.	5 min R = 4,125
		3 g/l BSA	1,5	6,75	2,125	N.T.	N.T.	N.T.	5 min R = 4,625
SEPTALKAN trial 2	100,00%	3 g/l BSA + 3 ml erythrocytes	1,5	6,5	2,5	N.T.	N.T.	N.T.	5 min R= 4
		3 g/l BSA	1,5	6,75	2,5	N.T.	N.T.	N.T.	5 min R= 4,25
Formaldéhyde	1,40%	PBS	2	7	5,125	5	4,625	N.T.	
Control of infectivity trial 1	N.A.	PBS	N.A.	7	N.T.	N.T.	N.T.	6,875	
Control of infectivity trial 1	N.A.	3 g/l BSA	N.A	7	N.T.	N.T.	N.T.	6,625	
Control of infectivity trial 1	N.A.	3 g/l BSA + 3 ml erythrocytes	N.A	6,5	N.T.	N.T.	N.T.	6,75	
Control of infectivity trial 2	N.A.	PBS	N.A	7	N.T.	N.T.	N.T.	6,875	
Control of infectivity trial 2	N.A.	3 g/l BSA	N.A	7	N.T.	N.T.	N.T.	6,625	
Control of infectivity trial 2	N.A.	3 g/l BSA + 3 ml erythrocytes	N.A	6,5	N.T.	N.T.	N.T.	6,75	
Cells sensitivity to the virus	SEPTALKAN	N.A.	Untreated cells	N.T.	N.T.	N.T.	N.T.	7	
	10^{-1}	N.A.	Treated cells	N.T.	N.T.	N.T.	N.T.	6,25	

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Table A3 — Raw results for the SEPTALKAN product with 3 g/l BSA + 3 ml/l sheep erythrocytes against HIV

Trial 1

	Concentration	Organic soil load	Exposure time	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	100,00%	3 g/l BSA + 3 ml erythrocytes	5 min	4444	3323	0000	0000	0000	0000	0000	0000	0000	0000
				4444	3303	0000	0000	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	3333	3333	1112	1010	0000	0000	0000
				4444	4444	4444	3333	3333	1100	0000	0000	0000	0000
	100,00%	3 g/l BSA	5 min	4444	1211	0000	0000	0000	0000	0000	0000	0000	0000
				4444	1000	0000	0000	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	3322	1010	1100	0000	0000	0000
				4444	4444	4444	4444	3333	1111	1100	0000	0000	0000
SEPTALKAN cytotoxicity	100,00%	3 g/l BSA + 3 ml érythrocytes	N.A.	1133	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
				4111	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
Formaldehyde	1,40%	PBS	5	4444	4444	4444	3333	1011	0000	0000	0000	0000	N.T.
				4444	4444	4444	3331	1100	0000	0000	0000	0000	
			15	4444	4444	4444	2111	1211	0000	0000	0000	0000	N.T.
				4444	4444	4444	1111	0000	0000	0000	0000	0000	
			30	4444	4444	4444	2133	1000	0000	0000	0000	0000	N.T.
				4444	4444	4444	3333	0000	0000	0000	0000	0000	
Formaldehyde (cytotoxicity)	1,40%	PBS	N.A.	4444	1200	0000	0000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
				4444	0011	0000	0000						
Control of infectivity	N.A.	PBS	0	4444	4444	4444	4444	3333	3331	1120	0000	0000	0000
				4444	4444	4444	4444	3333	3333	1000	0000	0000	0000
			60	4444	4444	4444	4444	3333	1111	1101	0000	0000	0000
				4444	4444	4444	4444	1333	1111	0000	0000	0000	0000
Control of infectivity	N.A.	3 g/l BSA	0	4444	4444	4444	4444	3333	2111	1111	0000	0000	0000
				4444	4444	4444	4444	3333	1201	0100	0000	0000	0000
			60	4444	4444	4444	4444	3333	2222	1000	0000	0000	0000
				4444	4444	4444	4444	3333	1111	0000	0000	0000	0000
Control of infectivity	N.A.	3 g/l BSA + 3 ml erythrocytes	0	4444	4444	4444	4444	3332	1111	0000	0000	0000	0000
				4444	4444	4444	4444	2222	1111	0000	0000	0000	0000
			60	4444	4444	3333	3333	2222	1111	0011	0000	0000	0000
				4444	4444	3333	3333	2222	1111	0000	0000	0000	0000

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Trial 2

	Concentration	Organic soil load	Exposure time	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	100,00%	3 g/l BSA + 3 ml erythrocytes	5 min	4444	1120	1000	0000	0000	0000	0000	0000	0000	0000
				4444	1111	0000	0000	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	3333	3233	1112	0001	0000	0000	0000
				4444	4444	4444	3333	2222	0011	1000	0000	0000	0000
	100,00%	3 g/l BSA	5 min	4444	3344	0000	0000	0000	0000	0000	0000	0000	0000
				4444	4444	0000	0000	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	3333	1111	1110	0000	0000	0000
				4444	4444	4444	4444	3333	1110	0000	0000	0000	0000
SEPTALKAN cytotoxicity	100,00%	3 g/l BSA + 3 ml érythrocytes	N.A.	3311	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
				1111	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
Formaldehyde	1,40%	PBS	5	4444	4444	4444	3333	1011	0000	0000	0000	0000	N.T.
				4444	4444	4444	3331	1100	0000	0000	0000	0000	
			15	4444	4444	4444	2111	1211	0000	0000	0000	0000	N.T.
				4444	4444	4444	1111	0000	0000	0000	0000	0000	
			30	4444	4444	4444	2133	1000	0000	0000	0000	0000	N.T.
				4444	4444	4444	3333	0000	0000	0000	0000	0000	
Formaldehyde (cytotoxicity)	1,40%	PBS	N.A.	4444	1200	0000	0000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
				4444	0011	0000	0000						
Control of infectivity	N.A.	PBS	0	4444	4444	4444	4444	3333	3331	1120	0000	0000	0000
				4444	4444	4444	4444	3333	3333	1000	0000	0000	0000
			60	4444	4444	4444	4444	3333	1111	1101	0000	0000	0000
				4444	4444	4444	4444	1333	1111	0000	0000	0000	0000
Control of infectivity	N.A.	3 g/l BSA	0	4444	4444	4444	4444	3333	2111	1111	0000	0000	0000
				4444	4444	4444	4444	3333	1201	0100	0000	0000	0000
			60	4444	4444	4444	4444	3333	2222	1000	0000	0000	0000
				4444	4444	4444	4444	3333	1111	0000	0000	0000	0000
Control of infectivity	N.A.	3 g/l BSA + 3 ml erythrocytes	0	4444	4444	4444	4444	3332	1111	0000	0000	0000	0000
				4444	4444	4444	4444	2222	1111	0000	0000	0000	0000
			60	4444	4444	3333	3333	2222	1111	0011	0000	0000	0000
				4444	4444	3333	3333	2222	1111	0000	0000	0000	0000

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Validations:

Product	Concentration	Organic soil load	Time exposure	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	80,00%	3 g/l BSA + 3 ml erythrocytes	5 min	4444	4444	2112	0000	0000	0000	0000	0000	0000	0000
				4444	4444	1000	0000	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	3333	2220	1100	0000	0000	0000
				4444	4444	4444	4444	3333	2220	1110	0000	0000	0000
	10,00%	3 g/l BSA + 3 ml erythrocytes	5 min	4444	4444	4344	2222	2222	1101	0000	0000	0000	0000
				4444	4444	4433	2222	2222	1000	0000	0000	0000	0000
			Viral control	4444	4444	4444	3334	3333	2221	1010	0000	0000	0000
				4444	4444	4444	4444	3333	2222	1100	0000	0000	0000

Product	Concentration	Organic soil load	cytotoxicity	Lg $DICT_{50}$		Reduction > 4 lg	
				0	5 min		
SEPTALKAN	80,00%	3 g/l BSA + 3 ml erythrocytes	1,5	6,875	3,125	5 min R = 3,75	inactive
	10,00%	3 g/l BSA + 3 ml erythrocytes	1,5	7	6	5 min R = 1,000	inactive

Sensitivity of cells to the HIV:

Product	dilution	Organic soil load		Dilutions								
				-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	10^{-1}	3 g/l BSA + 3 ml erythrocytes	Untreated cells	4444	4444	3333	2333	1112	1101	0000	0000	0000
				4444	4444	3333	3333	1110	1100	0000	0000	0000
			Treated Cells	4444	4444	3333	1122	1100	0000	0000	0000	0000
				4444	4444	3333	1111	1111	0000	0000	0000	0000

TEST REPORT

VIRUCIDAL ACTIVITY OF THE SEPTALKAN PRODUCT AGAINST BVD VIRUS

For: **ALKAPHARM**
PARC BIOCITECH
102 AVENUE GASTON ROUSSEL
93230 ROMAINVILLE

Date of request: 07/20/2012

Study number: n°201V13-2012

VIRUCIDAL ACTIVITY:

According to the NF EN 14476+A1 (January 2007) standard – chemical antiseptics and disinfectants – virucidal quantitative suspension tests for chemical disinfectants and antiseptics used in human medicine.

Tests using the SEPTALKAN product, on the BVD (Viral Bovine Diarrhea), virus used as a model for biocidal testing on hepatitis C virus.

This test report included 13 pages.

Study completion date: 10/28/2012

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

Professeur Georges HERBEIN
Professor of Medicine – MD-PhD in virology
Scientific consultant

SUMMARY

1. PERFORMING LABORATORY
2. PRODUCT IDENTITY
3. EXPERIMENTAL CONDITIONS
4. VALIDATIONS
5. VIRUCIDAL ASSAYS
6. CONCLUSIONS
7. TECHNICAL APPENDIX

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

1 PERFORMING LABORATORY

APEX BIOSOLUTIONS
18, rue Alain SAVARY
25000 BESANÇON
FRANCE

2 PRODUCT IDENTITY

SEPTALKAN

Batch n°: 2463F2563A

Expiration date: non communicated

Manufacturer: ALKAPHARM

Manufacturing date: non communicated

Storage conditions: as recommended by the manufacturer

Active substances: didecyl dimethyl ammonium chloride ≤2,5%, alkylbenzyl dimethyl ammonium chloride ≤9,5%

Appearance: clear.

Product diluent recommended by the manufacturer for use: none, ready-to-use product

Date of delivery of the product: 06/26/2012

Date of tests: from 08/02/2012 to 09/24/2012

3 EXPERIMENTAL CONDITIONS

Temperature used during the assays: 20°C ± 1°C

Titration units: log $TCID_{50}$

Exposure time: 5 minutes

Test concentration: 100 %

Diluent used for the product: distilled sterile water

Viral strain: BVDV strain, grown on BT cells, at 37°C, under 5% CO2 atmosphere

Organic soil load: 3 g/l albumin bovine serum + 3 ml sheep erythrocytes (dirty conditions)

Product stability: good

Stop solution: cold shock

Viral titer:

Viral titers are expressed in log $TCID_{50}$ per ml, according to the SPEARMAN-KÄRBER method. Titer of BVDV suspension assay = 7,375 log $TCID_{50}$.

