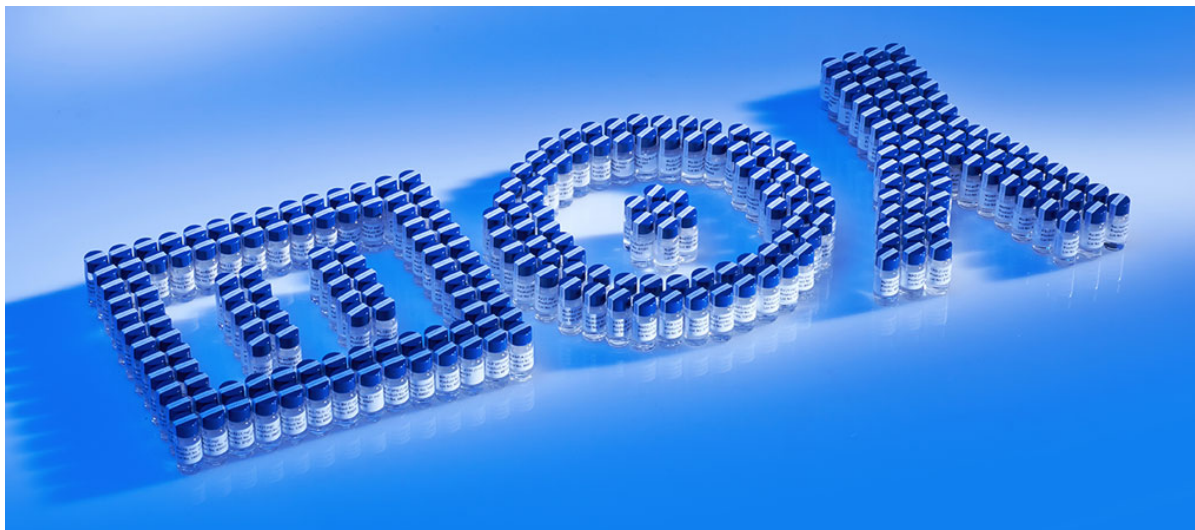


DIARECT Newsletter

Summary

- New in Autoimmunity: Biotinylated Antigens
- New Infectious Disease Antigens: *Bartonella henselae* and *Babesia microti*

Dear Madam or Sir,

As in the past years, DIARECT will attend the largest international trade fair for medical industry, MEDICA, with over 4,900 exhibitors, taking place in Düsseldorf, Germany from November 13 - 16, 2017. More than 127,000 expected visitors will have the opportunity to connect with global clinical market leaders in laboratory medicine and learn about cutting-edge technologies. To read more about the event click here.

DIARECT would like to invite all attendees to stop by our **booth D27 in the temporary hall 3A**.

We are pleased to introduce new additions to our current product lines

- Autoimmunity, including recently launched biotinylated autoantigens
- Infectious Disease Serology
- Allergy

To get an overview of all our products, including the antigens to be presented at this year's MEDICA, have a look at our new product list, available at this link: product list

Current issue of our Newsletter gives you a first impression and description of the newest additions to our extensive product portfolio.

We are looking forward to seeing you in Düsseldorf.
DIARECT AG

New in Autoimmunity: Biotinylated Antigens

Bead-based technologies for *in-vitro* diagnosis provide several advantages compared to traditional single protein measurement techniques (as the gold standard: ELISA).These include high sensitivity, low sample volume, lower costs, all of which allow rapid turnover while generating reproducible data, through ease of performance and elimination of the need for trained personnel and specialized procedures. Multiplex analysis is used to simultaneously look for multiple targets in one sample applying highly standardized assay procedures with automation and high throughput capability.
Increasing awareness of multifactorial nature of various diseases and pathological states calls for simultaneous, time-saving and cost-effective measurement of multiple analytes, also at sample volumes, which are too small for traditional immunoassays. Multiplex systems could provide a cost-effective solution to the omnipresent demand for productivity increases and have the potential to dramatically accelerate biological and biomedical research by enabling the comprehensive analysis of patient samples to become inexpensive, routine and widespread. The rapidly evolving methodologies already widely used in basic research are slowly penetrating the *in-vitro* diagnostic market.

However, limitations of protein microarrays such as inadequate sensitivity, reproducibility, and scalability and especially higher costs of manufacture prevent these approaches from completely replacing standard techniques.

In bead-based arrays (suspension or liquid arrays) usually 5.6 µm polystyrene particles are used, and the protocol is similar to a classic sandwich ELISA. The use of magnetic beads enables the automation of washing steps as well as construction of fully automated analyzers.

