Product Catalogue

Diagnostics for the Determination of Autoantibodies, for Infectious Serology and Allergology

Indirect Immunofluorescence — ELISA — RIA — Westernblot
EUROASSAY — EUROLINE — EUROPLUS — EUROArray
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EUROIMMUN was founded in September 1987 and today has its headquarters in Luebeck, Germany. Branches are situated in Groß Groenau near Luebeck (Schleswig-Holstein), in Dassow (Mecklenburg-Western Pomerania), Rennersdorf (Upper Lusatia, Saxony), and in Pegnitz (Upper Franconia, Bavaria). Further EUROIMMUN subsidiaries can be found in Canada (Mississauga), China (Beijing, Hangzhou), Great Britain (Pontypool in Wales), Italy (Padua), Lebanon (Beirut), Poland (Wroclaw), Switzerland (Lucerne), Singapore, South Africa (Capetown), Turkey (Istanbul) and the USA (New Jersey). At present EUROIMMUN has 775 employees in Germany, 990 worldwide. The company is ISO-certified (EN ISO 9001:2008, EN ISO 13485:2003/CMDCAS).

EUROIMMUN produces reagents for medical laboratory diagnostics. In the foreground are test systems for the determination of various antibodies in patient serum in the diagnosis of autoimmune diseases, infectious diseases and allergies.

The test methods employed are predominantly indirect immunofluorescence, microplate ELISA, various blot techniques (Westernblot, EUROASSAY, EUROLINE, EUROLINE-WB) and all molecular biology techniques. The company is based on worldwide-patented state-of-the-art production methods and microanalysis techniques and is one of the world’s leading manufacturers of medical laboratory diagnostics.

The BIOCHIPs are one of EUROIMMUN’s many inventions: paper-thin sheets of glass are coated with cells or tissue sections and then cut automatically into millimetre-sized fragments which are subsequently glued onto slides using a fully automated device. This BIOCHIP technology allows extreme miniaturization and standardization of immunobiochemical analyses. With BIOCHIP Mosaics made from 30 or more different organ sections, cell substrates or defined antigens (EUROPLUS) only minimum incubation efforts are necessary to obtain a detailed antibody profile.

In EUROIMMUN enzyme immunoassays (ELISA) defined antigens, purified using state-of-the-art biotechnological processes, are employed as the antigen substrate. Some of these antigens are synthesized in the company’s molecular biology laboratories. EUROIMMUN ELISA are characterized by their excellent stability, simple handling and short incubation times, and they are ideal for automated use. All reagents are delivered ready-to-use and are exchangeable between different lots. EUROIMMUN offers the largest and most differentiated arsenal of enzyme immunoassays worldwide for the diagnosis of autoimmune and infectious diseases.
The innovative procedures **EUROASSAY** and **EUROLINE** developed by EUROIMMUN follow the same test principle as the ELISA methods, with the use of the BIOCHIP technology. At EUROIMMUN purified antigens are printed in parallel lines at defined positions on membrane strips. Following incubation, users can evaluate results visually without additional equipment. These reagents allow in particular the differentiation of antibodies that are not clearly defined microscopically by indirect immunofluorescence. EUROASSAY and EUROLINE are also employed in laboratories where no sophisticated laboratory instruments are available.

EUROIMMUN produces an extensive range of **Westernblot strip** test systems and corresponding reagents for confirmation of positive fluorescence and ELISA results as well as clarification of difficult-to-interpret results in autoimmune diagnostics, infectious serology and allergology. Refined electrophoreitical processes have been developed to allow the precise separation of diagnostically relevant proteins from one another. Lot-specific evaluation templates are produced for the evaluation of band patterns. The program “**EURO-LineScan**” enables fully automated evaluation of membrane-based test systems and simplifies the archiving of results with large sample series.

One of the company’s main strengths is its technical expertise. This encompasses not just the manufacture and sale of medical laboratory diagnostics, but also the diagnostic application of the products in a reference laboratory which provides highly differential diagnostics. This reference laboratory has set standards in Germany, and worldwide is unequalled in the whole field of autoimmune diagnostics. The diagnostic spectrum of the laboratory also covers the areas of infectious serology and serological allergy diagnostics. The reference laboratory receives hundreds of serum samples daily from all over Germany as well as from many other countries. It helps EUROIMMUN customers to secure their results: a large proportion of serum samples sent to EUROIMMUN for evaluation are analysed free of charge in order to maintain high standards in the laboratories of EUROIMMUN customers. Customers can obtain further technical information from experienced scientists in the company, with whom they can also discuss serological problem cases. The “Institute for Quality Assurance”, an institution newly founded by EUROIMMUN, organises unbiased quality assessments and provides advice in the area of quality management. Moreover EUROIMMUN has established the “Institute for experimental Immunology”, which is engaged in basic research.

In October 2010, the **EUROIMMUN workforce** included 150 university and college graduates, among these biologists, biochemists, chemists, engineers and medical doctors (48 of them holding a doctor’s degree). Medical technicians are particularly strongly represented with 103 people, corresponding to EUROIMMUN’s activities, as well as biology/chemistry laboratory technicians (81). At present the company is training 55 young people as biology laboratory assistants, industrial clerks, IT specialists, electronic system technicians, electronic technicians for devices and systems, industrial mechanics, lathe operators and cooks as well as business information technology specialists and business economists (dual system). At EUROIMMUN great value is placed on advising customers and prospective customers in a factual, technical and commercially restrained manner and fully supporting them in the use of our diagnostically demanding products. EUROIMMUN products are backed by an energetic and competent sales force, qualified information material, didactic test instructions and scientifically based, but nevertheless understandable advertisements in technical journals. Advertising material is produced in-house using the latest desktop publishing methods, right up until the fully digitalised ready-for-exposure documents. The most important publications and posters are translated into many languages. EUROIMMUN has set up an informative homepage on the internet (www.euroimmun.com) which is visited extensively internationally.

**Over 3,000 laboratories worldwide use EUROIMMUN diagnostics.** 400 of these are in Germany. The company’s development is shaped by continuous growth. Although the diagnostic market in Germany stagnated and has become particularly strongly competitive, EUROIMMUN has been able to continue its strong expansion. The company is achieving an ever increasing independence from the German market, since more and more products are sold abroad. With their quality and standardization, **EUROIMMUN products** are capturing the leading position in the world.
TECHNIQUES FOR THE SEROLOGICAL INVESTIGATION OF ANTIBODIES
Indirect Immunofluorescence: An Easy and Modern Method

Principle of the Test
- For the determination of autoantibodies or antibodies against infectious agents, cells, tissue sections or purified, biochemically characterized substances are used as antigen substrates.
- If the sample is positive, specific antibodies in the diluted serum sample attach to the antigens coupled to a solid phase.
- In a second step, the attached antibodies are stained with fluorescein-labelled anti-human antibodies and visualized with the fluorescence microscope.
- Positive samples can be titrated in steps. The most suitable titration interval is provided by the dilution factor 3.16 (square root of 10). In this way, every second step represents in its denominator an integral power of 10 (1:10, 1:32, 1:100, 1:320, 1:1000, 1:3200, 1:10000 etc.).

Indirect Immunofluorescence: A Standardized Technique for the Determination of Autoantibodies and Antibodies against Infectious Agents
- High specificity: positive and negative samples produce a large difference in signal strength. Each bound antibody shows a typical fluorescence pattern depending on the location of the individual antigens.
- The entire antigen spectrum of the original substrate is available, thus allowing the detection of a large number of antibodies and achieving a higher detection rate.
- Immunofluorescence enables simultaneous detection of antibodies against several biochemically different antigens on one single biological substrate.
- The indirect immunofluorescence test is the analytical method of choice when it would be too difficult or too complicated to prepare the test antigens individually for enzyme immunoassays.