4 VALIDATION

a) Methodology

The BT cells were exposed to the SEPTALKAN product and a weak cytotoxic effect was observed at the dilution 10^{-1}.

b) Sensibility assays

The BVDV was titrated on cell cultures untreated with the SEPTALKAN product (indicator cell line), and titrated on cell cultures treated with the SEPTALKAN product.

	Viral titer (log $TCID_{50}$)		
Product dilution	Untreated cell cultures	treated cell cultures	**Difference of viral titer (log $TCID_{50}$)**
SEPTALKAN 10^{-2}	7,5	6,75	**0,75**

According to the European standard EN 14476 + A1, the SEPTALKAN product used at the dilution of 10^{-1} does not have an effect on the BVDV titration method (the difference between viral titers must be < 1,0 log).

c) Validations of the cold shock method on the virucidal effect of the SEPTALKAN product:

Product concentration	**Organic soil load**	**Viral titer (log $TCID_{50}$)**	**Difference with the viral suspension assay**
SEPTALKAN 100 %	3 g/l BSA	trial 1 : 7,25	0,125
		trial 2 : 7,5	0,125
SEPTALKAN 100 %	3 g/l BSA + 3 ml sheep erythrocytes	trial 1 : 7,5	0125
		trial 2 : 7,375	0,0

The method is validated for a difference in viral titers ≤ 0,5 log.

d) Inactivation assays of the virus with a control solution (1,4 % formaldehyde)

	Viral titer (log $TCID_{50}$)	**Viral titer reduction (log $TCID_{50}$)**
Viral suspension control	7,375	
With 1,4%formaldehyde		
Inactivation exposure 5 min	7,375	0,000
Inactivation exposure 15 min	6,750	0,625
Inactivation exposure 30 min	6,500	**0,875**

The viral titer reduction (difference between the titers of the viral suspension treated with formaldehyde and the viral suspension control) must be between -0,5 and -2,5 log after 30 min of exposure. In the assays, the viral titer reduction is 0,875 log, indicated that the BVDV is inactivated after 30 min of exposure with the formaldehyde solution.

5 VIRUCIDAL ASSAYS

Trial 1

The viral test suspension was titrated at **7,25 log DICT$_{50}$** prior the assays(3 g/l BSA) and **7,5 log DICT$_{50}$** (3 g/l BSA + 3 ml sheep erythrocytes)

PRODUCT	ORGANIC SOIL LOAD	Concentration (v/v)	Time exposure	Temperature	Viral titer (log TCID$_{50}$)	Viral titer reduction
SEPTALKAN	3 g/l BSA + 3 ml sheep erythrocytes	100 %	5 min	20°C	3,000	**4,5**
	3 g/l BSA	100 %	5 min	20°C	2,375	**4,875**

Trial 2

The viral test suspension was titrated at **7,5 log TCID$_{50}$** prior the assays (3 g/l BSA) and **7,375 log TCID$_{50}$** prior the assays (3 g/l BSA + 3 ml sheep erythrocytes).

PRODUCT	ORGANIC SOIL LOAD	Concentration (v/v)	Time exposure	Temperature	Viral titer (log TCID$_{50}$)	Viral titer reduction
SEPTALKAN	3 g/l BSA + 3 ml sheep erythrocytes	100 %	5 min	20°C	2,75	**4,625**
	3 g/l BSA	100 %	5 min	20°C	2,25	**5,25**

The concentrations of the product demonstrated a virucidal activity on the virus tested if the viral titer reduction is ≥ 4,0 log.

The assays were validated as required by the European standard EN 14476+A1:

- The viral titers of the suspension tests were sufficient in order to observe a reduction of 4 log after time exposure with the product. The viral titer of the BVDV was 7,375 log $TCID_{50}$.
- The virus was inactivated with the control solution of formaldehyde after 30 min of exposure : the reduction observed was of 0,875 log for the BVDV.
- The product does not have a cytotoxic effect on the cells. The dirty conditions do not affect the infectious capacity of the BVDV.
- The SEPTALKAN product does not affect the infectious capacity of the virus: the differences in viral titers between the virus inoculated on cells and the virus inoculated on the cells treated with the SEPTALKAN product was ≤ 1,0 log (0,75 log).
- The difference between the viral suspension assay titer and the viral suspension titer in the cold shock validation was ≤ 0,5 log.

6 CONCLUSIONS

The assays performed with the SEPTALKAN product (batch#n°2463F2563A) demonstrated that:

- the **SEPTALKAN product** **demonstrated a virucidal activity on the BVDV from the concentration 100%,** as required by the European standard EN 14476+A1, following a **5 minutes** exposure period, at 20°C, in dirty conditions
- **as the BVD virus is employed as a viral model for efficacy testing of biocides for the hepatitis C virus (HCV), then the SEPTALKAN product has a virucidal activity on HCV in the test conditions (5 min, 20°C, dirty conditions), too.**

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

7 TECHNICAL APPENDIX

Cell line: BT cells (ATCC ref. CRL-1390, batch n°58078520)

Viral strain: virus BVD (ATCC ref. VR-534, batch n°10W)

Buffers and reagents:

- Buffer PBS: sodium chloride, Dominique DUTSCHER, ref. 19032391, batch # n°836751; sodium phosphate dibasic, Sigma Aldrich, ref. S5136, batch # n° BCBC7067V; sodium phosphate monobasic, Sigma Aldrich, ref. S5011, batch # n° 1019K01021V
- DMEM media, Sigma Aldrich, ref. D5796, batch n° RNBB9336
- Fetal calf sera, Sigma Aldrich, ref. F7524, batch n° 098K3397
- MEM media, Sigma Aldrich, ref. 0268, batch n° 040M8301

Reagents:

- Bovine albumin serum (powder), Sigma Aldrich, ref. 05479, batch STBB7838V
- Sheep erythrocytes, Oxoïd, ref. SR 0051E, batch n° 4234000

Inactivation solution:

- formaldehyde, Sigma Aldrich, ref. F-1635, batch n° BCBB3510

Table A1 - Titration of BVDV by cytopathic effect, with the Spaerman-Kärber method:

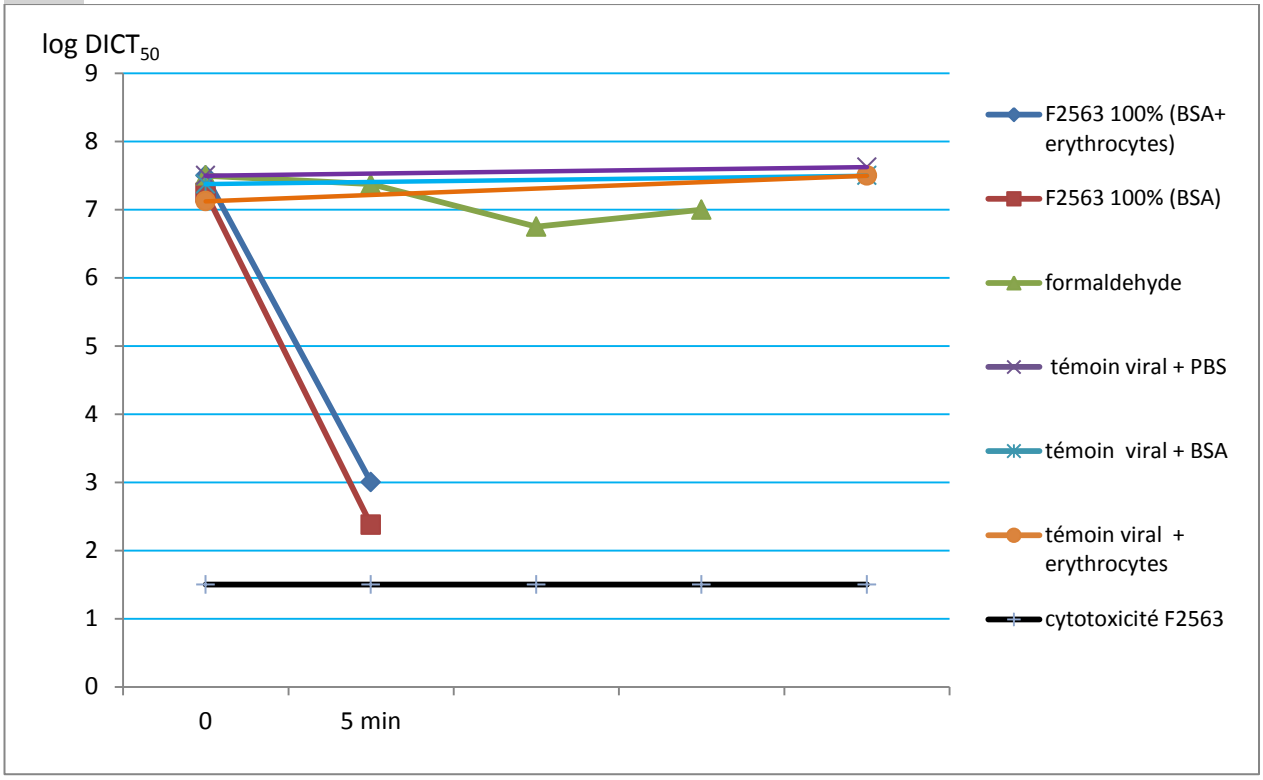
log $TCID_{50}$ = 7,375

Dilution (- log)	Results	% of positive results
-3	44444444	100
-4	44444444	100
-5	33323122	100
-6	22212221	100
-7	01221111	87,5
-8	00000000	0
-9	00000000	0
-10	00000000	0
Sum of the % of the positive wells		487,5

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Figure 1 – chart of the results:

