

Produktinformation



Forschungsprodukte & Biochemikalien



Zellkultur & Verbrauchsmaterial



Diagnostik & molekulare Diagnostik



Laborgeräte & Service

Weitere Information auf den folgenden Seiten! See the following pages for more information!



Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic in



C€ 0483

SeroCT™ IgA (RT)

Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of specific IgA antibodies to *Chlamydia trachomatis* in human serum

Instruction Manual

Test kit for 96 determinations REF: 1183-01

For In Vitro Diagnostic Use For professional use only Store at 2-8°C. **Do Not Freeze**



Savyon® Diagnostics Ltd.

3 Habosem St. Ashdod 77610 ISRAEL

Tel. +972.8.8562920 Fax: +972.8.8523176

E-mail: support@savyondiagnostics.com

Intended Use

The SeroCT™ RT IgA kit is intended for the detection of IgA antibodies specific to *C. trachomatis* in human serum. The Savyon® SeroCT™ RT IgA kit is a new generation qualitative ELISA test which is based on *Chlamydia trachomatis* specific synthetic peptides. SeroCT™ RT is used as an aid in the diagnosis of *C. trachomatis* specific infection.

SeroCT™ RT IgA is intended to be run and interpreted in conjunction with the Savyon[®] SeroCT™ RT IgG kit.

For In Vitro Diagnostic Use.

Introduction

Chlamydia is a gram-negative obligate intracellular bacteria that causes acute and chronic diseases in mammalian and avian species. The genus Chlamydia is comprised of four species: *C.trachomatis*, *C.pneumoniae*, *C.psittaci* and *C. pecorum* (1-4).

C.trachomatis is divided into 15 serovars (5-8). Serovars A, B, Ba and C are agents of trachoma (9), the leading cause of preventable blindness endemic in third world countries. Serovars L_1 - L_3 are the agents of lymphogranuloma venereum. Serovars D-K are the common cause of sexually transmitted genital infection worldwide: cervicitis, endometritis/ salpingitis (10) in females and urethritis (11) in both males and females. Endometritis/salpingitis can lead to tubal occlusion with a higher risk of extrauterine pregnancy and infertility. Genital infection may cause an acute and

persistent infection occasionally without any clinical symptoms. Generally, these infections are treatable once they are diagnosed. However without any treatment the infection may progress to a severe chronic inflammation leading to infertility, ectopic pregnancy, induced abortion or child delivery. Moreover, infants to infected mothers may be infected during birth, leading to conjunctivitis or pneumonia (12-14).

The serology of *C. trachomatis* is more interesting in cases of chronic infections than in acute infections.

C.pneumoniae is an important respiratory pathogen in humans and causes up to 10% of community-acquired pneumonia cases. It has been associated with acute respiratory diseases, pneumonia, asthma, bronchitis, pharyngitis, acute chest syndrome of sickle cell disease, coronary heart disease, and Guillain-Barre syndrome (15-17).

C.psittaci infects a diverse range of host species from molluscs to birds to mammals and also causes severe pneumonia. In animals, *C.psittaci* and *C.pecorum* are capable of inducing diverse disease syndromes like pneumonia, enteritis, polyserositis, encephalitis and conjunctivitis.

Serological testing, now an established approach in many countries, has been shown to provide a comprehensive answer for the detection of *C. trachomatis* infection. In suspected deep-seated infection, serum sampling reduces the necessity for invasive procedures, which are required for direct antigen detection. In cases of lower urogenital infections, collection limitations such as effectiveness of scrape sampling procedure, specimen handling and transportation difficulties have to be weighed. Above all, the issue remains that most Chlamydia infections are asymptomatic. Therefore an infection may persist for a long time, ascend the upper genital tract, causing deep and chronic infections, and increase the probability of false negative results in direct antigen detection.

Serological testing for *Chlamydia trachomatis*, through the detection of various specific antibodies, is today an effective and highly accepted option (10,11,18,19). New and accurate technologies apply the immuno markers IgM, IgA and IgG to characterize the presence and stage of infection.