EUROIMMUN’s Innovations for the Standardization and Modernization of Indirect Immunofluorescence
- Activation technique: physically or chemically activated cover glasses are coated with cultured cells or tissue sections. Frozen tissue sections are fixed to the glass surface by covalent bonding, increasing adhesion more than 100 times and thus preventing the substrates from being detached.
- BIOCHIP Technology: cover glasses coated with biological substrates are cut into millimetre-sized fragments (BIOCHIPs) on a machine. This makes it possible to obtain ten or more first-class preparations of homogeneous quality per tissue section, in the case of cultured cell substrates even several thousands.
- BIOCHIP Mosaics™: using several BIOCHIPs coated with different substrates side by side on one and the same reaction field, antibodies against various organs or infectious agents can be investigated simultaneously. Detailed antibody profiles can thus be established with comparatively little effort, allowing the reciprocal determination of the results on different substrates.
- TITERPLANE™ Technique: samples or reagents are applied to the reaction fields of a reagent tray. The BIOCHIP Slides are then placed into the recesses of the reagent tray, where all BIOCHIPs come into contact with the fluids, and the individual reactions commence simultaneously. As the fluids are confined in a closed space, there is no need for the use of a conventional „humidity chamber“.
Indirect Immunofluorescence: An Easy and Modern Method

Chemically Activated Cover Glasses for Histochemistry

- For diagnostics of organ-specific or tissue-specific autoantibodies frozen tissue sections of various organs are used. However, formerly, the morphology of tissues suffered during incubation in aqueous medium, tissue parts occasionally became detached from slides, and the interpretation of results was difficult.
- Using the activation technique for the first time in histology, we have applied solid phase techniques. Firstly, the surface of cover glasses is coated with spontaneously reactive aldehyde groups. In a second step, the tissue sections are applied to the chemically activated cover glasses (Stöcker, W: European Patent No. 0117262; U.S. Patent No. 4,647,543). Free amino groups of the tissue sections, especially of the hydroxylsine contained in the collagen, bind to the carrier material by covalent bonding.
- This results in an increased adhesion of frozen tissue sections more than a hundredfold and prevents them from being detached during incubation. Furthermore, in some cases the activation technique results in a significantly better conservation of tissue structures, especially in organs which previously exhibited a generally low level of adhesion. Therefore, the tests can be evaluated with considerably greater confidence.

Determination of Low-Avidity Antibodies

- An alternative principle for the serological diagnosis of fresh infections has been established by investigating the antibody avidity.
- The first reaction of the immune system following an infection is the formation of low-avidity antibodies. As the infection proceeds, increasingly antigen-adapted IgG is formed, and avidity grows. As long as high-avidity IgG is not yet detected in the serum, it can be assumed that the infection is still in an early stage.
- To identify low-avidity antibodies in a patient’s serum, two immunofluorescence tests are performed in parallel: one test is carried out in the conventional way, the other one includes urea treatment between incubations with patient’s serum and peroxidase-labelled anti-human IgG, resulting in the detachment of low-avidity antibodies from the antigens.
- Low-avidity antibodies are present if the fluorescence intensity is significantly reduced (two intensity levels or more) by urea treatment.
- The following test kits for avidity determination are available: Toxoplasma gondii, Rubella virus, West-Nile virus, CMV, EBV-EA, EBV-CA.

EUROPLUS™ System: Combination of conventional immunofluorescence substrates and monospecific tests

- In EUROPLUS™ immunofluorescence tests antibody detection is performed using both tissue sections/cell substrates and monospecifically reacting antigens.
- Antibodies detected in IFT screening tests can therefore be differentiated or confirmed with one and the same reaction field. In some cases, the antigens help to extend the antigen spectrum, offering a wider range for screening.
- BIOCHIPs coated with purified or recombinant antigens are used as monospecific substrates.
- In case of a positive result the antigens fluoresce green in defined areas under the microscope.
- In some EUROPLUS™ test systems several different antigens are coated on one BIOCHIP in separate antigen rows. In this manner, several monospecific analyses can be performed using a single BIOCHIP.

Fixation of frozen tissue sections to glass surfaces by covalent bonding.

low-avid Ab against EBV-CA

high-avid Ab against EBV-CA

without urea  with urea

EUROPLUS™: BIOCHIP combination of tissue sections/cell substrates (left) and purified antigens (right).
Indirect Immunofluorescence: An Easy and Modern Method

New: Hydrophobic Slides
- EUROIMMUN has developed a specific slide surface with hydrophobic properties.
- This prevents the droplets from spreading on the slide surface. The slides are now suitable for manual incubation using TITERPLANE Technique and non-manual incubation on automated systems.
- The reaction fields must not be marked with a Cytomation Pen anymore.
- Price and order number are the same as for conventional EUROIMMUN slides (please add „hydrophobic“ when ordering)
- Hydrophobic slides are available on request for many products.

AP16 IF Plus by DAS: Automated Solution for all EUROIMMUN Immunofluorescence Tests
- Various validated parameters. Slide definitions and test files available.
- CE conformity for device/test system combination.
- Capacity: 16 slides, 80 samples, 200 dilutions.
- Programmable for 8 methods per run.
- 12 dilution series freely programmable.
- Automated sample dilution, sample and reagent dispersion, incubation and washing of slides.
- Laboratory software interface.
- The incubation protocol for result documentation is automatically created from the worklist.
- Barcode reader available on request.
- Very simple operation.

Fluorescence microscope EUROStar III Plus and EUROIMMUN cLED
- EUROStar III Plus is specifically tailored to the requirements of indirect immunofluorescence. The conventional complex illumination fittings have been replaced by the stunningly simple EUROStar Bluelight system.
- The LED has a life expectancy of 50,000 hours – which is 500 times longer than a mercury vapour lamp. Its light intensity is maintained at a constant level by electronic regulation. Thus, the microscope requires almost no maintenance.
- Our technicians regularly check the light intensity of your EUROStar III Plus and issue a certificate to support your quality management system.
- LEDs require only a tenth of the electrical power of a 50-watt HBO lamp, at a comparable brightness. EUROStar Bluelight provides instant full light output every time after being switched on. It does not emit any ultraviolet radiation and is explosion-proof.
- Switching between the camera and the eyepieces is unnecessary due to the convenient 50/50 beam splitter.
- With its halogen transmitted-light source, EUROStar III Plus is suited for bright-field, darkfield and, optionally, for phase contrast microscopy.
- With the EUROIMMUN cLED, the EUROStar Bluelight technology is also available as a separate component to upgrade other microscope types.
Slides for indirect immunofluorescence can be produced with single substrates or BIOCHIP Mosaics™ from up to 45 different substrates according to your individual requirements: thyroid gland, parathyroid gland, pancreas, adrenal gland, ovary, placenta*, testis, spermatozoa, pituitary gland, hypothalamus*, hippocampus, cerebrum, cerebellum, brain stem, pons*, lobus temporalis, substantia nigra*, peripheral nerve, spinal cord, optic nerve (AQP-4, NMO IgG) eye, granulocytes (fixed with EOH, HCHO or MOH), DNA-bound lactoferrin, lymphocytes, monocytes*, thrombocytes, kidney (primate, rat, mouse), lung*, liver (primate, rat, mouse), mouth mucosa, stomach (corpus, antrum), jejunum, colon, intestinal goblet cells, umbilical cord, mamma, lacrimal gland, parotid gland, prostate, vesicula seminalis, skeletal muscle, F-actin (VSM47), heart muscle, thymus, lipocytes, cartilage*, epidermis, oesophagus (primate, rat), tongue, lip, melanocytes, HEp-2 cells, HEp-20-10 cells, HUVEC, Crithidia luciliae sensitive etc. Adenovirus, Afipia felis*, Bartonella henselae, B. quintana, Bordetella parapertussis, B. pertussis, Borrelia afzelii, B. burgdorferi sensu stricto (strains CH, USA), B. garinii, Campylobacter coli*, C. jejuni, Candida albicans, C. glabrata*, C. krusei*, C. parapsilosis*, C. tropicalis*, Chikungunya virus, Chlamydia pneumoniae, C. trachomatis, C. psittaci, CMV, Coxsackievirus (A7, A9, A16, A24, B1 to B6), Dengue virus type 1 to 4, EBV-CA, EBNA, EBV-EA, Echinococcus granulosus, ECHO virus, Hantavirus, Haemophilus influenzae*, Helicobacter pylori, HHV-6, HSV-1, HSV-2, Influenza virus A (strains H3N2, H1N1, H5N1), Influenza virus B, Japanese encephalitis virus, Klebsiella pneumoniae*, Legionella bozemanii*, L. dumoffii*, L. gormanii*, L. jordanis*, L. longbeachae, L. micdadei*, L. pneumophila (serotypes 1 to 14), Leishmania donovani, Listeria monocytogenes (1/2a and 4b)*, measles virus, mumps virus, Mycoplasma hominis, M. pneumoniae, Parainfluenza virus type 1 to 4, Rift valley fever virus*, RSV, rubella virus, Saccharomyces cerevisiae, SARS-CoV, Sindbis virus, TBE virus, TO.R.C.H. profile, Toxoplasma gondii, Treponema pallidum, T. phagedaenis, Ureaplasma urealyticum, VZV, West Nile virus, Yellow fever virus, Yersinia enterocolitica (O:3, O:4, O:6 and O:9)*. **EUROPLUS:** HEp-2/liver + RNP/Sm, Sm, SS-A, SS-B, Scl-70, rib. P-proteins, Jo-1; granulocytes + MPO, PR3; primate stomach (parietal cells) + intrinsic factor; primate liver (endomysium) + gliadin (GAF-3X); rat kidney + AMA M2; thyroid gland + thyroglobulin; renal glomeruli and tubules + GBM + PLA2R; BP180 (NC16A-4X); Borrelia burgdorferi and afzelii + OspC and VlsE; EBV-CA + gp125 + p19; Plasmodium falciparum (HRP-2, MSP-2); P. vivax (MSP, CSP). **Transfected cells:** rPAg 1+2 (pancreas antigen 1+2), AQP-4, glutamate receptor (type NMDA, AMPA), Caspr2, Lgi1, AMPA, GABABR, PLA2R, desmoglein 1+3, BP230gc, Crimean Congo fever virus (GPC, N). * Currently not available in the European Union.
The Indirect Immunofluorescence Test, Performed Using the TITERPLANE™ Technique