Trial 1



Trial 2

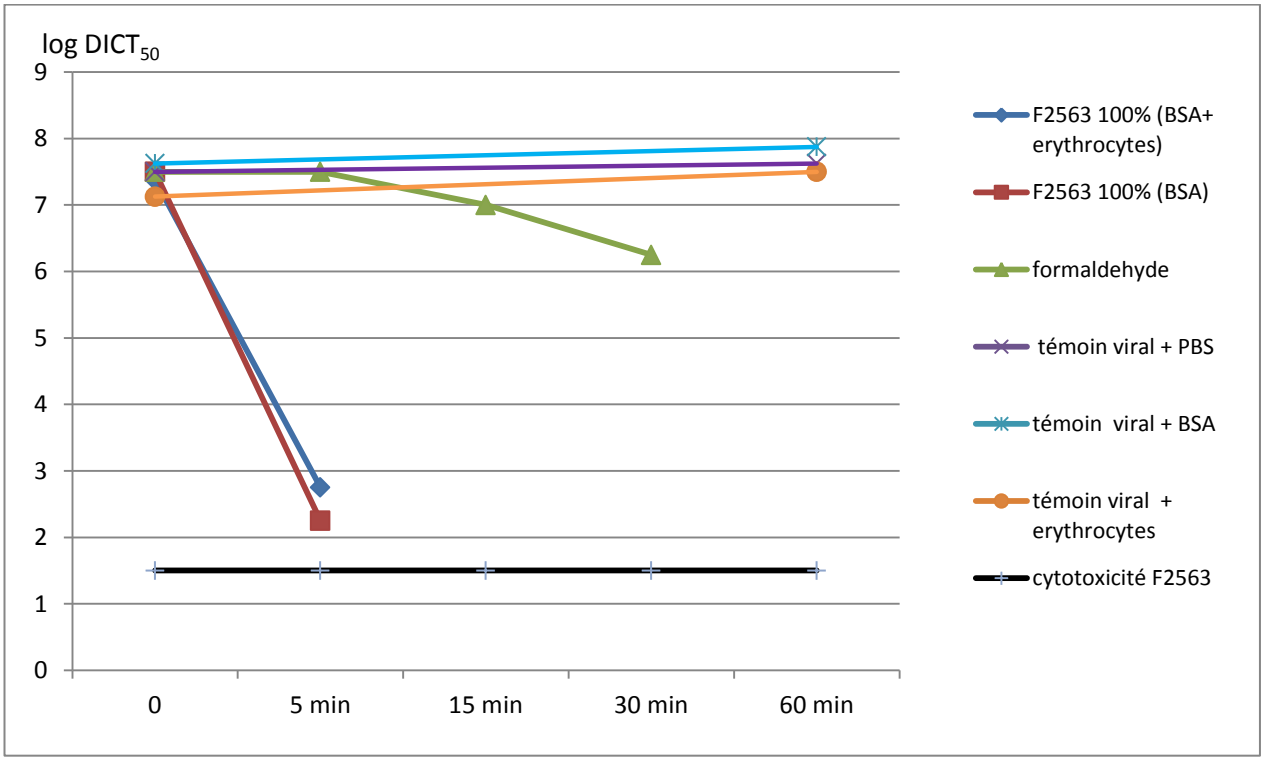


Table A2 — Results for the SEPTALKAN product against the BVDV in dirty conditions (3 g/l BSA + 3 ml erythrocytes)

Product	Concentration	Organic soil load	Cytotoxicity level	Lg $DICT_{50}$					Reduction
				0	5 min	15 min	30 min	60 min	
SEPTALKAN trial 1	100,00%	3 g/l BSA + 3 ml erythrocytes	1,5	7,5	3	N.T.	N.T.	N.T.	5 min R = 4,5
		3 g/l BSA	1,5	7,25	2,375	N.T.	N.T.	N.T.	5 min R = 4,875
SEPTALKAN trial 2	100,00%	3 g/l BSA + 3 ml erythrocytes	1,5	7,375	2,75	N.T.	N.T.	N.T.	5 min R = 4,625
		3 g/l BSA	1,5	7,5	2,25	N.T.	N.T.	N.T.	5 min R = 5,25
Formaldehyde Trial 1	1,40%	PBS	2,5	7,5	7,375	6,75	6,5	N.T.	
Formaldehyde Trial 2	1,40%	PBS	2,5	7,5	7,5	7	6,25	N.T.	
Control of infectivity Trial 1	N.A.	PBS	N.A.	7,5	N.T.	N.T.	N.T.	7,625	
Control of infectivity Trial 1	N.A.	3 g/l BSA	N.A	7,375	N.T.	N.T.	N.T.	7,5	
Control of infectivity Trial 1	N.A.	3 g/l BSA + 3 ml erythrocytes	N.A	7,125	N.T.	N.T.	N.T.	7,5	
Control of infectivity Trial 2	N.A.	PBS	N.A	7,5	N.T.	N.T.	N.T.	7,625	
Control of infectivity Trial 2	N.A.	3 g/l BSA	N.A	7,375	N.T.	N.T.	N.T.	7,5	
Control of infectivity Trial 2	N.A.	3 g/l BSA + 3 ml erythrocytes	N.A	7,125	N.T.	N.T.	N.T.	7,5	
Cells sensitivity to the virus	10^{-2}	N.A.	Untreated cells	N.T.	N.T.	N.T.	N.T.	7,5	
		N.A.	Treated cells	N.T.	N.T.	N.T.	N.T.	6,75	

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Table A3 — Raw results for the SEPTALKAN product with 3 g/l BSA + 3 ml/l sheep erythrocytes against BVDV

Trial 1

	Concentration	Organic soil load	Exposure time	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	100%	3 g/l BSA + 3 ml erythrocytes	5 min	4444	4444	0101	0000	0000	0000	0000	0000	0000	0000
				4444	4444	1100	0000	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	4444	3333	2112	0000	0000	0000
				4444	4444	4444	4444	3333	3333	1122	0000	0000	0000
	100%	3 g/l BSA	5 min	4444	4444	0000	0000	0000	0000	0000	0000	0000	0000
				4444	3404	0000	0000	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	3333	2222	1122	0000	0000	0000
				4444	4444	4444	4444	3333	1221	2010	0000	0000	0000
SEPTALKAN cytotoxicity	100,00%	3 g/l BSA + 3 ml erythrocytes	N.A.	2331	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
				2312	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
Formaldehyde	1,40%	PBS	5	4444	4444	4444	4444	4444	2332	1011	0000	0000	0000
				4444	4444	4444	4444	4444	3334	1111	0000	0000	0000
			15	4444	4444	4444	4444	3233	1111	0110	0000	0000	0000
				4444	4444	4444	4444	3333	1111	0000	0000	0000	0000
			30	4444	4444	4444	3434	3333	2222	0000	0000	0000	0000
				4444	4444	4444	3331	3333	2222	0000	0000	0000	0000
Formaldehyde (cytotoxicity)	1,40%	PBS	N.A.	4444	3343	0000	0000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
				4444	3343	0000	0000						
Control of infectivity	N.A.	PBS	0	4444	4444	4444	4444	4444	3333	2333	0000	0000	0000
				4444	4444	4444	4444	4444	4343	2222	0000	0000	0000
			60	4444	4444	4444	4444	4444	3333	3333	0001	0000	0000
				4444	4444	4444	4444	4444	3333	3333	0000	0000	0000
Control of infectivity	N.A.	3 g/l BSA	0	4444	4444	4444	4444	4444	3333	2013	0000	0000	0000
				4444	4444	4444	4444	4444	3333	3333	0000	0000	0000
			60	4444	4444	4444	4444	4444	4444	1211	0000	0000	0000
				4444	4444	4444	4444	4444	4444	2323	0000	0000	0000
Control of infectivity	N.A.	3 g/l BSA + 3 ml erythrocytes	0	4444	4444	4444	4444	4444	3332	0001	0000	0000	0000
				4444	4444	4444	4444	4444	2222	1122	0000	0000	0000
			60	4444	4444	4444	4444	4444	4444	3223	0000	0000	0000
				4444	4444	4444	4444	4444	4444	3333	0000	0000	0000

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Trial 2

	Concentration	Organic soil load	Exposure time	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	100%	3 g/l BSA + 3 ml erythrocytes	5 min	4444	4444	0100	0000	0000	0000	0000	0000	0000	0000
				4444	4444	0001	0000	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	4444	2222	1111	0000	0000	0000
				4444	4444	4444	4444	4444	1211	1011	0000	0000	0000
	100%	3 g/l BSA	5 min	4444	4444	0000	0000	0000	0000	0000	0000	0000	0000
				4444	0044	0000	0000	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	2222	2221	1111	1000	0000	0000
				4444	4444	4444	4444	2222	2222	1011	0000	0000	0000
SEPTALKAN cytotoxicity	100,00%	3 g/l BSA + 3 ml erythrocytes	N.A.	4444	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
				4444	0000	0000	0000	0000	0000	0000	0000	0000	N.T.
Formaldehyde	1,40%	PBS	5	4444	4444	4444	4444	3333	3333	1211	0000	0000	0000
				4444	4444	4444	4444	3434	2332	1111	0000	0000	0000
			15	4444	4444	4444	3333	2222	1111	1110	0000	0000	0000
				4444	4444	4444	4444	2322	1111	1000	0000	0000	0000
			30	4444	4444	4444	3434	3333	0012	0000	0000	0000	0000
				4444	4444	4444	3331	3333	1112	0000	0000	0000	0000
Formaldehyde (cytotoxicity)	1,40%	PBS	N.A.	4444	4444	0000	0000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
				4444	4433	0000	0000						
Control of infectivity	N.A.	PBS	0	4444	4444	4444	4444	4444	3333	2333	0000	0000	0000
				4444	4444	4444	4444	4444	4343	2222	0000	0000	0000
			60	4444	4444	4444	4444	4444	3333	3333	0001	0000	0000
				4444	4444	4444	4444	4444	3333	3333	0000	0000	0000
Control of infectivity	N.A.	3 g/l BSA	0	4444	4444	4444	4444	4444	3333	2013	0000	0000	0000
				4444	4444	4444	4444	4444	3333	3333	0000	0000	0000
			60	4444	4444	4444	4444	4444	4444	1211	0000	0000	0000
				4444	4444	4444	4444	4444	4444	2323	0000	0000	0000
Control of infectivity	N.A.	3 g/l BSA + 3 ml erythrocytes	0	4444	4444	4444	4444	4444	3332	0001	0000	0000	0000
				4444	4444	4444	4444	4444	2222	1122	0000	0000	0000
			60	4444	4444	4444	4444	4444	4444	3223	0000	0000	0000
				4444	4444	4444	4444	4444	4444	3333	0000	0000	0000

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Validations:

Product	Concentration	Organic soil load	cytotoxicity	Lg $DICT_{50}$		Reduction > 4 lg	
				0	5 min		
SEPTALKAN	90,00%	3 g/l BSA + 3 ml erythrocytes	1,5	7,5	3,75	5 min R = 3,75	INACTIVE
SEPTALKAN	50,00%	3 g/l BSA + 3 ml erythrocytes	1,5	7,5	7,375	5 min R = 0,125	INACTIVE

Sensitivity of cells to the BVDV:

Product	dilution	Organic soil load		Dilutions								
				-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	10^{-2}	3 g/l BSA + 3 ml érythrocytes	Untreated cells	4444	4444	4444	3333	2231	1111	0000	0000	0000
				4444	4444	4444	3443	1122	1111	0000	0000	0000
			Treated Cells	4444	4444	4444	3333	3333	1010	0000	0000	0000
				4444	4444	4444	3333	3333	000	0000	0000	0000

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

TEST REPORT

VIRUCIDAL ACTIVITY OF THE SEPTALKAN PRODUCT AGAINST THE PRV VIRUS ACCORDING TO THE EN 14476 STANDARD

For: **ALKAPHARM**
PARC BIOCITECH
102 AVENUE GASTON ROUSSEL
93230 ROMAINVILLE

Date of request: 07/20/2012

Study number: n°201V12-2012

VIRUCIDAL ACTIVITY:

According to the NF EN 14476+A1 (January 2007) standard – chemical antiseptics and disinfectants – virucidal quantitative suspension tests for chemical disinfectants and antiseptics used in human medicine.