Specific IgM is indicative of acute Chlamydia infections. Absence does not; however preclude the presence of ongoing infection, especially in recurrent and chronic cases. The use of specific IgA as a marker for active Chlamydia infection has been shown to have an important role because of its short half-life time, while persisting as long as antigenic stimulation exists. IgA, however, is more suitable for post therapy follow-up. IgG is a marker for Chlamydia positive immune response in either current, chronic or past infections.

Serological cross-reactions occur between the three different species of Chlamydia. Most of the serological diagnostic assays for Chlamydia use either purified elementary bodies microimmunofluorescence (MIF) and ELISA tests, lipopolysaccharides (LPS) or purified major outer membrane protein (MOMP), as antigens. Genus specific epitopes are present in all the above antigens, therefore low species specificity is observed. Moreover, a large proportion of the population has been exposed to C.pneumoniae (with no clinical signs), the prevalence of anti- Chlamydia antibodies is very high. Therefore, the differentiation between

C.pneumoniae and C. trachomatis specific antibodies using conventional serological screening tests (MIF, ELISA, EIA etc.) is insufficient.

Savyon® Diagnostics Ltd has developed an assay in which *C.trachomatis* species specific epitopes, derived from different serotypes, are used in an Enzyme-Linked Immunosorbent Assay (ELISA). The test excludes cross-species reactive epitopes and enables more accurate and more specific determination of *C.trachomatis* IgG and IgA antibodies.

Principle of the Test

- SeroCT[™] RT Plates are coated with C. trachomatis specific peptides.
- Serum to be tested is diluted and incubated with the precoated SeroCT™ RT plate 30 minutes at Room Temperature (RT). In this step *C. trachomatis* specific antibodies are bound to the immobilized *C. trachomatis* specific peptides.
- Non-specific antibodies are removed by washing.
- Anti-human IgA conjugated to horseradish peroxidase (HRP) is added and incubated 30 minutes at Room Temperature. In this step the HRP-conjugate is bound to the prebound antigen-antibody complex.
- Unbound conjugate is removed by washing.
- Upon the addition of TMB-substrate, the substrate is hydrolyzed by the peroxidase, yielding a blue solution of the reduced substrate.
- Upon the addition of the stop solution, the blue color turns yellow and should be read by an ELISA reader at a wavelength of 450 / 620nm.
- The absorbance is proportional to the amount of the specific antibodies which are bound to the immobilized peptides.

Summary of Procedure: Manual/Automation*

Wells of microtiter plate coated with C.trachomatis antigens

Add 2 x 100µl of Cut Off Control Add 1 x 100µl each of Negative Control, Positive Control and diluted specimens

Cover plate and incubate 30 minutes at Room Temperature

Wash 5 times with Wash Buffer

Add 100µl of Ready to Use HRP-Conjugate

Cover plate and incubate 30 minutes at Room Temperature

Wash 5 times with Wash buffer

Add 100µl of TMB-Substrate

Cover plate and incubate 30 min at room temperature

Add 100µl of Stop Solution

Read absorbance at 450/620nm

Calculate and interpret results

*Automation procedure:

20 minutes sample's incubation 5 wash cycles

M1183-01E 11-10/12

Kit Contents: for Manual use/Automated use

Test Kit for 96 determinations Catalog No.: A1183-01M / A1183-01D

 C. trachomatis antigen - coated microtiter plate: 96 break-apart wells (8x12) coated with C. trachomatis specific peptides, packed in an aluminum pouch containing a desiccant card.

1 plate / 1 plate

- Concentrated Wash Buffer (20x): A PBS -Tween buffer.
 bottle, 100 ml / 1 bottle, 100 ml
- 3. **Serum Diluent-RT (Blue):** A ready to use buffer solution. Contains less than 0.05% Proclin as preservative.

1 bottle, 30 ml / 1 bottle, 60 ml

4. Ready to Use HRP-Conjugate (Green): Horseradish Peroxidase (HRP) conjugated anti-human IgA (alpha chain specific). Contains less than 0.05% Proclin as a preservative.

2 bottles, 4 ml each & 2 bottles, 7 ml each

 Cut Off Control: A ready to use C.trachomatis IgA serum used for cut off determination. Contains less than 0.1% Sodium Azide and less than 0.05% Proclin as preservatives.