(Reaction fields 5x5mm)

The TITERPLANE™ Technique was developed by EUROIMMUN in order to standardize immunological analyses: Samples or labelled antibodies are applied to the reaction fields of a reagent tray. The BIOCHIP Slides are then placed into the recesses of the reagent tray, where all BIOCHIPs of the slide come into contact with the fluids, and the individual reactions commence simultaneously. Position and height of the droplets are exactly defined by the geometry of the system. As the fluids are confined in a closed space, there is no need for the use of a conventional “humidity chamber”. It is possible to incubate any number of samples next to each other and simultaneously under identical conditions.

Prepare: Check the reagent tray: Are the reaction fields hydrophilic and the surrounding coating hydrophobic? If not, rub with a wet paper towel, using normal household detergent or Extran MA 01 (Merck) if necessary, and rinse thoroughly with water. For occasional disinfection, immerse for 1 h in 3% Sekusept Extra (Henkel) in water. Open the individual packets containing the BIOCHIP Slides only after they have reached room temperature. Do not touch the BIOCHIPs. Mark BIOCHIP Slides as required with a felt pen.

Dilute: Dilute serum samples according to the user’s test protocol. Include positive and negative controls with every test procedure. Mix control sera before use.

Pipette: Apply 30 µl of diluted serum to each reaction field of the reagent tray, avoiding air bubbles. Transfer all samples to be tested before starting the incubation (up to 200 droplets). Use a polystyrene pipetting template.

Incubate: Start reactions by fitting the BIOCHIP Slides into the corresponding recesses of the reagent tray. Ensure that each sample makes contact with its BIOCHIP and that the individual samples do not come into contact with each other. Incubate for 30 min at room temperature.

Wash: Rinse the BIOCHIP Slides with a flush of PBS-Tween using a beaker, and immerse them immediately afterwards in a cuvette containing PBS-Tween for at least 5 min.

Pipette: Apply 20 µl of fluorescein-labelled anti-human immunoglobulin (conjugate) onto each reaction field of a clean reagent tray. Add all drops (reagent for a maximum of 50 slides) before continuing incubation. Use a stepper pipette. The labelled anti-human serum should be mixed with a pipette before use. To save time, conjugate can be pipetted onto separate reagent trays during incubation with the diluted serum.

Incubate: Remove one BIOCHIP Slide from the PBS-Tween and within five seconds blot only the back and the long edges with a paper towel and immediately put the BIOCHIP Slide into the recesses of the reagent tray. Do not dry the areas between the reaction fields. Check for correct contact between the BIOCHIPs and liquids. Then continue with the next BIOCHIP Slide. From now on, protect the slides from direct sunlight. Incubate for 30 min at room temperature.

Wash: Rinse the BIOCHIP Slides with a flush of PBS-Tween using a beaker and put them in a cuvette containing PBS-Tween for at least 5 min. 10 drops of Evans Blue (150 µl) for each 150 ml phosphate buffer can be added for counterstaining.

Embed: Place glycerol/PBS onto a cover glass – drops of 10 µl per reaction field. Use a polystyrene embedding template. Remove one BIOCHIP Slide from the PBS-Tween and dry the back and all four edges as well as the surface around, but not between, the reaction fields with a paper towel. Put the BIOCHIP Slide, with the BIOCHIPs facing downwards, onto the prepared cover glass. Check immediately that the cover glass is properly fitted into the recesses of the slide. Correct the position if necessary. Now proceed in the same way with the next BIOCHIP Slide.

Evaluate: Read the fluorescence under the microscope.
The Indirect Immunofluorescence Test, Performed Using the TITERPLANE™ Technique

Pipette:
- 10 µl per field (3 x 3 mm)
- 30 µl per field (5 x 5 mm)
- 70 µl per field (7 x 9 mm)

Incubate: 30 min

Wash:
- 1 s flush
- 5 min cuvette

Pipette:
- 10 µl per field (3 x 3 mm)
- 20 µl per field (5 x 5 mm)
- 60 µl per field (7 x 9 mm)

Incubate: 30 min

Wash:
- 1 s flush
- 5 min cuvette

Embed:
- 10 µl per field (3 x 3 mm)
- 10 µl per field (5 x 5 mm)
- 20 µl per field (7 x 9 mm)

Evaluate: fluorescence microscopy

Techniques for the Serological Investigation of Antibodies
### Recommended Serum Dilutions for Indirect Immunofluorescence – Autoimmunity –

<table>
<thead>
<tr>
<th>Antibodies against</th>
<th>Substrate</th>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
<th>IgAGM</th>
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<tbody>
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<td>acetylcholine receptor</td>
<td>*skeletal muscle, monkey / heart, monkey</td>
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<td>actin</td>
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<td>basic myelin protein (BMP)</td>
<td>cerebellum, monkey / nerves, monkey / intestinal tissue, fetal monkey</td>
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<td>10 + 100</td>
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<td>bile canaliculi</td>
<td>Hepatitis Mosaic**</td>
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<td>Dermatology Mosaic (transfected cells)</td>
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<tr>
<td>brain: grey matter</td>
<td>cerebellum, monkey / intestinal tissue, fetal monkey</td>
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<td>10</td>
<td>10</td>
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<tr>
<td>brain: white matter</td>
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<td>gastrin-producing cells</td>
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<td>gliadin</td>
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*) In addition to the preferential analysis or as a plausibility check.
**Hepatitis Mosaic: liver, monkey/heart, monkey / HEp-2 cells/liver, rat/kidney, rat/stomach, rat.
### Recommended Serum Dilutions for Indirect Immunofluorescence