Tests using the SEPTALKAN product, on the PRV (Pseudo Rabies Virus- suid herpes virus type 1), used as model for biocidal testing on hepatitis B virus.

This test report included 13 pages.

Study completion date: 10/03/2012

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

Professeur Georges HERBEIN
Professor of Medicine – MD-PhD in virology
Scientific consultant

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

SUMMARY

1. PERFORMING LABORATORY
2. PRODUCT IDENTITY
3. EXPERIMENTAL CONDITIONS
4. VALIDATIONS
5. VIRUCIDAL ASSAYS
6. CONCLUSIONS
7. TECHNICAL APPENDIX

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

1 PERFORMING LABORATORY

APEX BIOSOLUTIONS
18, rue Alain SAVARY
25000 BESANÇON
FRANCE

2 PRODUCT IDENTITY

SEPTALKAN

Batch n°: 2463F2563A

Expiration date: non communicated

Manufacturer: ALKAPHARM

Manufacturing date: non communicated

Storage conditions: as recommended by the manufacturer

Active substances: didecyl dimethyl ammonium chloride ≤2,5%, alkylbenzyl dimethyl ammonium chloride ≤9,5%

Appearance: clear.

Product diluent recommended by the manufacturer for use: none, ready-to-use product

Date of delivery of the product: 06/26/2012

Date of tests: from 08/02/2012 to 09/24/2012

3 EXPERIMENTAL CONDITIONS

Temperature used during the assays: 20°C ± 1°C

Titration units: log $TCID_{50}$

Exposure time: 5 minutes

Test concentration: 100 %

Diluent used for the product: distilled sterile water

Viral strain: PRV strain, grown on VERO cells, at 37°C, under 5% CO2 atmosphere

Organic soil load: 3 g/l albumin bovine serum + 3 ml sheep erythrocytes (dirty conditions)

Product stability: good

Stop solution: cold shock

Viral titer:

Viral titers are expressed in log $TCID_{50}$ per ml, according to the SPEARMAN-KÄRBER method. Titer of PRV suspension assay = 7,25 log $TCID_{50}$.

4 VALIDATION

a) Methodology

The VERO cells were exposed to the SEPTALKAN product and a weak cytotoxic effect was observed at the dilution 10^{-2}.

b) Sensibility assays

The PRV was titrated on cell cultures untreated with the SEPTALKAN product (indicator cell line), and titrated on cell cultures treated with the SEPTALKAN product.

	Viral titer (log $TCID_{50}$)		
Product dilution	Untreated cell cultures	treated cell cultures	**Difference of viral titer (log $TCID_{50}$)**
SEPTALKAN 10^{-3}	7,625	6,875	**0,75**

According to the European standard EN 14476 + A1, the SEPTALKAN product used at the dilution of 10^{-3} does not have an effect on the PRV titration method (the difference between viral titers must be < 1,0 log).

c) Validations of the cold shock method on the virucidal effect of the SEPTALKAN product:

Product concentration	**Organic soil load**	**Viral titer (log $TCID_{50}$)**	**Difference with the viral suspension assay**
SEPTALKAN 100 %	3 g/l BSA	Trial 1 : 7,5	-0,25
		Trial 2 : 7,375	-0,125
SEPTALKAN 100 %	3 g/l BSA + 3 ml sheep erythrocytes	Trial 1 : 7,25	0,0
		Trial 2 : 7,25	0,0

The method is validated for a difference in viral titers ≤ 0,5 log.

d) Inactivation assays of the virus with a control solution

	Viral titer (log $TCID_{50}$)	**Viral titer reduction (log $TCID_{50}$)**
Viral suspension control	7,125	
With 1,4%formaldehyde		
Inactivation exposure 5 min	6,875	0,250
Inactivation exposure 15 min	6,125	1,000
Inactivation exposure 30 min	5,500	**1,625**

The viral titer reduction (difference between the titers of the viral suspension treated with formaldehyde and the viral suspension control) must be between -0,5 and -2,5 log after 30 min of exposure. In the assays, the viral titer reduction is 1,625 log, indicated that the PRV is inactivated after 30 min of exposure with the formaldehyde solution.

5 VIRUCIDAL ASSAYS

Trial 1

The viral test suspension was titrated at **7,5 log DICT$_{50}$** prior the assays(3 g/l BSA) and **7,25 log DICT$_{50}$** (3 g/l BSA + 3 ml sheep erythrocytes)

PRODUCT	ORGANIC SOIL LOAD	Concentration (v/v)	Time exposure	Temperature	Viral titer (log TCID$_{50}$)	Viral titer reduction
SEPTALKAN	3 g/l BSA + 3 ml sheep erythrocytes	100 %	5 min	20°C	3,250	**4,000**
	3 g/l BSA	100 %	5 min	20°C	2,750	**4,750**

Trial 2

The viral test suspension was titrated at **7,25 log TCID$_{50}$** prior the assays (3 g/l BSA) and **7,375 log TCID$_{50}$** prior the assays (3 g/l BSA + 3 ml sheep erythrocytes).

PRODUCT	ORGANIC SOIL LOAD	Concentration (v/v)	Time exposure	Temperature	Viral titer (log TCID$_{50}$)	Viral titer reduction
SEPTALKAN	3 g/l BSA + 3 ml sheep erythrocytes	100 %	5 min	20°C	3,000	**4,250**
	3 g/l BSA	100 %	5 min	20°C	2,500	**4,875**

The concentrations of the product demonstrated a virucidal activity on the virus tested if the viral titer reduction is ≥ 4,0 log.

The assays were validated as required by the European standard EN 14476+A1:

- The viral titers of the suspension tests were sufficient in order to observe a reduction of 4 log after time exposure with the product. The viral titer of the PRV was 7,25 log $TCID_{50}$.
- The virus was inactivated with the control solution of formaldehyde after 30 min of exposure: the reduction observed was of 1,625 log for the PRV.
- The product does not have a cytotoxic effect on the cells. The dirty conditions do not affect the infectious capacity of the PRV.
- The SEPTALKAN product does not affect the infectious capacity of the virus: the differences in viral titers between the virus inoculated on cells and the virus inoculated on the cells treated with the SEPTALKAN product was ≤ 1,0 log (0,75 log).
- The difference between the viral suspension assay titer and the viral suspension titer in the cold shock validation was ≤ 0,5 log.

6 CONCLUSIONS

The assays performed with the SEPTALKAN product (batch#n°2463F2563A) demonstrated that:

- the **SEPTALKAN product** **demonstrated a virucidal activity on the PRV from the concentration 100%,** as required by the European standard EN 14476+A1, following a **5 minutes** exposure period, at 20°C, in dirty conditions
- **as the PRV virus is employed as a viral model for efficacy testing of biocides for the hepatitis B virus (HBV), then the SEPTALKAN product has a virucidal activity on HBV in the test conditions (5 min, 20°C, dirty conditions), too.**

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

7 TECHNICAL APPENDIX

Cell line: VERO cells (RD-Biotech réf. 84009, lot n°110118-110V)

Viral strain: PRV virus (lot n° 3/2091233/151812- Friedrich Loeffler Institut)

Buffers and reagents:

- Buffer PBS: sodium chloride, Dominique DUTSCHER, ref. 19032391, batch # n°836751; sodium phosphate dibasic, Sigma Aldrich, ref. S5136, batch # n° BCBC7067V; sodium phosphate monobasic, Sigma Aldrich, ref. S5011, batch # n° 1019K01021V
- DMEM media, Sigma Aldrich, ref. D5796, batch n° RNBB9336
- Fetal calf sera, Sigma Aldrich, ref. F7524, batch n° 098K3397
- MEM media, Sigma Aldrich, ref. 0268, batch n° 040M8301

Reagents:

- Bovine albumin serum (powder), Sigma Aldrich, ref. 05479, batch STBB7838V
- Sheep erythrocytes, Oxoïd, ref. SR 0051E, batch n° 4234000

Inactivation solution:

- formaldehyde, Sigma Aldrich, ref. F-1635, batch n° BCBB3510

Table A1 - Titration of PRV by cytopathic effect, with the Spaerman-Kärber method:

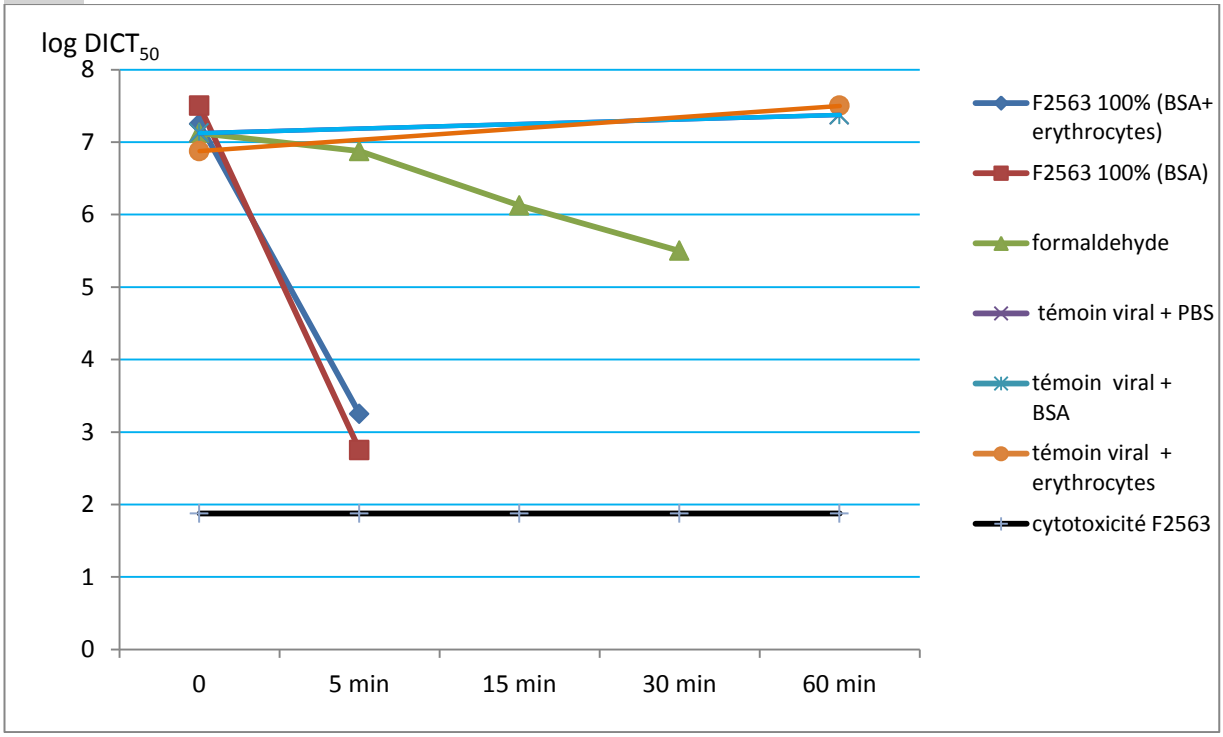
log $TCID_{50}$ = 7,25

Dilution (- log)	Results	% of positive results
-3	44444444	100
-4	44444444	100
-5	33344444	100
-6	22332222	100
-7	11021120	75
-8	00000000	0
-9	00000000	0
-10	00000000	0
Sum of the % of the positive wells		475