1 vial, 2.5 ml / 1 vial, 2.5ml

6. Negative Control: A ready to use *C. trachomatis* IgA negative human serum. Contains less than 0.05% Proclin and less than 0.1% Sodium Azide as preservatives.

1 vial, 2 ml / 1 vial, 2ml

7. **Positive Control:** A ready to use *C. trachomatis* IgA positive human serum. Contains less than 0.05% Proclin and less than 0.1% Sodium Azide as preservatives.

1 vial, 2 ml / 1 vial, 2ml

8. **TMB-Substrate:** A ready to use solution contains 3, 3' 5, 5' tetramethylbenzidine as a chromogen and peroxide as a substrate.

1 bottle, 14 ml / 1 bottle, 16 ml

9. **Stop Solution:** A ready to use solution. Contains 1M H₂SO₄. **1 bottle, 15 ml / 1 bottle, 16 ml** 10. **Plate cover: 1 unit / none**

Frate cover: 1 unit / n

11. Instruction Manual:

1/1

Materials Required But Not Supplied

- 1. Clean test tubes for dilution of patients' sera.
- Adjustable micropipettes, or multichannel pipettes (5-50, 50-200 and 200-1000μl ranges) and disposable tips.
- 3. One liter volumetric flask.
- 4. One 50ml volumetric cylinder.
- 5. Wash bottle.
- 6. Absorbent paper.
- 7. Vortex mixer.
- 8. ELISA-reader with 450/620nm filter.
- 9. Distilled or double deionized water.

Warning and Precautions

For In Vitro Diagnostic Use

 This kit contains human sera, which have been tested by FDA and CE approved techniques, and found to be negative for HBV antigen and for antibodies to HCV and to HIV 1 & 2. Since no known method can offer complete assurance that products derived from human blood do not transmit infection, all human blood components supplied in this kit must be handled as potentially infectious serum or blood, according to the recommendations published in the CDC/NIH manual "Biosafety in Micro Biological and Biomedical Laboratories", 1988.

- TMB-Substrate solution is an irritant material to skin and mucous membranes. Avoid direct contact.
- 3. Diluted sulfuric acid $(1M H_2SO_4)$ is an irritant agent for the eyes and skin. In case of contact with eyes, immediately flush area with water and consult a physician.).
- All the components of this kit have been calibrated and tested by lot. It is not recommended to mix components from different lots since it might affect the results.

Storage and Shelf-Life of Reagents

 All the reagents supplied should be stored at 2-8°C. The unopened reagent vials are stable until the expiration date indicated on the kit pack. Exposure of originally stoppered or sealed components to ambient temperature for a few hours will not cause damage to the reagents.

DO NOT FREEZE!

- 2. Once the kit is opened, its shelf life is 90 days.
- Unused strips must be resealed in the aluminum pouch with the desiccant card, by rolling the open end and sealing tightly with tape over the entire length of the opening.
- 4. Each vial of HRP Conjugate can only be used twice. Please discard after the second time.
- 5. Crystals may form in the 20x concentrated Wash Buffer during cold storage, this is perfectly normal. Redissolve the crystals by warming the buffer to 37°C before diluting. Once diluted, the solution may be stored at 2-8°C up to twenty one days.

Serum Collection

Prepare sera from aseptically collected samples using standard techniques. Heat inactivated sera should not be used. The use of lipemic, turbid or contaminated sera is not recommended. Particulate material and precipitates in sera may cause erroneous results. Such specimens should be clarified by centrifugation or filtration prior to the test.

Storage

Specimens should be stored at 2-8°C and tested within 7 days (adding of 0.1% Sodium Azide is highly recommended). If a longer storage period is anticipated, aliquot and store the specimens below -20°C. Avoid repeated thawing and freezing.

Test Procedure for Manual Use

The procedure below is for manual use, please see attached Appendix for automation use

A. Preparation of Reagents

- Bring all components and clinical specimens to be tested to room temperature. Mix well the Cut Off Control, Negative Control, Positive Control and the clinical specimens before use.
- Determine the total number of specimens to be tested. In addition to the specimens, the following must be included in each test: two wells of Cut Off Control, and one well of each Negative Control and Positive Control.
- Withdraw the microtiter plate from its aluminum pouch by cutting one end near the seal. Leave the required number of strips (according to the number of specimens to be tested) in the 96 well frame.
- Dilute the Concentrated Wash Buffer 1/20 with doubledeionized or distilled water. For example, in order to prepare one liter of Wash Buffer, add 50ml of the Concentrated Wash Buffer to 950ml of double-deionized or distilled water.