#### – Autoimmunity –

<table>
<thead>
<tr>
<th>Antibodies against (IgA, IgG, IgM, IgAG)</th>
<th>Substrate</th>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
<th>IgAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>glutamic acid decarboxylase (GAD)</td>
<td>cerebellum, monkey / pancreas, monkey</td>
<td>10 + 100</td>
<td>100</td>
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<tr>
<td>goblet cells</td>
<td>goblet cells (culture)</td>
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<tr>
<td>Golgi apparatus</td>
<td>HEP-2 cells / liver, monkey</td>
<td>100 + 1000 + 10000</td>
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<tr>
<td>GP2 (rPaG2)</td>
<td>CIBD Mosaic</td>
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<td>hair follicle</td>
<td>epidermis, monkey</td>
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<td>heart muscle</td>
<td>skeletal muscle, monkey / heart, monkey</td>
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<td>heart valves</td>
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<td>histones</td>
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<td>100 + 1000 + 10000</td>
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</tr>
<tr>
<td>Hu</td>
<td>cerebellum, monkey / nerves, monkey / intestinal tissue, fetal monkey</td>
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<td>hypothalamus</td>
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<td>intercalated discs</td>
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<td>intestinal epithelial tissue</td>
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<td>intrinsic factor</td>
<td>*stomach, monkey / intrinsic factor</td>
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<tr>
<td>Jo-1</td>
<td>*HEP-2 cells / liver, monkey</td>
<td>100 + 1000 + 10000</td>
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<tr>
<td>keratin</td>
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<td>keratin, RA-associated (filaggrin)</td>
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<tr>
<td>Ku</td>
<td>*HEP-2 cells / liver, monkey</td>
<td>100 + 1000 + 10000</td>
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<td>lacrimal gland (excretory ducts and acini)</td>
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<td>laminin</td>
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<tr>
<td>lamins</td>
<td>HEP-2 cells / liver, monkey</td>
<td>100 + 1000 + 10000</td>
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<tr>
<td>lipocytes</td>
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<td>liver membrane (LMA)</td>
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<td>liver-specific protein (LSP)</td>
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<tr>
<td>M2</td>
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<tr>
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<td>NOR</td>
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<td>nRNP</td>
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</table>

*) In addition to the preferential analysis or as a plausibility check.

**) Hepatitis Mosaic: liver, monkey/heart, monkey/HEP-2 cells/liver, rat/kidney, rat/stomach, rat.
### Recommended Serum Dilutions for Indirect Immunofluorescence – Autoimmunity –

<table>
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<tr>
<th>Antibodies against</th>
<th>Substrate</th>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
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<td>pANCA</td>
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<td>pancreas acini (Crohn's disease autoantigen)</td>
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<td>pancreas islets</td>
<td>pancreas, monkey (1st step: 18 hours)</td>
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<td>pancreas, excretory duct epithelium</td>
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<td>parietal cells</td>
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<td>parotid gland</td>
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<td>peripheral nerves</td>
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<td>pituitary gland, anterior lobe</td>
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* In addition to the preferential analysis or as a plausibility check.
## Recommended Serum Dilutions for Indirect Immunofluorescence
### – Infectious Serology –

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<th>IgG</th>
<th>IgM</th>
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<td>HIV-1/2</td>
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<td>Legionella pneumophila (all serotypes)</td>
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<td>West-Nile virus</td>
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Diagnostically Relevant Autoantibodies

Systemic Autoantibodies against Ig- AGM A G M

- **BASIS SPECTRUM**
  - Ig- AGM A G M ANA (cell nuclei) I/F global testing
  - Ig- AGM A G M dsDNA-Ncx ELISA
  - Ig- AGM A G M DNA-IF
  - Ig- AGM A G M RNA-IF
  - Ig- AGM A G M SSA-B
  - Ig- AGM A G M SSB
  - Ig- AGM A G M Scl-70
  - Ig- AGM A G M Jo-1
  - Ig- AGM A G M CENP-A
  - Ig- AGM A G M CENP-B
  - Ig- AGM A G M PM-Scl75
  - Ig- AGM A G M PM-Scl100
  - Ig- AGM A G M CENPA
  - Ig- AGM A G M CENPB
  - Ig- AGM A G M RP11
  - Ig- AGM A G M RP155

- **SYST. LUPUS ERYTHEMATOSUS (SLE)**
  - Ig- AGM A G M ANA (cell nuclei) I/F global testing
  - Ig- AGM A G M dsDNA-Ncx ELISA
  - Ig- AGM A G M DNA-IF
  - Ig- AGM A G M RNA-IF
  - Ig- AGM A G M SSA-B
  - Ig- AGM A G M SSB
  - Ig- AGM A G M Scl-70
  - Ig- AGM A G M Jo-1
  - Ig- AGM A G M CENP-A
  - Ig- AGM A G M CENPB
  - Ig- AGM A G M RP11
  - Ig- AGM A G M RP155

- **SHARP’S SYNDROME MCTD**
  - Ig- AGM A G M Ut-nRNP (70K, A, C)
  - Ig- AGM A G M SSA-B
  - Ig- AGM A G M SSB
  - Ig- AGM A G M Scl-70
  - Ig- AGM A G M Jo-1

- **POLYMYSITIS, DERMATOMYOSITIS**
  - Ig- AGM A G M ANA (cell nuclei) I/F global testing
  - Ig- AGM A G M Myositis Profile 3 EUROLINE (M-2, Ku, PM-Scl100, PM-Scl75, SRF, Jo-1, PL-7, PL-12, OJ, Ej, Ro-52)

- **OTHER AUTOANTIBODIES**
  - Ig- AGM A G M centrioles
  - Ig- AGM A G M MSA-1 (NuM, spindle fibres)
  - Ig- AGM A G M MSA-2 (mitochondria)
  - Ig- AGM A G M MSA-3 (chromosome-ass. antigen)
  - Ig- AGM A G M centromer F protein (CENP-F)
  - Ig- AGM A G M centromer G protein (CENP-G)
  - Ig- AGM A G M ribosomes
  - Ig- AGM A G M Golgi apparatus
  - Ig- AGM A G M lysosomes
  - Ig- AGM A G M cytoskeleton
  - Ig- AGM A G M actin
  - Ig- AGM A G M vimentin
  - Ig- AGM A G M cytokeratin
  - Ig- AGM A G M tropomyosin
  - Ig- AGM A G M vinculin
  - Ig- AGM A G M desmin
  - Ig- AGM A G M laminin (basal membranes)
  - Ig- AGM A G M collagen type VII
  - Ig- AGM A G M elastin
  - Ig- AGM A G M vessel endothelium

- **THERAPY CONTROL**
  - Ig- AGM A G M interferon alpha
  - Ig- AGM A G M interferon beta
  - Ig- AGM A G M antihistone
  - Ig- AGM A G M diDNA RIA
  - Ig- AGM A G M CIC-C1q ELISA

Grey boxes: standard analysis  *) ANCA diagnostics in acute cases within one hour, at any hour  **) Use special procedure for taking sample(s)  ***) Send frozen sample(s)
Antibodies for Infectious Serology

**Antibodies against**

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<th>Ig-</th>
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<th>BACTERIA A-Z</th>
<th>VIRUSES A-Z</th>
<th>FUNGI A-Z</th>
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<td>Afipia felis</td>
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<td>219b</td>
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<td>Bartonella henselae</td>
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<tr>
<td>219c</td>
<td></td>
<td>Bartonella quintana</td>
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<td>219d</td>
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<td>Bordetella parapertussis</td>
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<td>219e</td>
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<td>Saccharomyces cerevisiae</td>
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<td>219g</td>
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<td>Borrelia burgd. s. strict</td>
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<td>219h</td>
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<td>Borrelia burgd. (USA)</td>
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<td>Chlamydia psittaci</td>
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<td>Chlamydia trachomatis</td>
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<td>Diphtheria toxoid</td>
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<td>Haemophilus influenzae</td>
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<td>Helicobacter pylori</td>
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<td>219r</td>
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<td>Klebsiella pneumoniae</td>
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<td>219x</td>
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<td>Legionella pneumophila</td>
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<td>Legionella pneumophila</td>
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<td>219z</td>
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</tbody>
</table>