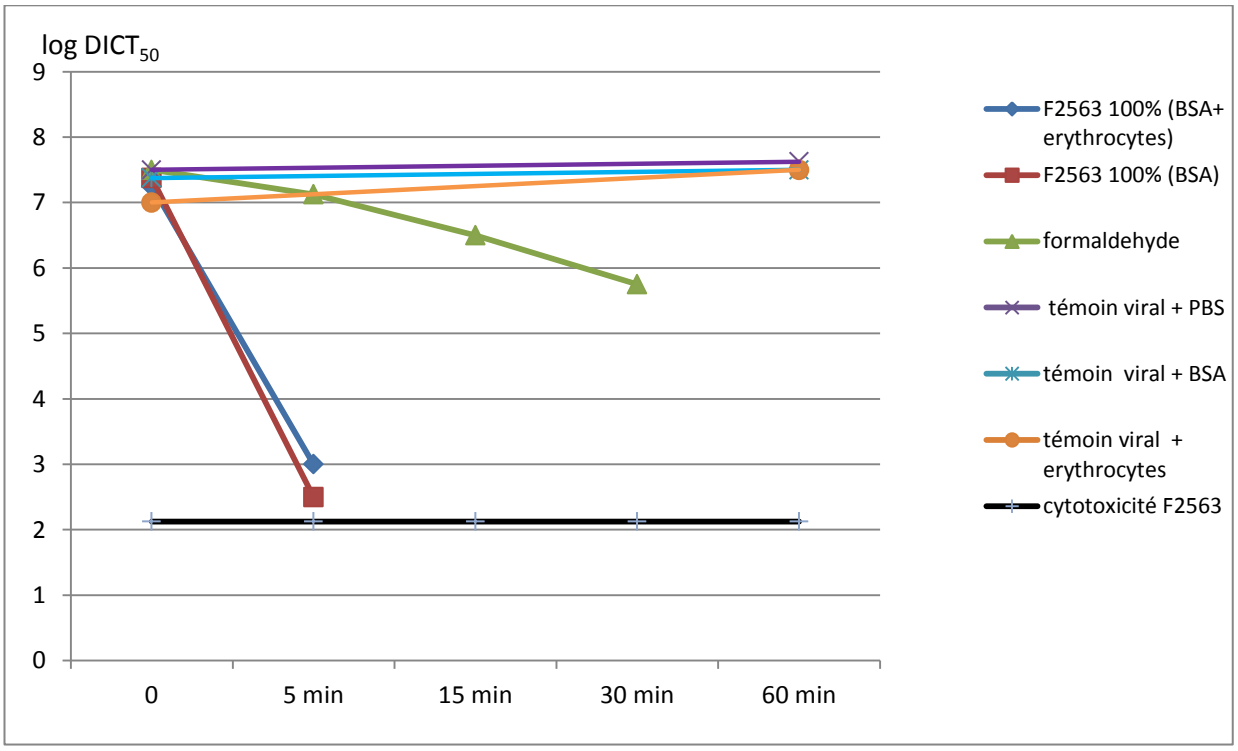
18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Figure 1 – Chart of the results :

Trial 1



Trial 2



18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Table A2 — Results for the SEPTALKAN product against the PRV in dirty conditions (3 g/l BSA + 3 ml erythrocytes)

Product	Concentration	Organic soil load	Cytotoxicity level	Lg $DICT_{50}$					Reduction
				0	5 min	15 min	30 min	60 min	
SEPTALKAN trial 1	100,00%	3 g/l BSA + 3 ml erythrocytes	1,5	7,25	3,25	N.T.	N.T.	N.T.	5 min R = 4
		3 g/l BSA	1,5	7,5	2,75	N.T.	N.T.	N.T.	5 min R = 4,75
SEPTALKAN trial 2	100,00%	3 g/l BSA + 3 ml erythrocytes	1,5	7,25	3	N.T.	N.T.	N.T.	5 min R = 4,25
		3 g/l BSA	1,5	7,375	2,5	N.T.	N.T.	N.T.	5 min R = 4,875
Formaldehyde Trial 1	1,40%	PBS	2,5	7,125	6,875	6,125	5,5	N.T.	
Formaldehyde Trial 2	1,40%	PBS	2,5	7,5	7,125	6,5	5,75	N.T.	
Control of infectivity Trial 1	N.A.	PBS	N.A.	7,125	N.T.	N.T.	N.T.	7,375	
Control of infectivity Trial 1	N.A.	3 g/l BSA	N.A	7,125	N.T.	N.T.	N.T.	7,375	
Control of infectivity Trial 1	N.A.	3 g/l BSA + 3 ml erythrocytes	N.A	6,875	N.T.	N.T.	N.T.	7,5	
Control of infectivity Trial 2	N.A.	PBS	N.A	7,5	N.T.	N.T.	N.T.	7,625	
Control of infectivity Trial 2	N.A.	3 g/l BSA	N.A	7,375	N.T.	N.T.	N.T.	7,5	
Control of infectivity Trial 2	N.A.	3 g/l BSA + 3 ml erythrocytes	N.A	7	N.T.	N.T.	N.T.	7,5	
Cells sensitivity to the virus	10^{-3}	N.A.	Untreated cells	N.T.	N.T.	N.T.	N.T.	7,625	
		N.A.	Treated cells	N.T.	N.T.	N.T.	N.T.	6,875	

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Table A3 — Raw results for the SEPTALKAN product with 3 g/l BSA + 3 ml/l sheep erythrocytes against PRV

Trial 1

	Concentration	Organic soil load	Exposure time	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	100% en BSA+ érythrocytes	3 g/l BSA + 3 ml erythrocytes	5 min	4444	3333	2200	0000	0000	0000	0000	0000	0000	0000
				4444	3244	1111	0000	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	2233	1211	1100	0000	0000	0000
				4444	4444	4444	4444	3333	1112	1111	0000	0000	0000
	100% en BSA	3 g/l BSA	5 min	4444	2222	1100	0000	0000	0000	0000	0000	0000	0000
				4444	1112	0000	0000	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	3333	2222	1111	1211	0000	0000	0000
				4444	4444	4444	2333	2221	1111	2111	0000	0000	0000
SEPTALKAN cytotoxicity	100,00%	3 g/l BSA + 3 ml erythrocytes	N.A.	4444	2200	0000	0000	0000	0000	0000	0000	0000	N.T.
				4444	1000	0000	0000	0000	0000	0000	0000	0000	N.T.
Formaldehyde	1,40%	PBS	5	4444	4444	4444	3333	1111	1111	1000	0000	0000	0000
				4444	4444	4444	3333	1111	1111	0011	0000	0000	0000
			15	4444	4444	4444	2222	1111	1011	0000	0000	0000	0000
				4444	4444	4444	2222	1111	1100	0000	0000	0000	0000
			30	4444	4444	2222	1111	1111	0000	0000	0000	0000	0000
				4444	4444	3333	1111	1111	0000	0000	0000	0000	0000
Formaldehyde (cytotoxicity)	1,40%	PBS	N.A.	4444	3333	1110	0000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
				4444	3434	0000	0000						
Control of infectivity	N.A.	PBS	0	4444	4444	4444	3333	3333	1111	1111	0000	0000	0000
				4444	4444	4444	3333	3333	1122	1000	0000	0000	0000
			60	4444	4444	4444	4444	2221	1111	1111	0000	0000	0000
				4444	4444	4444	4444	1111	1111	1110	0000	0000	0000
Control of infectivity	N.A.	3 g/l BSA	0	4444	4444	4444	4444	3333	2222	1110	0000	0000	0000
				4444	4444	4444	4444	3322	2221	1100	0000	0000	0000
			60	4444	4444	4444	4444	3332	2222	1110	0000	0000	0000
				4444	4444	4444	4444	3333	2222	1111	0000	0000	0000
Control of infectivity	N.A.	3 g/l BSA + 3 ml erythrocytes	0	4444	4444	4444	4444	2222	1111	1000	0000	0000	0000
				4444	4444	4444	4444	1111	1111	1100	0000	0000	0000
			60	4444	4444	4444	4444	2222	1111	1111	0000	0000	0000
				4444	4444	4444	4444	3223	1111	1111	0000	0000	0000

Trial 2

	Concentration	Organic soil load	Exposure time	Dilutions									
				-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	100%	3 g/l BSA + 3 ml erythrocytes	5 min	4444	3333	0000	0000	0000	0000	0000	0000	0000	0000
				4444	3311	1111	0000	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	4444	3331	1111	1100	0000	0000	0000
				4444	4444	4444	4444	3333	1111	1111	0000	0000	0000
	100%	3 g/l BSA	5 min	4444	1111	0000	0000	0000	0000	0000	0000	0000	0000
				4444	1111	0000	0000	0000	0000	0000	0000	0000	0000
			Viral control	4444	4444	4444	3333	1111	1111	1111	0000	0000	0000
				4444	4444	4444	2333	1111	1111	0111	0000	0000	0000
SEPTALKAN cytotoxicity	100,00%	3 g/l BSA + 3 ml erythrocytes	N.A.	4444	0003	0000	0000	0000	0000	0000	0000	0000	N.T.
				4444	2211	0000	0000	0000	0000	0000	0000	0000	N.T.
Formaldehyde	1,40%	PBS	5	4444	4444	4444	4444	3333	1222	1110	0000	0000	0000
				4444	4444	4444	4444	3443	1112	1100	0000	0000	0000
			15	4444	4444	4444	3333	1111	1111	0000	0000	0000	0000
				4444	4444	4444	3333	1111	1111	0000	0000	0000	0000
			30	4444	4444	4444	3333	3333	0000	0000	0000	0000	0000
				4444	4444	4444	3333	3333	0011	0000	0000	0000	0000
Formaldehyde (cytotoxicity)	1,40%	PBS	N.A.	4444	2222	1100	0000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
				4444	2222	1000	0000						
Control of infectivity	N.A.	PBS	0	4444	4444	4444	4444	3333	1111	1111	0000	0000	0000
				4444	4444	4444	4444	3333	1111	1111	0000	0000	0000
			60	4444	4444	4444	4444	3333	2222	1111	1000	0000	0000
				4444	4444	4444	4444	3333	2222	1111	0000	0000	0000
Control of infectivity	N.A.	3 g/l BSA	0	4444	4444	4444	4444	3333	2222	1110	0000	0000	0000
				4444	4444	4444	4444	3322	2221	1111	0000	0000	0000
			60	4444	4444	4444	4444	4444	3233	1111	0000	0000	0000
				4444	4444	4444	4444	4444	2222	1111	0000	0000	0000
Control of infectivity	N.A.	3 g/l BSA + 3 ml erythrocytes	0	4444	4444	4444	2222	1111	1111	1110	0000	0000	0000
				4444	4444	4444	2332	1111	1111	0001	0000	0000	0000
			60	4444	4444	4444	3334	3332	1111	1111	0000	0000	0000
				4444	4444	4444	4444	2133	1111	1111	0000	0000	0000

