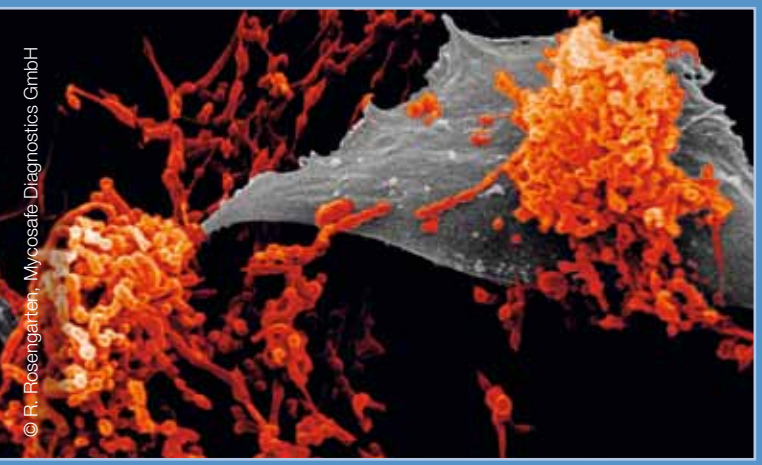
Your Power for Health

greiner bio-one



© R. Rosengarten, Mycosafe Diagnostics GmbH

CytoInspect™

Test Kit
for the Detection and Identification of Mycoplasma Species in Biologics

www.gbo.com/bioscience

CytoInspect™ - Flexible, Sensitive and Robust Mycoplasma Testing

The Threat of Mycoplasma Contamination

Mycoplasma contamination during biopharmaceutical manufacture is a significant threat to the production of high quality biotechnological products. Contamination with mycoplasmas, the smallest known class of self-replicating microorganisms, can compromise final product safety. Moreover, product yield can be reduced, leading to an increase in total costs.

Traditional mycoplasma detection assays are based on time-consuming, standard microbiological colony and culturing methods. Although officially approved, these methods present many disadvantages to the user including length of time required to complete an assay (28 days+), an inability to culture all mycoplasmas and the risk of cross contamination associated with live bacteria cultivation.

CytoInspect™ Mycoplasma Detection Kit

The CytoInspect™ assay from Greiner Bio-One utilises DNA microarray technology for detection and species-specific identification of mycoplasmas. With results delivered in 5 hours, CytoInspect™ is a rapid and effective alternative to culture-based mycoplasma assays. The assay shows superior sensitivity and specificity to traditional mycoplasma detection tests.

Fast Results and Flexible Throughput

The CytoInspect™ assay has been optimised for an efficient workflow, with results delivered in 5 hours (see below). Fast results mean that a contamination problem can be rapidly assessed and action taken, saving both time and money. The CytoInspect™ kit is part of a complete assay system, consisting of the CytoInspect™ DNA Extraction Kit, the CytoInspect™ kit, microarray scanning using the CheckScanner™ and automated results analysis with the CheckReport™Software. Day to day flexibility is assured, as the system can handle up to 48 in-parallel sample analyses.

CytoInspect™ at a Glance

- Fast results in 5 hours
- Identification of 40 mycoplasma species
- Detection of all mycoplasmas using a universal probe
- Validated sensitivity of < 10 CFU/ml
- Comprehensive on-chip controls
- Use of patent-pending CytoInspect™ DNA Extraction Kit for high efficiency mycoplasma isolation, allowing use of suspension culture matrices with up to 10^9 cells/ml
- Externally validated under GMP conditions
- Validated according to Ph. Eur. monographs 2.6.7, 2.6.21 and Ph. Jap. chapter 9
- Rapid, automated digital results analysis and report generation
- Microarray-based mycoplasma detection system
- CheckReport™Software developed acc. to FDA electronic records regulations (21 CFR part 11)

Working schedule for CytoInspect™

Mycoplasma identification in 5 hours

1 Sample collection

Duration: 10 minutes

2 DNA extraction

Duration: 60 minutes

3 PCR

Duration: 3 hours

4 Hybridisation

Duration: 30 minutes

External Test Validation

CytoInspect™ has been externally validated under GMP conditions in accordance with the regulatory guidelines for nucleic acid amplification technique (NAT)-based mycoplasma tests (European Pharmacopoeia monographs 2.6.7, 2.6.21 and Japanese Pharmacopoeia Chapter 9). Extensive validation assures confidence in the results generated by the CytoInspect™ assay.