B. Incubation of sera samples and controls

- 5. Dilute each patient serum 1/11 with the supplied Serum Diluent-RT as follows: Add $25\mu l$ of patient serum to $250\mu l$ of Serum Diluent-RT.
- Pipette Cut Off Control in duplicate: 100μl into each well. Pipette 100μl of each: Negative Control, Positive control, and 1/11 diluted sera into separate wells of the test strip.
- 7. Cover the strips with a plate cover and incubate for 30 minutes at room temperature (22°C-28°C).
- 8. Discard the liquid content of the wells.
- 9. **Washing step:** Fill each well with Wash Buffer (300 350µl) up to the end of the well and discard the liquid, repeat this step 4 times, for a total of 5 washing steps.
- 10.Dry the strips and frame by gently tapping them over clean absorbent paper.

C. Incubation with Conjugate

Each vial of HRP Conjugate can only be used twice.

- 11.Pipette 100µl of ready to use HRP Conjugate into each well
- 12. Cover the strips with a plate cover and incubate for 30 minutes at room temperature (22°C-28°C).
- 13. Discard the liquid content and wash as described in steps 9-10.

D. Incubation with TMB - Substrate

- 14. Dispense 100μl TMB-Substrate into each well, cover the strips with a plate cover and incubate at room temperature (22°C-28°C) for **30 minutes**.
- 15. Stop the reaction by adding $100\mu l$ of Stop Solution (1M H_2SO_4) to each well.

E. Determination of Results

16. Determine the absorbance at 450/620nm and record the results. Determination should not exceed 30 minutes following stopping of chromogenic reaction.

Note: Any air bubbles should be removed before reading. The bottom of the ELISA plate should be carefully wiped

Test Procedure for Automated Use

The vials and reagents' volume have been adapted for automation applications.

A. Preparation of Reagents

- Bring all components and the clinical specimens to be tested to room temperature. Mix well the Cut-Off Control, Negative Control, Positive Control and the clinical specimens before use.
- Determine the total number of specimens to be tested. In addition to the specimens, the following must be included in each test: Two wells of Cut-Off Control and one well of each Negative Control and Positive Control.
- Withdraw the microtiter plate from its aluminum pouch by cutting one end near the seal. Leave the required number of strips (according to the number of specimens to be tested) in the 96 well frame.
- Dilute the Concentrated Wash Buffer 1/20 with doubledeionized or distilled water. For example, in order to prepare one liter of wash buffer, add 50ml of the Concentrated Wash Buffer to 950ml of double-deionized or distilled water.

B. Incubation of sera samples and controls

- 5. Dilute each patient serum 1/11 as follows: Dispense 250µl of **Serum Diluent-RT** to each sample's tube. Add 25µl patient serum to each sample's tube.
- Dispense 100μl each of Negative Control and Positive Control, 2x 100μl (duplicate) of Cut Off Control and 1/11 diluted serum samples into separate wells of the test strip.
- Incubate for 20 minutes at room temperature (22-28°C).
- 8. Eliminate assay drift caused by this operation.
- Washing step: Perform 5 X 500µl wash cycles using Savyon's Wash Buffer.
- 10. Perform 2 aspirate cycles with aspirate sweep.

C. Incubation with conjugate

Each vial of HRP Conjugate can only be used twice.

- 11. Dispense $100\mu l$ of Ready-to-Use HRP-conjugate into each well.
- 12.Incubate for 30 minutes at room temperature (22-28°C).
- 13. Wash as described in steps 9-10.

D. Incubation with TMB - Substrate

- 14. Dispense 100μ l TMB-Substrate into each well and incubate at room temperature (22-28°C) for **30** minutes in the dark.
- 15. Stop the reaction by adding $100\mu l$ of Stop Solution (1M H_2SO_4) to each well.

E. Determination of Results

16. Determine the absorbance at 450/620nm and record the results. Determination should not exceed 30 minutes following stopping of chromogenic reaction

Please note that each automation machine has specific technical commands. Please implement Savyon's automation procedure for this kit on the operation protocol of your automation machine.