**Comments:**

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</table>

**Grey:** standard analyses

*) Currently not available as IVD in the European Union

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**EUROIMMUN**

**Medizinische Labordiagnostika AG**

**Techniques for the Antibodies**

**Investigations for**

**EUROLINE**

**EUROIMMUN**

**Techniques for the Serological**

**Dr. Winfried Stöcker**
Antibodies for Allergology

Allergens Profiles: Antibodies of class IgE against

**GLOBAL TEST**

3840  □ Determination of total IgE (ELISA)

**INHALATION**

3110  □ Allergy Profile Inhalation
3111  □ Allergy Profile Pediatric Inhalation
3112  □ Allergy Profile Mediterranean Inhalation
3113  □ Allergy Profile Inhalation “South East Asia”
3116  □ Allergy Profile Inhalation “China”
3117  □ Allergy Profile Inhalation “Middle East”
3118  □ Allergy Profile Inhalation “Mix-Screen Turkey 1”
3119  □ Allergy Profile Inhalation “Turkey 1”
3120  □ Allergy Profile Inhalation “India”
3121  □ Allergy Profile Inhalation “Screen France”

**FOOD**

3410  □ Allergy Profile Food

**ATOXY, POLLEN-ASSOCIATED**

3710  □ Allergy Profile Atopy “Top Screen”
3711  □ Allergy Profile Pollen-Food

**EUROASSAY ALLERGY PROFILES**

3710  □ Allergy Profile Inhalation
3711  □ Allergy Profile Food

**INSECT VENOMS**

3720  □ Allergy Profile Insect Venoms

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**Techniques for the Serological Investigation of Antibodies**

**EUROIMMUN**

**Clinical Pathologist**

**Address**: ...

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**Alleroat™ 6 System: Antibodies of class IgE against 600 different allergens of the areas animal allergens, environmental allergens, food, grasses, herbal and flower pollen, house dust, insects, mites, moulds, parasites, pharmaceutical drugs, trees.**
EUROIMMUN Microplate ELISA

Principle of the Test
- Polystyrene microplate strips coated with purified, biochemically characterized antigens are used as solid phase containing bound antigens.
- If the sample is positive, specific antibodies in the diluted serum sample attach to the antigens coupled to the solid phase.
- In a second step, the attached antibodies are detected with peroxidase-labelled anti-human antibodies.
- In a third step, the bound antibodies are made visible using a chromogen/substrate solution which is capable of promoting a color reaction. The intensity of the color produced is proportional to the concentration of antibodies in the serum sample.
- Monospecific ELISA (enzyme immunoassays with a single antigen) provide a quantitative in vitro assay for the detection of antibodies.
- “Profile ELISA” provide a semiquantitative in vitro assay for the detection of different antibodies on a single microplate strip.
- The solid phase of “Pool ELISA” is coated with an antigen mixture for the semiquantitative detection of antibodies whose specificity must be subsequently investigated by monospecific assays.

Reliable and Economical Calibration/Evaluation
- In the case of a quantitative ELISA, calibration is performed using three calibration sera.
  Calibration serum 1: upper limit of the measurement range
  Calibration serum 2: upper limit of the normal range (cut-off value)
  Calibration serum 3: negative
- Only three wells are required for calibration, followed by serum samples. There is no need to incubate blank values or duplicate determinations.
- Semiquantitative ELISA are performed using only one calibrator.
- The calibration is performed in relative units per millilitre (RU/ml) or, if an international reference serum exists, in international units per millilitre (IU/ml).
- Each test can be optionally performed using a positive or negative control serum included in the test kit. Kit-specific reference ranges are provided for each calibrator and control serum.
- All calibrations can be easily performed with the usual ELISA software.

Easy, Quick and Economical Handling
- Microplate strips containing break-off wells (except Profile ELISA), each marked with an antigen abbreviation to avoid confusion of wells.
- Reagents ready for use (wash buffer: concentrate). Reagents are color-coded to ensure positive identification.
  - dark red: calibration serum 1
  - red: calibration serum 2
  - light red: calibration serum 3
  - dark blue: pos. control serum
  - green: neg. control serum
  - light blue: sample buffer
  - orange: anti-human IgA POD-conj.
  - green: anti-human IgG POD-conj.
  - red: anti-human IgM POD-conj.
  - turquoise: anti-human IgGM POD-conj.
  - yellow: anti-human IgAGM POD-conj.
- The sample buffer for infectious serology ELISA (detection of antibodies of class IgM) already contains an IgG/RF absorbent.
- Several tests can be combined on one and the same microplate, since the incubation times (30 min; 30 min; 15 min) are identical for all ELISA. Incubation at room temperature.
- Compatible with all commercial washer and reader systems.
Determination of Low-Avidity Antibodies

- An alternative principle for the serological diagnosis of fresh infections has been established by investigating the antibody avidity.
- The first reaction of the immune system following an infection is the formation of low-avidity antibodies. As the infection proceeds, increasingly antigen-adapted IgG is formed, and avidity grows. As long as high-avidity IgG is not yet detected in the serum, it can be assumed that the infection is still in an early stage.
- To identify low-avidity antibodies in a patient’s serum, two microplate ELISA are performed in parallel: one test is carried out in the conventional way, the other one includes urea treatment between incubations with patient’s serum and peroxidase-labelled anti-human IgG, resulting in the detachment of low-avidity antibodies from the antigens.
- Low-avidity antibodies are present if the ELISA extinction is significantly reduced by urea treatment. For an objective interpretation, the relative avidity index (RAI) can be calculated out of the measured values with and without urea incubation.
- Serum dilution 1:100, conjugate class anti-human IgG, POD-labelled.
- 3-point calibration, quantitative (IgG).
- The following test kits for avidity determination are available: Toxoplasma gondii, CMV, rubella virus, VZV, West-Nile virus, EBV-CA.

Antibody Determination in CSF

- Indication: local infections of the brain.
- CSF dilution 1:2, serum dilution 1:404. Conjugate classes anti-human IgG or IgM, POD-labelled.
- Easy to conduct: ready-for-use reagents.
- 4-point calibration, quantitative. Identical incubation conditions and times (room temperature; 60 min / 60 min / 15 min): all EUROIMMUN ELISA for CSF diagnostics can be combined without difficulty on one and the same microplate.
- The antibody concentration in the patient’s serum is determined in parallel to the antibody concentration in CSF on one and the same microplate. The CSF/serum quotient CSQ\textsubscript{path.-spec.} is calculated from both measured values.
- An intrathecal synthesis of specific antibodies is present if the CSF/serum quotient of the specific antibodies CSQ\textsubscript{path.-spec.} is significantly higher than the CSF/serum quotient of the whole IgG (CSQ\textsubscript{total}) or if necessary the CSQ\textsubscript{lim.}.
- For the automatic calculation of the CSQ\textsubscript{rel.} EUROIMMUN provides a specific Excel table free of charge.
- The following test kits for CSF diagnostics are available: Borrelia burgdorferi, Toxoplasma gondii, HSV-1, HSV-2, HSV-1/2 Pool, CMV, rubella virus, measles virus, mumps virus, VZV, TBE, EBV-CA.
- All test systems for CSF diagnostics can also be used only for serology.
- Perfectly adapted for the automated incubation in incubation devices.
ELISA Automation Using the EUROIMMUN Analyzers

EUROIMMUN Analyzer I and EUROIMMUN Analyzer I-2P: Broad Parameter Spectrum, Proven Reliability, Variable Throughput

- One for all: fully automated processing of all EUROIMMUN ELISA – autoimmune diagnostics, infectious serology and allergology – with only one system
- Flexibility for your benefit: open system with more than 800 validated EUROIMMUN parameters for serum, plasma and cerebrospinal fluid, over 50 or 30 parameters in parallel.
- Convenient, simple and reliable operation: e.g. by scanning QC certificates using a 2D-hand barcode scanner – ready-for-use reagents and preprogrammed assay protocols enable you to start immediately.
- Capacity and throughput: quick loading and efficient time management allow processing of up to 70 or 50 tests per hour – up to 7 or 3 plates, 180 or 144 samples at the start of a run.
- Security for patients: validated test systems and the comprehensive safety kit provide the basis for reliable diagnostics.
- Reliability and service: instruments and reagents from one manufacturer, quick and targeted support from our personnel for a smooth workflow in your laboratory.