Multiple Matrices Validated for Use with the CytoInspect™ DNA Extraction Kit

Mycoplasma DNA is efficiently extracted from samples using the patent-pending CytoInspect™ DNA Extraction Kit. The CytoInspect™ DNA Extraction protocol specifically enables the enrichment of mycoplasma DNA from cellular or acellular matrices. Importantly, use of the DNA Extraction Kit in conjunction with the CytoInspect™ assay has been validated for 3 separate matrices (see **Table 1**) and successfully used in practice for many more. The kit can be used to process sample volumes of 500 µl up to 50 ml, including cell cultures containing up to 10^9 cells/ml. CytoInspect™ is therefore useful for both in-process and end-product testing.

Table 1: CytoInspect™ Limit of Detection* for the 7 relevant Ph. Eur. mycoplasma strains validated as less than < 10 CFU/ml for three separate sample matrices

Mycoplasma Species	Tested Matrix		
	Cell Culture Media (Gibco RPMI 1640 + GlutaMAX)	Chinese Hamster Ovary (CHO) Cells (10^7/10ml)	Allantoic Fluid from Egg
A. laidlawii	< 1	< 5	< 5
M. fermentans	< 1	< 5	< 10
M. hyorhinis	< 1	< 8	< 9
M. gallisepticum	< 0.5	< 5	< 1
M. synoviae	< 1	< 3	< 5
M. arginini	< 2	< 8	< 9
S. citri	< 1	< 5	< 3

* The limit of detection was defined as the lowest concentration for which all samples (8 of 8 for a single dilution) were detected positive. In the case that an intermediate concentration had a lower detection rate, a conservative approach was adopted: the next higher concentration for which all samples (8 of 8) were detected positive was then specified as the LOD.

5 Washing

Duration: 10 minutes

6 Scanning

Duration: 10 minutes

7 Evaluation

Duration: 5 minutes

Mycoplasma Identification

Accurate Amplification with Touchdown PCR

Following DNA isolation, the CytoInspect™ assay uses touchdown PCR to specifically amplify a sequence of mycoplasma DNA from the 16S-23S intergenic spacer region (ITS) (**Figure 1**). The use of a touchdown protocol enhances the PCR amplification specificity and minimises the risk of mispriming. Careful bioinformatic analysis enables the use of a single set of fluorescently labelled primers, making the PCR reaction both specific and robust. dUTP is incorporated into the CytoInspect™ PCR Mastermix, enabling treatment of PCR reactions with Uracil-N-Glycosylase (UNG; not included in kit). UNG digestion of dUTP-containing amplification products thereby minimises the risk of cross contamination with amplicons from previous PCR reactions.

Mycoplasma Identification and Universal Detection

Mycoplasma species identification is achieved through use of the CytoInspect™ HTA™Slide* (DNA microarray). The CytoInspect™ consensus primers bind to mycoplasma-specific sequences. However the amplified ITS region contains species-specific sequences. Thus, fluorescently-labelled PCR products will hybridise to complementary sequences attached to the CytoInspect™ microarray (**Figure 2**). CytoInspect™ permits identification of the 40 most important mycoplasma species, these being the species found in over 99 % of all reported contaminations. In addition, a universal probe detects any species of mycoplasma present in the original sample (including *Acholeplasma sp., Spiroplasma sp. and Ureaplasma sp.*). Species determination can help identify the contamination source and thereby prevent further spread of an infection.

* The HTA™Slide platform is covered by U.S. Patent No. 8.007.744.