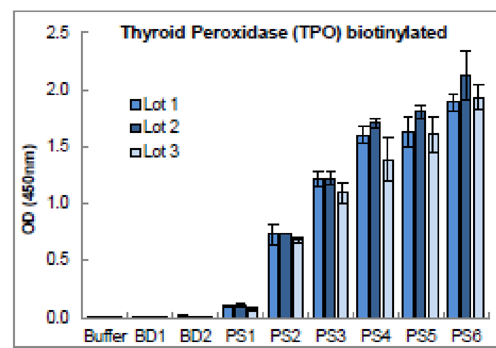
As in some second-generation ELISA technologies, bead-based arrays use biotin-conjugation of the antigen and subsequent conjugation to streptavidin-coated surfaces/beads. Using this approach the strength of attachment of antigens to the base is increased, which results in higher assay sensitivities (WHO 2006).

Biotin, formerly designated vitamin H or coenzyme R, is a water-soluble B-vitamin also known as vitamin B7, and is found in small amounts in numerous foods. It acts as an essential coenzyme for four carboxylases that catalyze essential steps in intermediary metabolism (Wallace *et al.* 1999).
Streptavidin is a homo-tetrameric protein (60 KDa) isolated from the actinobacterium *Streptomyces avidinii*. It has been reported to have binding ability similar to the one originally known from avidin, which was originally isolated from egg yolk (Green *et al.*1990).

Binding of biotin to streptavidin / avidin is one of the strongest non-covalent interactions known in nature, which results from several factors: the formation of multiple hydrogen bonds and van der Waals forces between biotin and the protein, together with surface polypeptide loops that bury the biotin within the protein. Structural alterations at the biotin binding site produce quaternary changes in the streptavidin tetramer, which promote formations in the twisted beta-sheets that link the tetramer subunits (Weber *et al.* 1989).

For validation of DIARECT's biotinylated antigens a bead-based ELISA was performed using magnetic beads (Dynabeads™ M-280 Streptavidin; Thermo Fisher Scientific) coupled with biotinylated antigens. Coupling was performed according to manufacturer's recommendations with suitable modifications made. The standard ELISA protocol using HRP-conjugated secondary antibodies was adapted to the use of magnetic beads which were effectively washed by taking advantage of magnetic separation. Optical density (OD) was determined using a standard plate reader.

Catalog No.	Product name
20100 / 20101	Thyroid Peroxidase (TPO) biotinylated
20200 / 20201	Thyroglobulin (non rec.) biotinylated
20300 / 20301	Ro/SS-A (60 kDa; rec.) biotinylated
20400 / 20401	Ro/SS-A (60 kDa; non rec.; bov.) biotinylated
20500 / 20501	Ro/SS-A (52 kDa) biotinylated
20600 / 20601	La/SS-B biotinylated
20700 / 20701	GBM biotinylated
20800 / 20801	GAD65 biotinylated



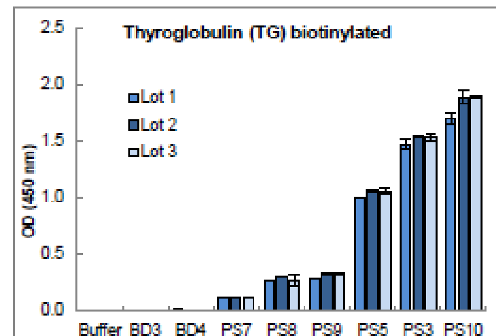


Figure: Three independent lots of TPO (upper graph) and TG (lower graph) were biotinylated and coupled onto streptavidin-coated microspheres. IgG specific responses to the antigens were detected from healthy donor (BD 1 – 4) and patient samples (PS 1 – 10), respectively (double determination). Buffer and non-biotinylated antigens (data available on request) were included as negative controls.

References:

Green et al. (1990) Methods Enzymol 184: 51-67
Wallace et al. (1999) Biochem J 30: 1-5
Weber et al. (1989) Science 243: 85–88
WHO (2006) Environmental Health Criteria 236: 1–333

For questions or additional information please feel free to contact us at:
Tel: +49 761 47979-0
Email: info@diarect.com

New Infectious Disease Antigens: *Bartonella henselae* and *Babesia microti*

The rod-shaped, gram-negative and facultative intracellular pathogen *Bartonella henselae* causes several forms of Bartonellosis, including Cat Scratch Disease (CSD) and Bacillary Angiomatosis (BA) (Anderson *et al.* 1995).