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Validations:

Product	Concentration	Organic soil load	cytotoxicity	Lg $DICT_{50}$		Reduction > 4 lg	
				0	5 min		
SEPTALKAN	90,00%	3 g/l BSA + 3 ml erythrocytes	1,875	7,5	3,75	5 min R = 3,75	INACTIVE
SEPTALKAN	50,00%	3 g/l BSA + 3 ml erythrocytes	1,875	7,375	7,375	5 min R = 0	INACTIVE

Sensitivity of cells to the PRV:

Product	dilution	Organic soil load		Dilutions								
				-2	-3	-4	-5	-6	-7	-8	-9	-10
SEPTALKAN	10^{-3}	3 g/l BSA + 3 ml érythrocytes	Untreated cells	4444	4444	3333	3333	1111	1111	1000	0000	0000
				4444	4444	3333	3333	1122	1111	0000	0000	0000
			Treated Cells	4444	4444	3333	2333	2222	1110	0000	0000	0000
				4444	4444	3333	2222	2112	0000	0000	0000	0000

18, rue Alain SAVARY - 25000 Besançon ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

UNI EN 16777:2019

QUANTITATIVE NON-POROUS SURFACE TEST WITHOUT MECHANICAL ACTION FOR THE EVALUATION OF VIRUCIDAL ACTIVITY OF CHEMICAL DISINFECTANT USED IN THE MEDICAL AREA

TEST PRODUCT F2563

Sample ID:	*19.529590.0003*
Description:	*F2563, Lot. n° F2563190916A*
Ref. AR:	*AR 2019/205/A CAP.2*
Starting date:	*30/09/2019*
Report emission date:	*08/11/2019*
Prepared by:	*Stefania Buso, Francesca Agetta*

Report digitally signed in accordance with Legislative Decrete No.82 of March, the 7th, 2005 and s.m.i
The test results contained in this Test Report refer only to the analyzed sample. This test report shall not be reproduced except in full, without written approval of Chelab laboratory.
CHELAB S.r.l. Socio Unico, Company subject to the direction and coordination of Mérieux NutriSciences Corporation
Head office: Via Fratta 25, 31023 Resana, Italy. Phone. + 39 0423.7177 / Fax + 39 0423.715058 www.merieuxnutrisciences.it
VAT nr. 01500900269, R.E.A Treviso n. 156079 Fully paid up € 103.480,00.

SAMPLE IDENTIFICATION

- Product Name: F2563
- Batch n°: F2563190916A
- Manufactured: n.a.
- Expiry date: n.a.
- Receiving date: 20/09/2019
- Storage condition: room temperature
- Product appearance: liquid, colorless
- Active substance and concentration: not available

EXPERIMENTAL CONDITIONS

- Test virus and number of passages:
  Vaccinia virus, strain Ankara, ATCC-VR-1508 (passage n° 6)
- Cell line and number of passages:
  BHK-21-cl 13, IZS-Brescia for the propagation of Vaccinia virus (passage n° 93+6)
- Product test concentrations: product as it is (100%)
- Stability and appearance of the mixture during the procedure: Stable, without precipitates
- Interfering substance: Albumin Bovine 3 g/l + 3 ml/l erythrocytes (dirty conditions)
- Contact times: 5 min ± 10 sec; 10 min ± 10 sec
- Test temperature: Room Temperature (between 18 ± 1 °C and 25 ±1 °C)
- Neutralization method: Filtration method with Microspin™ S400HR columns was applied during the test procedure. Notably, because of interference caused by volatile substances contained in the test product, filtration method was applied also during the procedure for testing cell susceptibility.
- Growth medium: EMEM, 10% FCS, 2 mM Glutamine, 1 mM Sodium Pyruvate,1% Pen-Strep mix.
- Maintenance medium: EMEM, 2% FCS.

Report digitally signed in accordance with Legislative Decrete No.82 of March, the 7th, 2005 and s.m.i
The test results contained in this Test Report refer only to the analyzed sample. This test report shall not be reproduced except in full, without written approval of Chelab laboratory.
CHELAB S.r.l. Socio Unico, Company subject to the direction and coordination of Mérieux NutriSciences Corporation
Head office: Via Fratta 25, 31023 Resana, Italy. Phone. + 39 0423.7177 / Fax + 39 0423.715058 www.merieuxnutrisciences.it
VAT nr. 01500900269, R.E.A Treviso n. 156079 Fully paid up € 103.480,00.

TEST RESULTS

See tables n° 1 to 2.

Table 1: Results of the test UNI EN 16777:2019 on Vaccinia virus contact time 5 min and 10 min

VIRUS	TEST	CONTACT TIME	SAMPLE	VIRUS TITRATION $logTCID_{50}$	ACCEPTANCE CRITERIA	RESULT
VACCINIA VIRUS	TITRATION OF VIRUS CONTROL	0 min	n.a.	5.375	/	/
		10 min	n.a.	5.75	/	/
	PRELIMINARY CYTOTOXICITY EFFECT	n.a.	DONE			
	CELL SUSCEPTIBILITY	n.a.	CONTROL	6.25	< 1	R = 0.125 PASS
			0.1%*	6.125		
	EFFICIENCY FOR SUPPRESSION OF DISINFECTANT ACTIVITY	n.a	100 %	6.125	≤ 0.5	R = 0.375 PASS
	VIRUCIDAL ACTIVITY	5 min	100 %	≤ 1.5	R ≥ 4	R ≥ 4.25 ACTIVE
		10 min	100 %	≤ 1.5	R ≥ 4	R ≥ 4.25 ACTIVE
	REFERENCE VIRUS INACTIVATION TEST	5 min	n.a.	3.5	R < 3	R = 2.25 PASS

* lowest apparently non cytotoxic dilution. n.a.: not applicable

Report digitally signed in accordance with Legislative Decrete No.82 of March, the 7th, 2005 and s.m.i
The test results contained in this Test Report refer only to the analyzed sample. This test report shall not be reproduced except in full, without written approval of Chelab laboratory.
CHELAB S.r.l. Socio Unico, Company subject to the direction and coordination of Mérieux NutriSciences Corporation
Head office: Via Fratta 25, 31023 Resana, Italy. Phone. + 39 0423.7177 / Fax + 39 0423.715058 www.merieuxnutrisciences.it
VAT nr. 01500900269, R.E.A Treviso n. 156079 Fully paid up € 103.480,00.

Table 2: Raw data of the test UNI EN 16777:2019 on Vaccinia virus contact time 5 min and 10 min

Test	Contact Time	Interfering substance	Concentration		dilutions log_{10}							
					2	3	4	5	6	7	8	9
TITRATION OF VIRUS CONTROL	0 min	BSA 3 g/l + DSB 3 ml/l	/		4444 4444	4444 4444	4444 4444	4444 4440	0000 0000	0000 0000	0000 0000	0000 0000
	10 min		/		4444 4444	4444 4444	4444 4444	4444 4444	4400 0000	0000 0000	0000 0000	0000 0000
EFFICIENCY FOR SUPPRESSION OF DISINFECTANT ACTIVITY	/	BSA 3 g/l + DSB 3 ml/l	100 %		4444 4444	4444 4444	4444 4444	4444 4444	4444 4000	0000 0000	0000 0000	0000 0000
VIRUCIDAL ACTIVITY	5 min	BSA 3 g/l + DSB 3 ml/l	100%	R1	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
				R2	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	10 min	BSA 3 g/l + DSB 3 ml/l	100%	R1	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
				R2	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Reference Inactivation Test	5 min	/	50 ppm		4444 4444	4444 4444	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Susceptibility (Control)	60 min	PBS	/		4444 4444	4444 4444	4444 4444	4444 4444	4444 4000	4000 0000	0000 0000	0000 0000
Susceptibility (Product)	60 min	PBS	0.1%		4444 4444	4444 4444	4444 4444	4444 4444	4444 4000	0000 0000	0000 0000	0000 0000
Test	Contact Time	Interfering substance	Concentration		Product dilutions (log_{10})							
					1	2	3	4	5	6	7	8
Cytotoxicity (Product)	n.a.	BSA 3 g/l + DSB 3 ml/l	100%		CCCC CCCC	CCCC CCCC	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000

R1: Test surface 1; R2: Test surface 2; n.a.: not applicable; C: cytotoxic

CONCLUSIONS

Based on UNI EN 16777:2019, under the test conditions applied, the test product resulted to have virucidal activity (R ≥ 4) against Vaccinia virus.

Report digitally signed in accordance with Legislative Decrete No.82 of March, the 7th, 2005 and s.m.i
The test results contained in this Test Report refer only to the analyzed sample. This test report shall not be reproduced except in full, without written approval of Chelab laboratory.
CHELAB S.r.l. Socio Unico, Company subject to the direction and coordination of Mérieux NutriSciences Corporation
Head office: Via Fratta 25, 31023 Resana, Italy. Phone. + 39 0423.7177 / Fax + 39 0423.715058 www.merieuxnutrisciences.it
VAT nr. 01500900269, R.E.A Treviso n. 156079 Fully paid up € 103.480,00.

TEST REPORT

SPORICIDAL ACTIVITY OF THE SEPTALKAN PRODUCT ACCORDING TO THE NF T72-281 STANDARD

For : ALKAPHARM
PARC BIOCITECH
102 AVENUE GASTON ROUSSEL
93230 ROMAINVILLE

Date of request: 01.02.2013

Study number: n° 032B03-2013-01

According to the NF T 72-281 (May 2009) Standard – aerial desinfection. Determination of the bactericidal, fungicidal, levuricidal and sporicidal activity.

Trials on 1 bacterial reference strain: *Clostridium difficile*.

This test report includes 7 pages.