Test Validation

For the test to be valid the following criteria must be met. If these criteria are not met the test should be considered invalid and should be repeated.

- 1. O.D. Positive Control \geq 0.8
- 2. Ratio O.D. Positive Control / O.D Cut Off Control > 2
- **3.** O.D.negative control < 0.3

Calculation of Test Results

- The average absorbance value of the Cut off serum run in duplicate should be calculated.
- In order to normalize the results obtained in different tests, the cut off index (COI) is calculated according to the following formula:

OD of the Serum Sample

COI =

OD Average of Cut Off Control

Interpretation of Results

Table 1:

COI	Result	Interpretation of Results
<1.0	Negative	No detectable IgA antibodies to C.trachomatis.
1-1.1	Borderline	Presence or absence of detectable (Borderline) levels of IgA antibodies to <i>C.trachomatis</i> cannot be determined. A second serum sample should be obtained after 14-21 days and tested. (When second serum sample is borderline the result should be considered as negative).
>1.1	Positive	Detectable levels of IgA antibodies to <i>C.trachomatis</i> .

Table 2: Interpretation of results based on IgG and IgA antibodies determination

Levels of C.trachomatis specific antibodies		Interpretation of Results
lgG	IgA	
Negative	Negative	Negative (or beyond the sensitivity of this test).
Positive	Negative or Borderline	May indicate past or current infection.
Borderline	Borderline	Second sample testing is required after 14-21 days. Repeated borderline results should be considered negative.
Positive	Positive	May indicate acute or chronic infection
Negative	Positive	May indicate acute or chronic infection

Performance Characteristics

Table 3: Sensitivity and Specificity

Sensitivity and Specificity of SeroCT RT IgA, was calculated using sera which have been defined by immunofluorescence assay Chlamydia IgA SeroFIATM (Savyon Diagnostics LTD.) as either negative or positive for C.Trachomatis. The study was carried out using 98 sera samples.

		SeroCT [™] RT-IgA	
SeroFIA [™]		Positive	Negative
Positive	26	26	0
Negative	72	0	72
Total	98	26	72

Sensitivity: 26/26 x 100 = 100% Specificity: 72/72 x 100 = 100%

Overall Agreement: 98/98x100 = 100%

Precision

Table 4: Intra-assay (within-run) precision of the SeroCTTM RT – IgA test is shown below:

Sample	No. of Replicates	Mean Value	CV%
Positive	10	1.433	7.0
Negative	10	0.082	11.4

Table 5: Inter-assay (between-run) precision of the SeroCTTM RT– IgA test is shown below:

Sample	No. of Replicates	Mean Value	CV%
Positive	10	0.813	4.1
Negative	10	0.073	9.0

Test Limitations

- No single serological test should be used for a final diagnosis. All clinical and laboratory data should be taken into account.
- Samples obtained too early during primary infection may not contain detectable antibodies. If Chlamydia infection is suspected, a second sample should be obtained 14-21 days later and tested in parallel with the original sample.

Bibliography

- Sarov, I.B., Shemer, A.Y., Manor, E., Zvilich, M., Lunenfeld, E., Piura, B., Chaim, W and Hagay, Z.(1989). Current topics in Chlamydia trachomatis Research. In: Serio, M.(Ed).Perspectives in Andrology; Raven Press, New York, 53: 355-366.
- Grayston, J.T., Kuo, C.C., Wang, S.P. and Altman J. (1986).
 The new *Chlamydia psitacci* strain, TWAR, Isolated in acute respiratory tract infections. New Eng. J. Med. 315: 161-168.
- Grayston, J. T., Kuo, C.C., Campbell, L.A. and Wang, S.P. (1989). Chlamydia pneumoniae sp. nov. for Chlamydia sp. strain TWAR. Int. J. Syst. Bacteriol. 39:88-90.