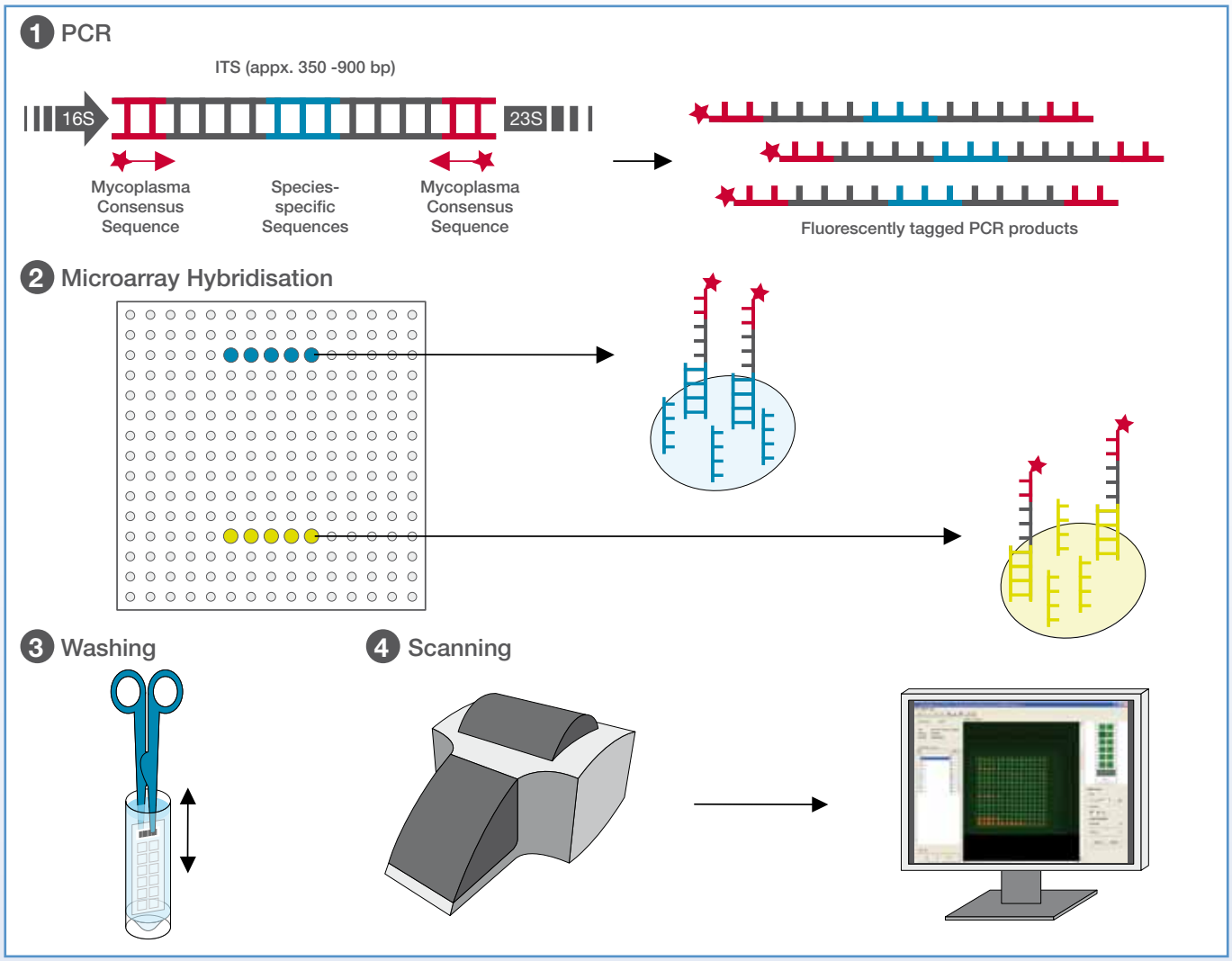


Figure 1: CytoInspect™ Mycoplasma Detection Overview. After sample DNA extraction, a sequence from the 16S - 23S intergenic spacer region (found in all mycoplasmas) is amplified with PCR using a highly conserved, fluorescently tagged primer pair. Labelled PCR products are then hybridised to complementary sequences fixed on the microarray. Species-specific DNA sequences are located within the amplified ITS region, allowing species identification. Each mycoplasma species is detected by five measuring points on the microarray. A universal probe allows detection of all mycoplasma species and provides secondary confirmation of species detection. Subsequent washing steps remove improperly bound probes. The bound and labelled probes are detected by excitation with monochromatic light with CheckScanner™. The CheckReport™Software automatically analyses CytoInspect™ results.

Specificity and Sensitivity

CytoInspect™ is a highly sensitive assay, with the validated LODs falling significantly below the 10 CFU/ml required by the pharmaceutical industry regulatory bodies for mycoplasma testing (**Table 1**). The phylogenetically-related bacterial species required to be tested by the European Pharmacopoeia were validated for CytoInspect™ as not resulting in false positive signals (**Table 2**). The mycoplasma-specificity of CytoInspect™ was further confirmed by extensive in-house testing with additional bacterial species, none of which resulted in false-positive signal production.