Modular System: A Highly Sophisticated Solution

- High convenience, fast and reliable loading through barcode identification of samples and reagents: automatic scanning when racks are inserted, reading of lot-specific QC data via 2D hand barcode scanner.
- Dilution area: 288 or 192 dilution positions (Deepwell, 2 ml).
- Liquid level detection (capacitive), multi-shot (dispensing) mode, automatic tip detection, clot detection.
- Pipetting possible during plate transport due to separation of transport and pipetting unit.
- 4 or 2 incubators with heating and shaking function, 4 or 3 incubators at room temperature.
- Standard Windows software: familiar user interface, all relevant statistical functions provided, available in different languages.
- Efficient use and convenient handling of tips and dilution plates through memory function.

A Convincing and Reliable Package: EUROIMMUN Analyzer, EUROIMMUN ELISA, EUROIMMUN Service

- Comprehensive test system validation for the EUROIMMUN Analyzer: all parameters validated in accordance with the 98/79/EC directive and based on EN ISO13485:2003/CMDCAS.
- All ELISA test systems are manufactured according to the European Quality Standards (IVD).
- National and international conformity (standardisation): CE, IVD, FDA and CMDCAS.
- Programming and set-up of automated system, including introduction to the system with customer training, performed by qualified personnel.
- Reliable and fast delivery of consumables and reagents.
- Connection to in-house computer system via communication protocol ASTM.
- Maintenance contract with EUROIMMUN on request.

*) soon available for the EUROIMMUN Analyzer I-2P
## Incubating the Microplate ELISA

- **microplate wells coated with antigens**

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>Pipette:</strong></td>
<td>100 µl per well</td>
</tr>
<tr>
<td>2</td>
<td><strong>Incubate:</strong></td>
<td>30 min</td>
</tr>
<tr>
<td>3</td>
<td><strong>Wash:</strong></td>
<td>300 µl wash buffer per well</td>
</tr>
<tr>
<td>4</td>
<td><strong>Pipette:</strong></td>
<td>100 µl per well</td>
</tr>
<tr>
<td>5</td>
<td><strong>Incubate:</strong></td>
<td>30 min</td>
</tr>
<tr>
<td>6</td>
<td><strong>Wash:</strong></td>
<td>300 µl wash buffer per well</td>
</tr>
<tr>
<td>7</td>
<td><strong>Pipette:</strong></td>
<td>100 µl per well</td>
</tr>
<tr>
<td>8</td>
<td><strong>Incubate:</strong></td>
<td>15 min</td>
</tr>
<tr>
<td>9</td>
<td><strong>Pipette:</strong></td>
<td>100 µl per well</td>
</tr>
<tr>
<td>10</td>
<td><strong>Evaluate:</strong></td>
<td>photometric measurement at a wavelength of 450 nm</td>
</tr>
</tbody>
</table>

**Legend:**
- Diluted samples
- Enzyme conjugate
- Chromogen/substrate solution
- Stop solution

---

**Note:** The steps above outline the process for incubating the microplate ELISA, including pipetting, incubation, washing, and evaluating the results.
EURONNNN M e d i z i n i s c h e L a b o r d i a g n o s t i k a A G

EUROASSAY: Line Blot in TITERPLANE™ Technique Format

Principle of the Test
- Membrane strips coated with thin parallel lines of several purified, biochemically characterized antigens are used as solid phase. The membrane strips are fixed as BIOCHIPs in the fields of microscope slides.
- If the sample is positive, specific antibodies in the diluted serum sample attach to the antigens coupled to the solid phase.
- In a second incubation step, the attached antibodies react with AP-labelled anti-human antibodies.
- In a third step, the bound antibodies are stained with a chromogen/substrate solution which is capable of promoting a color reaction. An intense dark band at the line of the corresponding antigen appears if the serum sample contains specific antibodies.
- The microscope slides are incubated using the TITERPLANE™ Technique: samples and reagents are applied to the reaction areas of a reagent tray. The slides are then placed into the recesses of the reagent tray, where all test strips of one slide come into contact with the liquids, and the individual reactions begin simultaneously.
- Depending on the spectrum of antigens used, it is possible to analyze several antibodies next to each other and simultaneously under identical conditions.

Easy Handling
- Several serum samples can be analyzed simultaneously on one and the same slide.
- Total time for performing the EUROASSAY test is about 100 minutes. During the washing procedure, reagents for the next incubation step can be applied to reagent trays.
- All incubation steps proceed at room temperature. Shaking the slides together with the reagent tray on a circulatory shaker ensures the best possible sensitivity.
- Low reagent consumption. Only 50 µl each of diluted serum and reagent are needed per test field.
- Reagents ready for use (wash buffer: concentrate).

Reliable and Simple Evaluation
- Since results are evaluated visually, there is no investment required for photometers, etc.
- The antigen bands are located at exactly defined positions, which means that evaluation of the test is much simpler than for Westernblots.
- Correct completion of the individual incubation steps in each test field is indicated by staining of the control band.
- Positive and negative results can be easily and reliably differentiated from each other. The intensity of the antigen bands correlates with the antibody titer.
- The antigens used are highly pure, mostly isolated by affinity chromatography. The membrane strips do not contain any superfluous proteins which might cause unspecific positive results.
- The incubated microscope slides can be stored for long periods. Results can be easily documented.
Incubating the EUROASSAY (TITERPLANE™ Technique)

Pipette: 50 µl per field

Incubate: 30 min shaking on a circulatory shaker (300 rpm)

Wash: 5 s flush 15 min cuvette

Pipette: 50 µl per field

Incubate: 30 min shaking on a circulatory shaker (300 rpm)

Wash: 5 s flush 15 min cuvette

Pipette: 50 µl per field

Incubate: 10 min shaking on a circulatory shaker (300 rpm)

Wash: flush with deionized or distilled water, air dry

Evaluate: visual assessment of color intensity
The EUROLINE: A New Technique for Extensive Antibody Profiles

Principle of the Test
- Membrane strips coated with thin parallel lines of several purified, biochemically characterized antigens are used as solid phase. The membranes are fixed as BIOCHIPs onto synthetic foil.
- If the sample is positive, specific antibodies in the diluted serum sample attach to the antigens coupled to the solid phase.
- In a second incubation step, the attached antibodies react with alkaline-phosphatase-labelled anti-human antibodies.
- In a third step, the bound antibodies are stained with a chromogen/substrate solution which is capable of promoting a color reaction. An intense dark band at the line of the corresponding antigen appears if the serum sample contains specific antibodies.
- Depending on the spectrum of antigens used, it is possible to analyze several antibodies next to each other and simultaneously under identical conditions.

Easy Handling, Reliable and Simple Evaluation
- A separate membrane strip is incubated for each serum sample.
- Total time for performing the analysis is about 105 minutes.
- The incubation can be automated using the EUROBlotMaster.
- All incubation steps proceed at room temperature.
- The antigen bands are located at exactly defined positions, which means that the evaluation of the test is much simpler than for Westernblots.
- Correct completion of the individual incubation steps is indicated by staining of the control band on each EUROLINE test strip.
- Positive and negative results can be easily and reliably differentiated from each other. The intensity of the antigen bands correlates with the antibody titer.
- The antigens used are highly pure, mostly isolated by affinity chromatography. The membrane strips do not contain any superfluous proteins which might cause unspecific positive results.
- The incubated EUROLINE test strips can be stored for long periods. Results can be easily documented.
- The program EUROLineScan from EUROIMMUN has been developed to enable quantitative evaluation of EUROLINE test strips, to facilitate management of data, and to provide detailed documentation of results. First, the incubated EUROLINE test strips are scanned using a flatbed scanner. EUROLineScan recognizes the position of the strips, even if they have been placed inexact, identifies the bands, and measures their intensity. The results are then saved together with the image data. A separate results sheet can be produced for each patient.