- Fukushi, H., and Hirai, K. (1992). Proposal of *Chlamydia pecorum* sp. nov. for Chlamydia strains derived from ruminants. Int. J. Syst. Bacteriol. 42:306-308.
- Stephens, R. S., Tam, M. R., Kuo, C. C. and Nowinski, R.C. (1982). Monoclonal antibodies to *Chlamydia trachomatis*: antibody specificities and antigen characterization. J. Immunol. 128:1083-1089.
- Stephens, R. S., Sanchez-Pescador, R., Wagar, E. A., Inouye, C. and Urdea, M.S. (1987). Diversity of *Chlamydia trachomatis* major outer membrane protein genes. J. Bacteriol. 169:3879-3885.
- Yuan, Y., Zhang, Y. X., Watkins, N. G. and Caldwell, H.D. (1989). Nucleotide and Deduced Amino Acid Sequences for the Four Variable Domains of the Major Outer Membrane Proteins of the 15 Chlamydia trachomatis Serovars. Infection and Immunity. 57:1040-1049. Copyright 1989, American Society for Microbiology.
- 8. Wang S.P., Kuo, C. C., Barnes, R.C., Stephens, R.S. and Grayston, J.T. (1985). Immunotyping of *Chlamydia trachomatis* with monoclonal antibodies. J. Infect Dis. **152**:791-800.
- Treharne J. D. (1985). The community epidemiology of trachoma. Rev Infect Dis. 7:760-763.
- Piura, B., Sarov, I., Sarov, B., Kleinman, D., Chaim, W. and Insler, V. (1985). Serum IgG and IgA antibodies specific for Chlamydia trachomatis in salpingitis patients as determined by the immunoperoxidase assay. Eur. J. Epidemiol 1: 110-116.
- the immunoperoxidase assay. Eur. J. Epidemiol 1: 110-116.

 11. Wang, S.P., Grayston, J.T., Kuo, C.C., Alexander, E.R., and Holmes, K.K. (1977). SeroDiagnosis of *Chlamydia trachomatis* infection with the microimmunofluorescence test. In: Nongonoccolcal urethritis and related infection, D. Hobson and K.K. Holmes (Eds), P. American Society for Microbiology, Washington DC. p. 237-248.
- Richard, L. S., Schachter, J. and Landers, D.V. z(1983).
 Chlamydial Infections in Obstetrics and Gynecology. Clinical Obstetrics and Gynecology 26:143
- Thompson III S. E., and Dretler R. H. (1982). Epidemiology and Treatment of Chlamydial Infections in Pregnant Women and Infants. Review of Infectious Diseases 4:S747
- Mardh A., Ripa, T., Svensson, L. and Westrom, S.(1977). Chlamydia Trachomatis Infection in Patients with Acute Salpingitis. Chlamydia Trachomatis and Acute Salpingitis. N. Engl. J. Med. No.24 296:1377-1379
- Grayston J. T., Campbell, L.A., Kuo, C.C., Mordhorst, C.H., Saikku, P., Thom, D.H. and Wang, S.P. (1990). A new respiratory tract pathogen. *Chlamydia pneumoniae* strain TWAR. J. Infect. Dis. 161:618-625.
- Hahn D. L., Dodge, R. W. and Golubjatnikow, R. (1991). Association of *Chlamydia pneumoniae* (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. JAMA 266: 225-230
- Saikku P., Mattila, K., Nieminen, M.S., Huttunen, J.K., Leinonen, M., Ekman, M.R., Makela, P.H., and Valtonen, V. (1988). Serological evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infection. Lancet II:983-986.
- 18. **Tsunekawa, T. and Kumamoto, Y.** (1989) A study of IgA and IgG titers of *C. trachomatis* in serum and prostatic secretion in chronic prostatitis. J.J.A. Inf. Dis. **63(2)**: 130-137.
- Kaneti, J., et al., (1988). IgG and gA antibodies specific for Chlamydia trachomatis in acute epididymitis. Europ. Urol. 14: 323-327.
- Sarov, I., Kleinman, D., Cevenini, R., Holcberg, G., Potashnik, G., Sarov, B. and Insler, V. (1986). Specific IgG and IgA antibodies to *Chlamydia trachomatis* in infertile women. Int. J. Fertil. 31 (3):193-197.



European Authorized Representative: Obelis s.a. Boulevard Général Wahis 53, B-1030 Brussels Tel: +32.2.732.59.54 Fax: +32.2.732.60.03 E-mail:mail@obelis.net