Table 2: Specificity of the CytoInspect™ assay: Cross-reactivity with phylogenetically-related bacteria. High concentrations of bacteria were added to samples and then assayed using CytoInspect™. None of the related bacteria resulted in a false positive signal.

Bacteria Species	Spiked Quantity	Positive Test	
		Species-Specific Probe	Universal Probe Positive
C. perfringens	10^5 CFU/ml	0/3	0/3
E. faecium	10^5 CFU/ml	0/3	0/3
L. casei	10^5 CFU/ml	0/3	0/3
St. epidermidis	10^5 CFU/ml	0/3	0/3
Str. agalactiae	10^5 CFU/ml	0/3	0/3
B. subtilis	10^5 CFU/ml	0/3	0/3

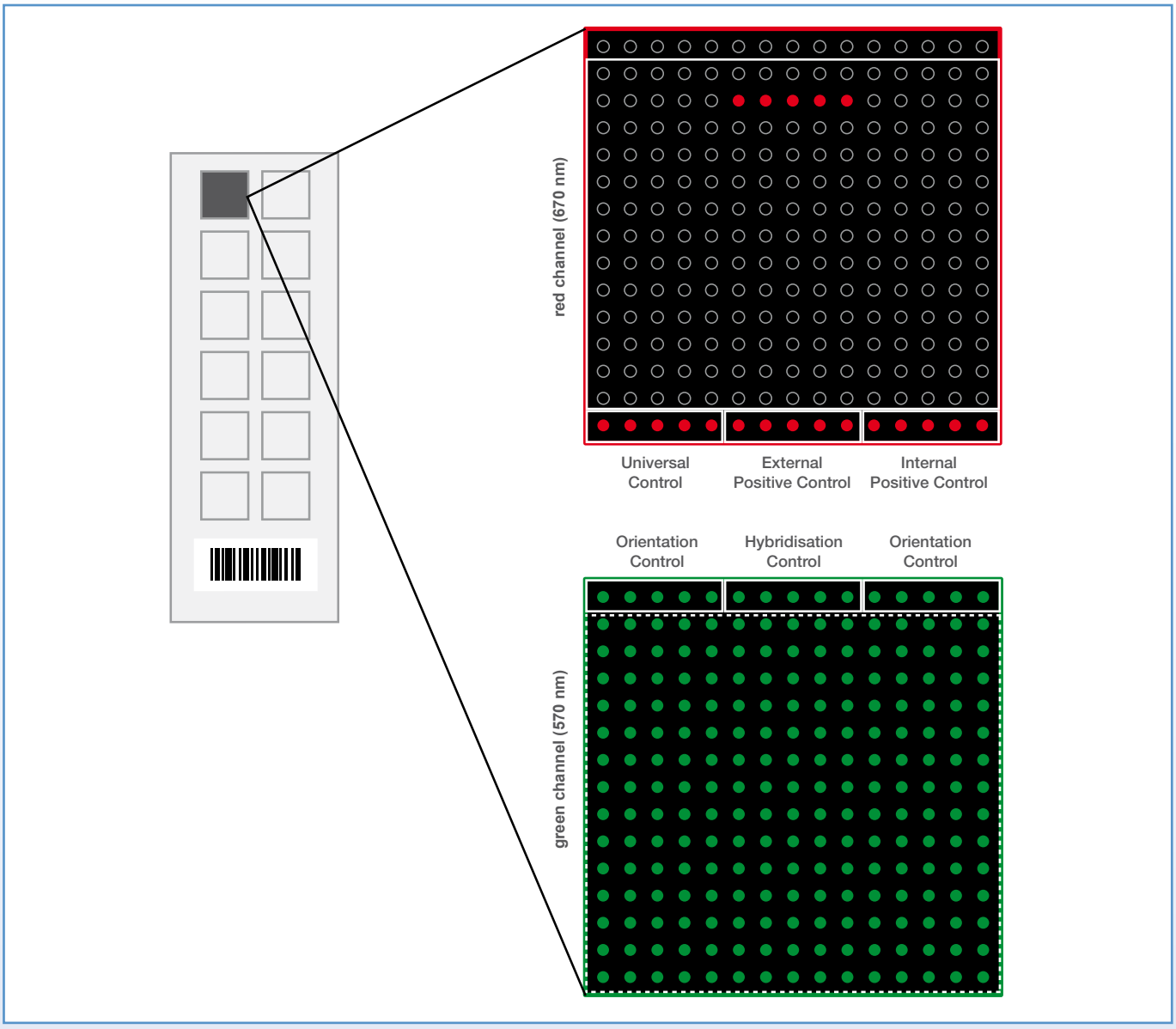


Figure 2: CytoInspect™ Chip Design and On-Chip Controls. A single CytoInspect™ microarray consists of 225 individually spotted DNA measuring points. The microarray is assessed using a laser scanner for both red and green fluorescence. The red channel includes the species identification probes (5 DNA measuring points per species), the universal mycoplasma species probes, and both the internal and external PCR controls. In the green channel, the printing and hybridisation controls are assessed and microarray orientation confirmed

Automated Data Analysis

Internal and External On-Chip Controls

As mandated by European Pharmacopeia guidelines for NAT-based testing, the CytoInspect™ microarray integrates a comprehensive range of controls (**Figure 2**), allowing monitoring of the entire assay. The protocol incorporates an internal positive control that tests for a successful DNA extraction. A DNA construct containing the primer binding sites used for mycoplasma amplification is added to a test sample before DNA extraction. After successful extraction and amplification, the internal positive control binds to the microarray at 5 DNA spots assessed in the red fluorescence channel. The CytoInspect™ PCR MasterMix contains an external positive control template which is bound by fluorescence-labelled primers and thereby subjected to PCR amplification. These labelled PCR products bind to 5 specific DNA 'External positive control' measuring points on the CytoInspect™ microarray, thereby allowing PCR quality evaluation. The green fluorescent channel is used to monitor printing quality of the DNA spots, hybridisation efficiency and orientation of the CytoInspect™ microarray within the CheckScanner™.