Study completion date: 06/19/2013

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

Professor Georges HERBEIN
Professor of Medicine – MD-PhD in virology
Scientific consultant

SUMMARY

- PEFORMING LABORATORY
- IDENTIFICATION OF THE PRODUCT
- EXPERIMENTAL CONDITIONS
- METHOD OF DESINFECTION BY SPRAYING AND WIPING UP
    - VALIDATIONS
    - RESULTS
- CONCLUSIONS
- TECHNICAL APPENDIX

1. PERFORMING LABORATORY

APEX BIOSOLUTIONS
18, rue Alain SAVARY
25000 BESANÇON
FRANCE

2. IDENTIFICATION OF THE PRODUCT

SEPTALKAN PRODUCT:

Batch N°: 2163F2563A

Expiration date: 04/2016

Manufacturer: ALKAPHARM

Manufacturing date: non communicated

Storage conditions: as recommended by the manufacturer

Active substances: didecyl dimethylammonium chloride and alkylbenzyl dimethyl ammonium chloride

Appearance of the product: clear

Diluent of the product: none, ready-to-use product

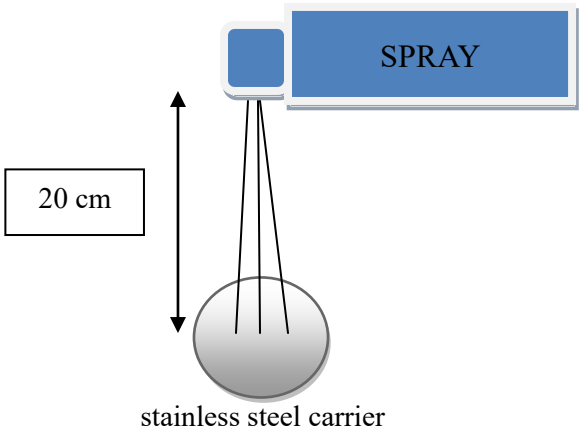
Date of delivery of the product: 06/26/2012

Date of tests: from 04/08/2013 to 04/22/2013

3. EXPERIMENTAL CONDITIONS

- Disinfection procedure : by manual spraying of the product, and wiping up with sterile wipes
- Protocol :

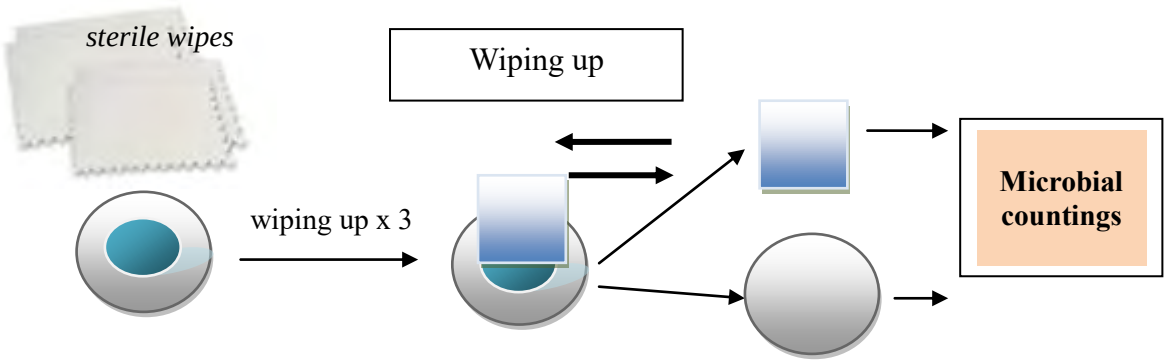
Step 1 :



Time of spraying : 2 sprays (4 seconds)
Weight of the sprays : 0,505 g

Recovery with wipes of the liquid sprayed (contact time wipe/product: 5 min)

Step 2 :



- Volume of the product sprayed: 2 sprays at 20 cm of the carrier
- no exposure time between the product and the microbial suspension on the stainless steel carrier, 5 min of time exposure between the wipe and the product
- wiping up of the carriers with the wipes (recovery of the product on the carrier)
- Spraying time: 4 seconds
- Culture conditions: on *Clostridium difficile agar*, at 37°C ± 1°C under anaerobic conditions.
- Strain : *Clostridium difficile* batch 10A (NC11209) - HPA.
- Microbial contamination of the carriers: with 50 µl of microbial spores in skim milk.
- Recovery diluent for the wipes : trypton salt solution (TS)

4. METHOD BY SPRAYING AND WIPING UP

(1) VALIDATIONS

Experimental conditions: 21°0 C ; 65% relative humidity

Stainless steel carriers: 2 cm diameter, 0,15 cm thick

Clostridium difficile

Microbial counts				
		VC1	VC2	
N	10^{-5}	223	256	2,39E+07
	10^{-6}	21	25	N1 = 2,40E+02
N2	10^{-6}	28	23	2,55E+01

Validations							
T	2,44E+06	**n1**	1,74E+02	**n2**	3,55E+01	**n3**	1,90E+02
$T>10^6$ x yes □ no		n1 > 0,5*N1 x yes □ no		n2 > 0,5 N2 x yes □ no		n3 > 0,5*N1 x yes □ no	

(2) RESULTS

Experimental conditions: 22°0 C ; 58% relative humidity

Stainless steel carriers: 2 cm diameter, 0,15 cm thick

Clostridium difficile

	Trials (results for the recovery liquid of the carriers)						Counts after wiping up the carriers	
	Trial 1		Trial 2		Trial 3			
Mb (87mL)	5	/	6	/	0	/	**disque 1**	23
Mb (10mL)	0	/	0	/	0	/	**disque 2**	15
10^0	0	0	0	0	0	0	**disque 3**	10
10^{-1}	0	0	0	0	0	0		
10^{-2}	0	0	0	0	0	0		
10^{-3}	0	0	0	0	0	0		
n'1	0	**d**	**5,18**					
n'2	1,6E+01	d≥3 x yes □ no						

	Trials (results for the recovery liquid of the wipes)					
	Trial 1		Trial 2		Trial 3	
Mb (87mL)	165	/	165	/	165	/
Mb (10mL)	165	/	165	/	142	/
10^0	20	15	21	18	15	16
10^{-1}	2	2	1	1	1	1
10^{-2}	1	1	0	0	1	1
10^{-3}	0	0	0	0	0	0
n'1	1,75E+03	**d**	**3,14**			
n'2	1,6E+01	d≥3 x yes □ no				

T = number of germs on the control carrier

n'1 = number of surviving germs in 100 ml of recovery liquid

n'2 = number of remaining germs on the carrier

5. CONCLUSION

The SEPTALKAN product showed a sporicidal activity against *Clostridium difficile* spores, with the spraying method (two sprays) according to the NF T 72-281 standard protocol:

- **On the stainless steel carriers after wiping up,**
- **In the wipes, after a 5 minutes contact time with the recovered product**

The protocol used (modified according to the pratical use conditions) allowed to reach 5,18 log of reduction on the carriers, after mechanical action by wiping up with sterile wipes. The reduction obtained with this modified protocol is greater than the mandatory one in the NF T 72-281 standard (reduction of at least 3 log).

This protocol allowed a reduction greater than 3 log in the wipes, after wiping up the carriers and after 5 minutes of contact time with the SEPTALKAN product.

TECHNICAL APPENDIX

Media:

Clostridium difficile agar, OXOID, ref. n° CM061, batch n° 1052043

Clostridium difficile supplement, Sigma Aldrich, ref. n°17122, batch n°BCBD0142

Anaerobic bags, OXOID

Recovery liquid

Trypton-Salt Solution (TS)

- Trypton, Laboratories CONDA, ref. 1612, batch n° 091229 ----------1,00 g/l
- Sodium chloride, PANREAC, ref n° 141659, batch n° 204679 ----------8,50 g/l

pH at 25°C : 7,0 ± 0,2

SKIM MILK

Skim milk, Régilait, batch n° L M F N 15:27.

WIPES

Cellulose and polyester wipes: ADS laminaire, Lymotech, ref.C-WIPES, 7 cm x 7 cm, steriles

STAINLESS STEEL CARRIERS – stainless steel disks**,** 2 cm diameter, 1.4301, 2B grade (MECAPOL).

TEST REPORT

FUNGICIDAL ACTIVITY OF THE SEPTALKAN PRODUCT ACCORDING TO THE NF T72-281 STANDARD

For: ALKAPHARM
PARC BIOCITECH
102 AVENUE GASTON ROUSSEL
93230 ROMAINVILLE

Date of request: 07.02.2013

Study number: n°032B02-2013-01

According to the NF T 72-281 (May 2009) Standard – aerial disinfection. Determination of the bactericidal, fungicidal, levuricidal and sporicidal activity.

Trials on 1 fungal reference strain: *Aspergillus fumigatus*.

This test report includes 7 pages.

Study completion date: 10/18/2013

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

Professor Georges HERBEIN
Professor of Medicine – MD-PhD in virology
Scientific consultant

SUMMARY

- PEFORMING LABORATORY
- IDENTIFICATION OF THE PRODUCT
- EXPERIMENTAL CONDITIONS
- METHOD OF DESINFECTION BY WIPING UP WITH IMPREGNATED WIPES
    - VALIDATION
    - RESULTS
- CONCLUSIONS
- TECHNICAL APPENDIX

18, rue Alain SAVARY • 25000 Besançon • Tel: 03.81.25.09.04 • Fax: 03.81.25.53.51• SARL • RCS BESANÇON • N° SIRET 51786053200012 • N° TVA intra FR 23517860532 • info@apexlabo.com

1. PERFORMING LABORATORY

APEX BIOSOLUTIONS
18, rue Alain SAVARY
25000 BESANÇON
FRANCE

2. PRODUCT IDENTITY

SEPTALKAN WIPES – READY-TO-USE

Batch N°: 20130401- 3161

Expiration date: 04/2016

Manufacturer: ALKAPHARM

Manufacturing date: non communicated

Storage conditions: as recommended by the manufacturer

Active substances: didecyl dimethylammonium chloride and alkylbenzyl dimethyl ammonium chloride

Product diluent recommended by the manufacturer for use: none – ready-to-use product

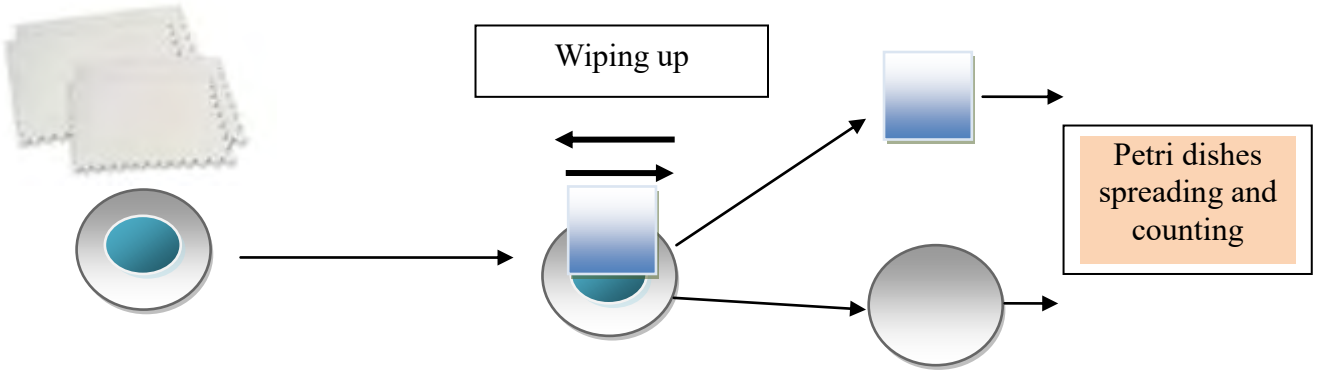
Date of delivery of the product: 07/05/2013.