CSD was first identified as clinical entity in 1950 in France (Debré *et al.* 1950). A candidate bacterium was observed in 1983 in CSD-patients (Wear *et al.* 1983) and named *Afipia felis* (Brenner *et al.* 1991). In 1992 Regnery *et al.* discovered a new etiologic agent for an infection similar to *A. felis* also identified as causative agent of the clinical syndrome bacillary angiomatosis (Stoler *et al.* 1983). These bacteria in an alpha subdivision of proteobacteria were called *Rochalimaea henselae*, and after later phylogenetic analysis, they were classified under the *Bartonella* genus (Brenner *et al.* 1991).

Due to difficulties in primary culturing of patient samples, indirect immunofluorescence analysis is difficult, which leads to variation in diagnostic test results. The use of recombinant antigens may therefore improve diagnostic accuracy. It is reported that up to 88 or 95% of patients with CSD present antigens against *B. henselae* in sera when tested by an ELISA (Kabeya *et al.* 2003).

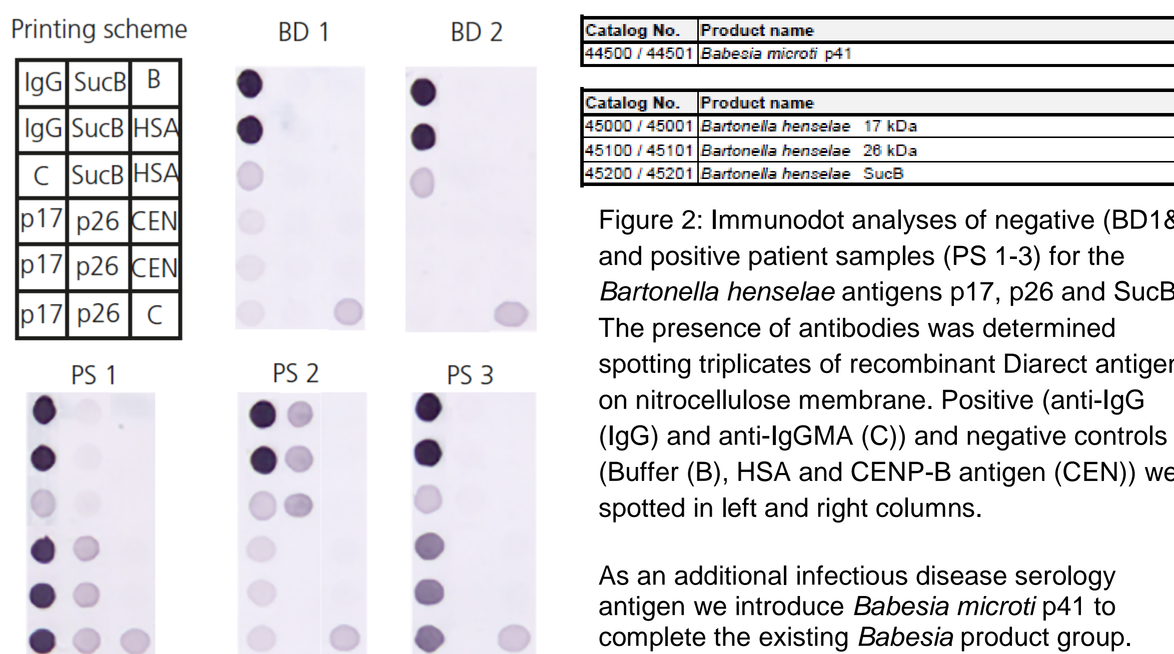
The key factors used as antigens in diagnostics are highly immunoreactive proteins produced in *B. henselae*. Through a Type IV secretory system, *B. henselae* proteins are transported into the host cells, provoking a vascular proliferation in infected endothelial cells (Hoey *et al.* 2009).

The first antigen found to be strongly reactive with sera from CSD patients was p17 (Sweger *et al.* 2000), and it is considered to be a good and species-specific marker for *B. henselae* during early stages of infection. The gene encoding this protein lies in the virB-operon of the Type IV secretion system (Hoey *et al.* 2009). The N-terminus of the protein is hydrophobic, while the rest of the sequence is hydrophilic or neutral and may represent the surface-exposed epitopes. It is presumed to be a bacterial membrane-associated protein containing lysine residues at the N-terminus that interact with phospholipids of the membrane, a typical motif for outer surface proteins. The hydrophoibic residues form a membrane-spanning or membrane anchor domain (Anderson *et al.* 1995). As a homolog of the virB5 family, it is strongly suggested to be involved in host recognition by the type IV secretion system,

most likely though protein-protein interactions involving amino acids within the C-terminus (Hoey *et al.* 2009).