Validation of Assay Robustness

CytoInspect™ is a highly robust assay. Alongside overall day to day and operator variability, key parameters of the PCR amplification, hybridisation and washing procedures have been additionally validated for robustness (see **Table 3**).

FDA-Compliant CheckReport™Software for Automated Analysis

After PCR products are hybridised to the CytoInspect™ microarray, the two-channel CheckScanner™ evaluates the fluorescent signals and transfers the data to the CheckReport™Software for automated analysis. CheckReport™Software utilises sophisticated algorithms to evaluate signal to noise ratios for fluorescence at each DNA spot. The presence / absence of mycoplasma species and the on-chip controls are automatically evaluated and results presented in user-friendly tabular format (see **Figure 3** for a screenshot of the results report). CheckReport™Software fulfills the requirements of the FDA electronic records regulations (21 CFR part 11) and produces QM-compliant electronic documentation. Moreover, CheckScanner™ and CheckReport™Software can be fully integrated into a laboratory information management system (LIMS).

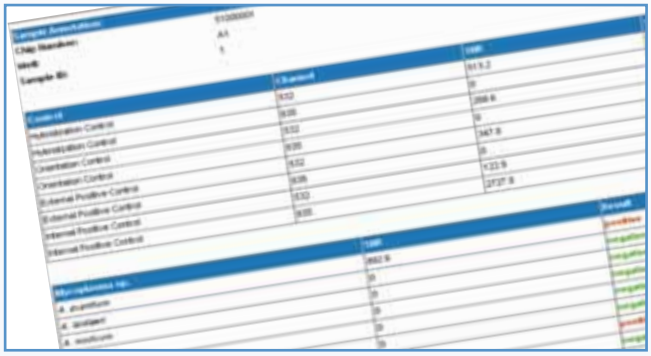


Figure 3: CheckReport™Software automated analysis of CytoInspect™ results. With an easy-to-use, intuitive software interface, CheckReport™Software allows users to quickly assess and understand Cytoinspect™ results. Barcodes enable painless tracking of both microarrays and associated results.

Table 3: Robustness of the CytoInspect™ Protocol. Various assay parameters were varied from standard values to simulate possible user errors. The effect of the parameter on final results was then evaluated. Tested mycoplasma species were those listed in Table 1 (10 CFU per sample and 3 samples per test). Delivery of expected result refers to either a mycoplasma-spiked assay producing a positive end-result on both the species-specific and universal mycoplasma probes or a mycoplasma-negative sample producing a negative end-result.