Date of tests: from 08/19/2013 to 09/02/2013

3. EXPERIMENTAL CONDITIONS

- Disinfection method : by mechanical disinfection with impregnated wipes with SEPTALKAN product
- Protocol:

Impregnated wipes with SEPTALKAN product



- 5 min of time exposure with the fungal suspension on the carriers
- Culture conditions : MEA (Malt Extract Agar).
- Strain: *Aspergillus fumigatus*.
- Microbial suspension: contamination of the stainless steel carriers with 50 µl of spores of *Aspergillus fumigatus* in sterile skim milk.

4. WIPING UP WITH IMPREGNATED WIPES

(1) VALIDATIONS

Experimental conditions: 22°5 C; 70% relative humidity

Stainless steel carriers: 2 cm diameter, 0,15 cm thick

COUNTS				
		VC1	VC2	
N	10^{-5}	>165	>165	7,75E+07
	10^{-6}	75	80	N1 = 77,5
N2	10^{-6}	81	85	83

VALIDATIONS							
T	1,07E+06	**n1**	78,5	**n2**	72,5	**n3**	71,5
$T>10^6$ x yes □ no		n1 > 0,5*N1 x yes □ no		n2 > 0,5 N2 x yes □ no		n3 > 0,5*N1 x yes □ no	

(2) RESULTS

Experimental conditions: 21°0 C; 64% relative humidity
Stainless steel carriers: 2 cm diameter, 0,15 cm thick

Counts of spores of *Aspergillus fumigatus* remaining on stainless steel carriers:

	TRIALS							
	Trial 1		**Trial 2**		**Trial 3**		**carrier**	
Mb (87mL)	2	/	0	/	4	/	**carrier 1**	1
Mb (10mL)	0	/	0	/	0	/	**carrier 2**	0
10^0	3	4	0	0	3	1	**carrier 3**	5
10^{-1}	0	0	0	0	0	0		
10^{-2}	0	0	0	0	0	0		
10^{-3}	0	0	0	0	0	0		
n'1	0	**d**	6,03					
n'2	6	**d≥4** **x yes □ no**						

Counts of spores of *Aspergillus fumigatus* remaining on wipes after wiping up (exposure time of 5 min):

	WIPES					
	Trial 1		**Trial 2**		**Trial 3**	
Mb (87mL)	12	/	25	/	8	/
Mb (10mL)	0	/	0	/	0	/
10^0	0	0	2	1	0	0
10^{-1}	0	0	0	0	0	0
10^{-2}	0	0	0	0	0	0
10^{-3}	0	0	0	0	0	0
n'1	0	**d**	**6,03**			
n'2	0	**d>4** **x yes □ no**				

5. CONCLUSION

The test protocol (modified according to the pratical use conditions) allowed to reach a reduction greater than 6 log (6,03 log reduction in the trials), by the use of steel carriers wiped up with impregnated wipes of SEPTALKAN product (batch n° 20130401- 3161) and for an exposure time of 5 min (exposure time between wipes and steel carriers contaminated with the fungal suspension). The reduction obtained is greater than the mandatory one in the NF T 72-281 standard (4 log reduction).

18, rue Alain SAVARY • 25000 Besançon • Tel: 03.81.25.09.04 • Fax: 03.81.25.53.51• SARL • RCS BESANÇON • N° SIRET 51786053200012 • N° TVA intra FR 23517860532 • info@apexlabo.com

TECHNICAL APPENDIX

Media:

MEA (Malt Extract Agar), Dutscher, ref. 777304, batch n°301031

DILUENT

Trypton-Salt Solution (TS)

- Trypton, Laboratories CONDA, ref. 1612, batch n° 091229 ----------1,00 g/l
- Sodium chloride, PANREAC, ref n° 141659, batch n° 204679 ----------8,50 g/l

pH at 25°C : 7,0 ± 0,2

SKIM MILK

Skim milk, Régilait, batch n° L M F N 15:27.

STAINLESS STEEL CARRIERS – stainless steel disks**,** 2 cm diameter, 1.4301, 2B grade (MECAPOL).

TEST REPORT

SPORICIDAL ACTIVITY OF THE SEPTALKAN PRODUCT BY AERIAL DISINFECTION, ACCORDING TO THE NF T72-281 STANDARD

For: ALKAPHARM
PARC BIOCITECH
102 AVENUE GASTON ROUSSEL
93230 ROMAINVILLE

Date ofrequest: 06.18.2014

Study number: n° 152B17-2014-01

According to the NF T 72-281 (May 2009) standard - aerial desinfection. Determination of the bactericidal, fungicidal, yeasticidal and sporicidal activity.

Trials performed on 1 reference strain: *Baci/lus subtilis.*

This test report included 7 pages.

Study completion date: 09/16/2014

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

Professor Georges HERBEIN
Professor of Medicine - MD-PhD in virology
Scientific consultant

1 PERFORMING LABORATORY

APEX BIOSOLUTIONS
18, rue Alain SAVARY
25000 BESANÇON
FRANCE

2 PRODUCT IDENTIFICATION

SEPTALKAN:

Batch n° 20 13030 170

Expiration date: 03/01/2016

Manufacturer: ALKAPHARM

Manufacturing date: non communicated

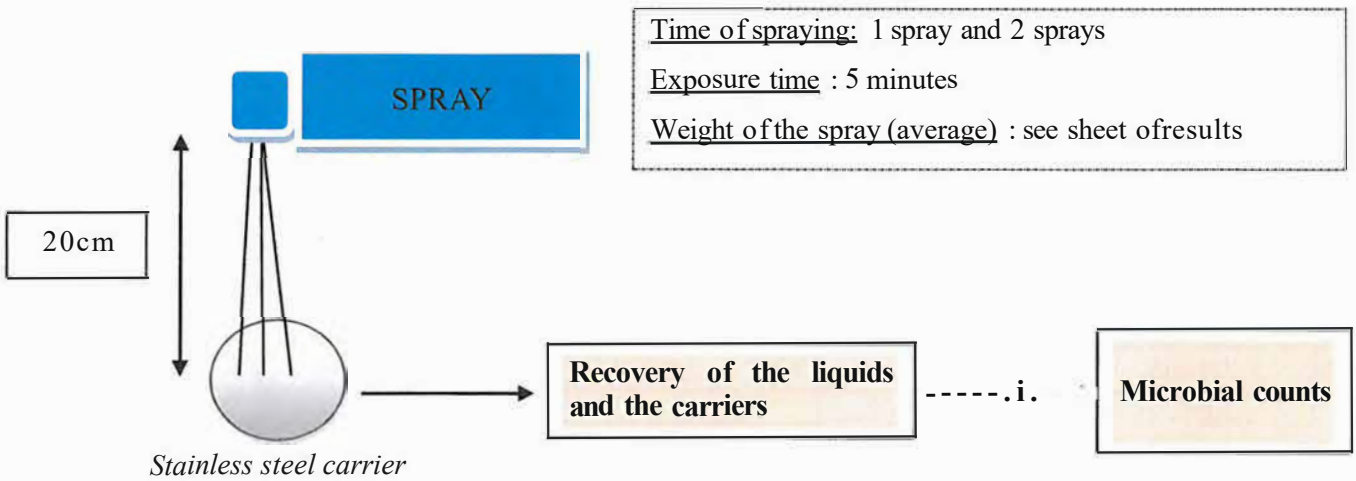
Storage conditions: as recommended by the manufacturer

Active compounds: didecyl dimethyl ammonium chloride ≤ 0,25% ; alkylbenzyl dimethyl ammonium chloride < 0,95%

Appearance of the product: clear, colorless

3 EXPERIMENTAL CONDITIONS

- Disinfection method : by manual spraying
- Protocol:



b) RESULTS

Bacillus subtilis

	Trial							
	Triall		Trial2		Trial3		Carrier	
Mb (87ml)	203	/	200	/	198	/	Carrier 1	55
Mb (10ml)	20	/	18	/	21	/	Carrier 2	21
10°	28	24	21	23	19	17	Carrier 3	29
10^{-1}	2	2	1	2	1	1		
10-2	0	0	0	0	0	0		
10^{-3}	0	0	0	0	0	0		
n'l	2,20E+03	d	2,89E+OO					
n'2	3,50E+01	d>3 o yes x no						

5 TESTS WITH TWO SPRAYS - VALIDATIONS AND RESULTS

c) VALIDATIONS

Experimental conditions: 25°5C ; 50% relative humidity
Stainless steel carriers: 4 cm diameter, 0,2 cm thick
Number of spraying : 2 (0,397 g)
Exposure time: 5 min.

No run off of the product outside the carrier was observed

Baci/lus subtilis

Microbial counts				
		VCl	VC2	
N	10^6	293	296	2,96E+08
	10-7	29	33	Nl = 3,lE+Ol
N2	10^{-6}	>330	>330	N2 = 3,5E+Ol
	10-7	35	35	

7 TECHNICAL APPENDIX

Media:

TSA *(Trypto* casein Soy Agar), Thermofisher, ref. n° 1176-8822, batch n° 3298495

DILUENT

Trypton-Salt solution

Composition per liter of distilled water:

- Trypton, Dominique Dutscher, ref. N° 777472, batch n° 090633---------- 1,00
- Sodium chloride, Dominique Dutscher, ref. 091560, batch n° 924561 -------- 8,50

pH at 25 °C : 7,0 ± 0,2

SKIMMED MILK:

Skimmed milk, Régilait, batch n° L H O S 06 :03.

Sterilisation 5 min at 121 °C.

MEMBRANES 0,45 µm, 47 mm, DUTSCHER, ref. 037045, batch n° G 1499906

FILTRATION SYSTEM MILLIPORE, ref. MŒAWG100, batch n° F4BA2952 1

STAINLESS STEEL CARRIERS- stainless steel disks, **4** cm diameter, 1.4301, 2B grade (CICAFIL).