Incubated EUROLINE test strips (Autoimmune Liver Diseases Profile, ANA Profile 3, Myositis Profile 3.)
EUROLINE Automation Using EUROBlotMaster and EUROLineScan

EUROBlotMaster
- Standardised incubation of immunoblot strips – higher precision and reproducibility.
- Automation of all EUROIMMUN immunoblot strips (EUROLINE, EUROLINE-WB, Westernblot)
- Over 65 validated parameters available (16 autoantibody, 28 infectious and 21 allergy parameters)
- Up to 30 strips per test run
- Easy operation
- Combination of different conjugates/tests in one run
- Walk-away function – fully automated from the start to the end of processing following loading
- Compatible with modern evaluation systems such as EUROBlotCamera and EUROLineScan

Automatic Evaluation of the Results Using EUROLineScan
- For all EUROIMMUN blot systems: EUROLINE, EUROLINE-WB, Westernblot.
- For all areas: autoimmune diagnostics, infectious serology and allergyology.
- EUROBlotCamera: digitalisation of strips while in the incubation tray.
- EUROLineScanner: digitalisation of strips using flatbed scanner.
- Fully automated identification, quantitation and assignment of bands.
- Option to modify results (changes are automatically documented).
- Complete results obtained just a few minutes after finishing the incubation.
- Fully automated administration and documentation of extensive individual data.
- Electronic archiving of all images and data (avoids the need to store potentially infectious blot strips).
- Online connection to laboratory software.
- Network compatible.
Techniques for the Serological Investigation of Antibodies

**Westernblot/EUROLINE-WB: Reliable Differentiation of Antibodies Present**

**Principle of the Test**

- Membrane strips containing electrophoretically separated antigen extracts are used as solid phase. The position of the proteins depends on their respective molecular masses.
- If the sample is positive, specific antibodies in the diluted serum sample attach to the antigens coupled to the membrane.
- In a second incubation step, the attached antibodies react with AP-labelled anti-human antibodies.
- In a third step, the bound antibodies are stained with a chromogen/substrate solution which is capable of promoting a color reaction. An intense dark band at the line of the corresponding antigen appears if the serum sample contains specific antibodies.
- Evaluating the band patterns on the incubated membrane strips involves differentiating non-specific from specific antibodies. The number and intensity of the specific bands is decisive for the result “positive/negative”.

**Easy Handling, Reliable Evaluation and High Diagnostic Significance**

- A separate membrane strip is incubated for each serum sample.
- Total time for performing the Western blot test is about 115 minutes.
- All incubation steps proceed at room temperature.
- The bands are assigned according to a lot-specific evaluation matrix provided. A separate lot is issued for each electrophoresis gel, helping to avoid errors in the assignment of the bands.
- Every test kit contains a membrane strip of the same lot incubated with a positive reference serum. Therefore, there is no need to incubate a positive control serum.
- The membrane strips are pre-numbered to prevent confusion. Laborious labelling is not necessary.
- Correct completion of the individual incubation steps for each membrane strip is indicated by staining of the control band at the bottom of the strip.
- Positive and negative reactions can be easily and reliably differentiated from each other. The intensity of the antigen bands correlates with the antibody titer.
- The Western blot is the method of choice when the objective is to confirm or differentiate positive results obtained in a screening test (indirect immunofluorescence or microplate ELISA).
- EUROLINE-WB is a combination of western blot and line blot techniques. Proteins from a whole antigen extract are electrophoretically separated according to molecular mass and transferred onto a nitrocellulose membrane. Highly purified native or recombinant antigens are then printed as lines onto the western blot strips (EUROLINE membrane chip).
- The program EUROLineScan from EUROIMMUN has been developed to enable quantitative evaluation of Western blot/EUROLINE-WB test strips, to facilitate management of data, and to provide detailed documentation of results. First, the incubated Western blot/EUROLINE-WB test strips are scanned using a flatbed scanner. EUROLineScan recognizes the position of the strips, even if they have been placed inexacte, identifies the bands, and measures their intensity. The results are then saved together with the image data. A separate results sheet can be produced for each patient.
Incubating the EUROLINE/Westernblot/EUROLINE-WB using the EUROBlotMaster or manually on a rocking platform

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
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<tbody>
<tr>
<td>Pipette</td>
<td>1.5 ml per channel</td>
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<tr>
<td>Incubate</td>
<td>5-15 min shaking, depending on test system</td>
</tr>
<tr>
<td>Aspirate off</td>
<td></td>
</tr>
<tr>
<td>Pipette</td>
<td>1.5 ml per channel</td>
</tr>
<tr>
<td>Incubate</td>
<td>30 min shaking</td>
</tr>
<tr>
<td>Wash</td>
<td>1.5 ml buffer, 5 min incubation, aspirate off</td>
</tr>
<tr>
<td>Pipette</td>
<td>1.5 ml per channel</td>
</tr>
<tr>
<td>Incubate</td>
<td>30 min shaking</td>
</tr>
<tr>
<td>Wash</td>
<td>1.5 ml buffer, 5 min incubation, aspirate off</td>
</tr>
<tr>
<td>Pipette</td>
<td>1.5 ml per channel</td>
</tr>
<tr>
<td>Incubate</td>
<td>10 min shaking</td>
</tr>
<tr>
<td>Wash</td>
<td>rinse with distilled water, aspirate off</td>
</tr>
<tr>
<td>Evaluate</td>
<td>with EUROLineScan or visual assessment</td>
</tr>
</tbody>
</table>

Applicable for most EUROLINE/Westernblot/EUROLINE-WB test kits
EUROIMMUN Radioimmunoassays (RIA/IRMA)

Test principle RIA (precipitation techniques)
- In the first incubation step patient sera are incubated with $^{125}$I-labelled antigen in polystyrene tubes. Any specific antibodies in the sera bind to the antigen.
- In the second incubation step the antigen-antibody complexes are precipitated using a precipitation agent.
- The precipitate is washed with buffer. After centrifugation and decanting of the supernatant, the radioactivity in the precipitate is counted using a gamma counter. The intensity of the radioactivity is proportional to the concentration of specific antibody in the patient serum.
- The antibody concentration is evaluated quantitatively using a calibration curve.

Test principle RIA (coated tubes)
- RIA tests (coated tubes) are competitive ligand assays for antibody and antigen determinations.
- The intensity of radioactive radiation is inversely proportional to the concentration of specific antibodies or antigens in the sample.
- The quantitative evaluation of the antigen/antibody concentration is carried out using a calibration curve.

Test principle IRMA (antigen determination)
- With this test principle, monoclonal antibodies which are bound directly or indirectly to the inner wall of polystyrene tubes are used.
- During the first incubation step, the patient sera to be investigated are incubated with the monoclonal $^{125}$I-labelled antibodies in the coated tubes.
- The antigen in the sample is bound by the immobilised antibodies and by the $^{125}$I-labelled antibodies. This results in a solid-phase bound sandwich complex.
- The unbound $^{125}$I-labelled antibodies are removed by washing and subsequently decanting. The intensity of radioactive radiation is proportional to the concentration of antigens in the patient serum.
- The quantitative evaluation of the antigen concentration is carried out using a calibration curve.