Parameter Tested	Parameter Range Tested	Delivery of Expected Result
PCR Amplification		
Amount of Taq Polymerase	-50 %	100 %
Amount of Template	±50 %	100 %
Influence of Matrix Genomic DNA (CHO Cells)	±100 %	100 %
Hybridisation		
PCR Product Volume per Reaction	±12.5 %	100 %
Buffer Volume per Reaction	±17 %	100 %
Incubation Time	±50 %	100 %
Hybridisation Temperature	-18 %, +27 %	100 %
Cross-Contamination Events	6 positive, 6 negative	100 %
Chip Washing		
Washing Time	±33 %	100 %
Washing Temperature	±10 %	100 %

CytoInspect™ Performance Data

Result Type	Qualitative with Species Identification
Time to Result	~5 hours
Validated Matrices	CHO cells; allantoic fluid from egg; cell culture media (Gibco RPMI 1640 + GlutaMAX)
Suspension Culture Cell Concentration	Up to 10^9/ml
Usable Sample Volumes	500 µl to 50 ml
PCR Specifications	Touchdown PCR protocol; dUTP in MasterMix for UNG treatment
Limit of Detection	< 1-10 CFU/ml for *A. laidlawii, M. arginini, M. gallisepticum, M. fermentans, M. hyorhinis, M. synoviae, S. citri*
Specificity	No cross reactivity with *Bacillus subtilis* *Clostridium difficile* *Clostridium perfringens* *Enterococcus faecalis* *Enterococcus faecium* *Escherichia coli* *Eubacterium nodatum* *Lactobacillus casei* *Peptostreptococcus micros* *Staphylococcus epidermidis* *Staphylococcus haemolyticus* *Staphylococcus saprophyticus* *Streptococcus agalactiae* *Streptococcus gordonii* *Streptococcus mitis* *Streptococcus mutans* *Streptococcus oralis* *Streptococcus pneumoniae* *Streptococcus pyogenes* *Veillonella parvula*

Ordering Information

CytoInspect™ Kit and Accessories

Cat. No.	464 060	464 070	516 070
Description	CytoInspect™ Identification of mycoplasma species	CytoInspect™ Identification of mycoplasma species	CytoInspect™ DNA Extraction Kit
Tests per case	test kit for 10 reactions	test kit for 60 reactions	extraction kit for 50 preparations

Your Power for Health

greiner bio-one

For further information, please visit our website **www.gbo.com/bioscience** or contact us:

Germany (Main office)
Greiner Bio-One GmbH
Maybachstraße 2
D-72636 Frickenhausen
Phone (+49) 70 22 948-0
Fax (+49) 70 22 948-514
E-Mail info@de.gbo.com

Austria
Greiner Bio-One GmbH
Phone (+43) 75 83 67 91-0
Fax (+43) 75 83 63 18
E-Mail office@at.gbo.com

Belgium
Greiner Bio-One BVBA/SPRL
Phone (+32) 24 61 09 10
Fax (+32) 24 61 09 05
E-Mail info@be.gbo.com

Brazil
Greiner Bio-One Brasil
Phone (+55) 19 34 68 96 00
Fax (+55) 19 34 68 96 01
E-Mail office@br.gbo.com

China
Greiner Bio-One Suns Co. Ltd.
Phone (+86) 10 83 55 19 91
Fax (+86) 10 63 56 69 00
E-Mail office@cn.gbo.com

France
Greiner Bio-One SAS
Phone (+33) 1 69 86 25 50
Fax (+33) 1 69 86 25 36
E-Mail infos@fr.gbo.com

Japan
Greiner Bio-One Co. Ltd.
Phone (+81) 3 35 05 88 75
Fax (+81) 3 35 05 88 79
E-Mail info@jp.gbo.com

Netherlands
Greiner Bio-One B.V.
Phone (+31) 1 72 42 09 00
Fax (+31) 1 72 44 38 01
E-Mail info@nl.gbo.com

UK
Greiner Bio-One Ltd.
Phone (+44) 14 53 82 52 55
Fax (+44) 14 53 82 62 66
E-Mail info@uk.gbo.com

USA
Greiner Bio-One North America Inc.
Phone (+1) 70 42 61 78 00
Fax (+1) 70 42 61 78 99
E-Mail info@us.gbo.com

Revision: July 2012 – F074 029