Outer membrane protein p26 was identified and characterized by Werner *et al.* (2006). The encoding gene has significant nucleotide identity with orthologs in *Brucellae* spp., *Bartonellae* spp., and several plant-associated bacteria. It is expressed during feline infection, contains a hydrophobic transmembrane region and four dominant antigenic sites (Werner *et al.* 2008).

Molecular characterization of the *Bartonella* spp. gene SucB revealed, that it encodes the immunogenic protein dihydrolipoamide-succinyltransferase, an enzyme of the alpha-ketoglutarate dehydrogenase complex (Gilmore *et al.* 2003; Kabeya *et al.* 2003). Analysis of the SucB protein crystal structure showed that the E2 subunit is composed of 23 subunits arranged with 432 point group symmetry. The catalytic domain catalyzes the transfer of a succinyl group from the S-succinyldihydrolipoyl moiety to coenzyme A. The protein is divided into an N-terminal domain, an intermediate linker domain, which is in contact with other proteins of the complex, and the enzyme domain in the C-terminus. The active site of the catalytic domain is located in the middle of a channel formed at the interface between two 3-fold related subunits (Knapp *et al.* 1998). Antigenic cross-reactivity of SucB with other *Bartonella* and *Brucella* species is well known, for example the protein was found to be 76.3% identical to the dihydrolipoamide succinyltransferase enzyme (SucB) of *Brucella melitensis* (Litwin *et al.* 2004).



Catalog No.	Product name
44500 / 44501	*Babesia microti* p41

Catalog No.	Product name
45000 / 45001	*Bartonella henselae* 17 kDa
45100 / 45101	*Bartonella henselae* 26 kDa
45200 / 45201	*Bartonella henselae* SucB

Figure 2: Immunodot analyses of negative (BD1&2) and positive patient samples (PS 1-3) for the *Bartonella henselae* antigens p17, p26 and SucB. The presence of antibodies was determined spotting triplicates of recombinant Diarect antigens on nitrocellulose membrane. Positive (anti-IgG (IgG) and anti-IgGMA (C)) and negative controls (Buffer (B), HSA and CENP-B antigen (CEN)) were spotted in left and right columns.

As an additional infectious disease serology antigen we introduce *Babesia microti* p41 to complete the existing *Babesia* product group.

References:

Anderson et al. (1995) J Clinical Microbiol. 9: 2358–2365
Brenner et al. (1991) J Clinical Microbiol. 29: 2450–2460
Debré et al. (1950) Bull Mem Soc Med Hop. 66: 76– 79
Gilmore et al. (2003) Infect Immun. 71: 4818–4822.
Hoey et al. (2009) Clin Vaccine immunol. 16: 282–284
Kabeya et al. (2003) Microbiol and Immunol. 47: 571–576
Knapp et al. (1998) J Mol Biol. 280: 655–668
Litwin et al. (2004) J Med Microbiol. 53: 1221–1227
Regnery et. al. (1992) The Lancet. 340: 557-558
Stoler et al. (1983) Am J Clin Pathol. 80: 714–718
Sweger et al. (2000) Clin Diagn Lab Immunol. 7: 251–257
Wear et al. (1983) Science. 221: 1403-1405
Werner et al. (2008) Comp Med. 58: 375–380
Werner et al. (2006) Clin Vaccine Immunol. 13: 830–836

For questions or additional information please feel free to contact us at:
Tel: +49 761 47979-0
Email: info@diarect.com

Distributors

To facilitate the world-wide sales of our products, DIARECT has distribution agreements with select companies.

If you are located in North America or Canada, DIARECT's products are exclusively distributed by:

SurModics IVD, Inc.
9924 West 74th Street
Eden Prairie, MN 55344
USA
Tel +1 952 500 7200
Toll-free +1 800 755 7793
Fax +1 952 500 7201
orders@surmodics.com
www.surmodicsivd.com

If you are located in China, DIARECT's products are exclusively distributed by:

Shanghai BioSun Sci&Tech Co., Ltd.

Room K, 5F, Bldg. No.1
825 Zhaojiabang Road
Shanghai 200032
P.R. China

Tel +86 21 54253791
Fax +86 21 64280007
biosun@biosun.cn
www.biosun.cn

Feedback

If you would like to receive more detailed information on DIARECT products, please follow this link to our contact and order form.

Contact Details

Address: Bötzinger Strasse 29 B, 79111 Freiburg, Germany
Phone: +49 761 479 79-0
Fax: +49 761 479 79-29
Email: info@diarect.com

DIARECT AG is a DIN EN ISO 9001 and 13485 certified company.
Copyright © 2005 - 2016 DIARECT AG. All rights reserved. www.diarect.com