Simple and economical handling, reliable analysis
- Simple test procedure.
- Synchronous processing of samples, including different tests in parallel.
- Ready-to-use reagents (possible exceptions: tracer, precipitation reagents).
- Different test formats for small and large sample series.
- Because of the large measurement range of EUROIMMUN RIA, further measurements with other sample dilutions are generally not necessary.
- Simple evaluation of test results.
- Each test can be optionally evaluated with controls which are supplied in the test kit. Test kit-specific reference ranges are given for all controls.
- EUROIMMUN radioimmunoassays show the following analytical characteristics:
  - High analytical specificity.
  - High detection sensitivity.
  - High clinical sensitivity and specificity.
  - Good reproducibility.
EUROIMMUN PRODUCTS FOR THE DETERMINATION OF AUTOANTIBODIES
Autoantibodies against Cell Nuclei (ANA)

Indirect Immunofluorescence Test: EUROPLUS™ ANA Mosaic 20

- Screening test for the detection of antibodies against cell nuclei (ANA).
- Indications: rheumatic diseases.
- Initial dilution 1:100; conjugate class anti-human IgG, FITC-labelled.
- Using HEp-2 cells many antibodies against cell nuclei can be analyzed, e.g. antibodies against DNA, histones, RNA, rRNP, Sm, SS-A, SS-B, nuclear dots, centromeres, nuclear membrane, nucleoli (PM-Scl, fibrillarin, RNA polymerase I, NOR), Scl-70, cyclin I and II, spindle fibers, midbody, centrioles.
- In addition, cytoplasmic autoantibodies are identified with HEp-2 cells: antibodies against ribosomes, Jo-1, mitochondria, Golgi apparatus, actin etc.
- The primate liver permits the verification of results between both substrates, makes the pre-differentiation of a large number of ANA possible, and helps to establish titer levels. Moreover, the primate liver often contains additional antigens, allowing the identification of further autoantibodies: antibodies against LMA, LSP, endomysium, bile ducts and endothelium cells, as well as cANCA und pANCA.
- The EUROPLUS™ system is a monospecific test that can be used to confirm the presence of autoantibodies against cell nuclei and cytoplasm with one and the same test kit. The following antigens are currently available as EUROPLUS™

Order No. FA 1510-####-20 G Formats page 127

Indirect Immunofluorescence Test: Innovative Cell Line from EUROIMMUN, HEp-20-10

- Screening test for detection of antibodies against cell nuclei.
- Indications: rheumatic diseases.
- Initial dilution 1:100; conjugate class anti-human IgG, FITC-labelled.
- Compared to standard HEp-2 cells, HEp-20-10 cells demonstrate 10-fold more mitotic cells. Antibodies against mitotic-specific structures (centromeres, spindle fibers, midbody, centrioles, NOR) can be more easily identified than with conventional preparations.
- At the same time, the cell line HEp-20-10 offers the full antigen spectrum for cell nuclei antibody diagnostics.
- The BIOCHIP with HEp-20-10 can be supplemented with additional substrates, for example, primate liver (ANA; Order No. FA1512-####-1G) as well as rat kidney and rat stomach (Order No. FA1802-####-3G).

Order No. FA 1522-#### G Formats page 128

EUROASSAY: Anti-ENA ProfilePlus

- Differentiation of anti-nuclear antibodies (ANA).
- Indications: rheumatic diseases.
- Serum dilution 1:100; conjugate class anti-human IgG, AP-labelled.
- 6 relevant anti-nuclear antibodies against “extractable nuclear antigens” (ENA) can be detected simultaneously and monospecifically: antibodies against rRNP/Sm, Sm, SS-A, SS-B, Scl-70, Jo-1.
- Native antigens, purified by affinity chromatography.
- On request EUROASSAY can be produced with special antigen combinations, for example, with dsDNA, histones, nucleosomes, PM-Scl, rRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, Jo-1, ribosomal P proteins, centromere protein B, M2, M4, M9, SLA/LP, LC-1, LKM-1.

Order No. DA 1590-####-1 G Formats page 78
EUROLINE: ANA Profile 3
- Differentiation of antibodies against cell nuclei (ANA).
- Indications: Sharp syndrome (MCTD), systemic lupus erythematosus, Sjögren’s syndrome, systemic sclerosis, poly/dermatomyositis, PBC.
- Serum dilution 1:100; conjugate class anti-human IgG, AP-labelled.
- With the EUROLINE ANA-Profile 3, fifteen autoantibodies can be determined: antibodies against nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, PM-Scl, Jo-1, centromere protein B, PCNA, dsDNA, nucleosomes, histones, ribosomal P-proteins, AMA M2.
- Antibodies against SS-A are characteristic markers for SLE and Sjögren’s syndrome. In contrast, antibodies against Ro-52 also occur in patients with other autoimmune diseases.
- Native antigens, purified by affinity chromatography (exception: centromere protein B, PM-Scl, Ro-52, PCNA).
- Further antigen combinations: page 80.
- Test strips can be automatically incubated and evaluated using the systems EUROBlotMaster und EUROWLineScan (see page 27).

EUROLINE: Myositis Profile 3
- Differentiation of myositis-associated antibodies against cell-nuclear and cytoplasmic antigens.
- Indications: poly/dermatomyositis.
- Serum dilution 1:100, conjugate class anti-human IgG, AP-labelled.
- With the EUROLINE Myositis Profile 3, eleven autoantibodies can be determined: antibodies against Mi-2, Ku, PM-Scl100, PM-Scl75, Jo-1, SRP, PL-7, PL-12, EJ, OJ and Ro-52.
- Test strips can be automatically incubated and evaluated using the systems EUROBlotMaster und EUROWLineScan (see page 27).

EUROLINE: Systemic Sclerosis Profile (Nucleoli)
- Differentiation of systemic sclerosis-associated antibodies against cell-nuclear antigens.
- Indications: systemic sclerosis (SSc, diffuse and limited form), overlap syndromes.
- Serum dilution 1:100, conjugate class anti-human IgG, AP-labelled.
- With the EUROLINE Systemic Sclerosis Profile (Nucleoli), thirteen autoantibodies can be determined: antibodies against Scl-70, CENP A, CENP B, RP11 (RNAP-III), RP155 (RNAP-III), fibrillarin, NOR-90, Th/To, PM-Scl100, PM-Scl75, Ku, PDGFR, Ro-52.
- Test strips can be automatically incubated and evaluated using the systems EUROBlotMaster und EUROWLineScan (see page 27).
Autoantibodies against Cell Nuclei (ANA)

Microplate ELISA: ANA Screen, Anti-ENA PoolPlus
- Screening test for predifferentiation of antibodies against cell nuclei (ANA) and cytoplasm components.
- Serum dilution 1:200, conjugate class anti-human IgG, POD-labelled.
- One microplate well incubated per patient.
- 1-point calibration, semiquantitative.
- Native antigens (exception: centromere, recombinant).
- The ANA Screen ELISA supplements the gold standard immunofluorescence. It is based on a mixture of 10 highly purified antigens, which provide higher sensitivity and specificity than the undefined cell extracts used by other manufacturers.
- Two ELISAs with different antigen combinations, adapted to particular indications or for follow-up of immunofluorescence patterns, are available.

Order No. | Formats
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EA 1590-9601-8 G | page 87

Microplate ELISA: SLE Profile 1/2, Anti-ENA ProfilePlus 1/2
- Differentiation of antibodies against cell nuclei (ANA) and cytoplasm components.
- Serum dilution 1:200; conjugate class anti-human IgG, POD-labelled.
- 8 or 6 relevant antibodies can be detected simultaneously.
- 1-point calibration, semiquantitative. Calibrator pool and negative controls each on a separate microplate strip (SLE Profiles and Anti-ENA ProfilePlus 2) or on the same microplate strip as the patient serum.
- Native antigens (exception: Ro-52, centromere and PM-Scl, recombinant).
- In total 4 different ELISAs with different antigen combinations, adapted to particular indications or for follow-up of immunofluorescence patterns, are available.

Order No. | Formats
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EA 1590-1208-2 G | page 87

Microplate ELISA: ANA Single-Antigen ELISAs
- Differentiation of antibodies against cell nuclei (ANA) and cytoplasm components.
- Indications: rheumatic diseases.
- Serum dilution 1:200; conjugate class anti-human IgG, POD-labelled.
- Antibodies against cell nuclei components can be determined quantitatively in RU/ml.
- 3-point calibration, quantitative.
- Identical incubation conditions and times: all single-antigen tests can be combined with each other on one microplate.
- Native antigens (exceptions: centromere and PM-Scl, recombinant).
- Single-antigen ELISAs available for detection of antibodies against cell nuclei and cytoplasm antigens: ssDNA, nucleosomes, dsDNA, histones, ribosomal P proteins, PM-Scl, nRNP/Sm, Sm, SS-A, SS-B, Scl-70, Jo-1, centromere.

Order No. | Formats
--- | ---
EA ####-9601 G | page 87
Autoantibodies against Double-Stranded DNA (dsDNA)

Indirect Immunofluorescence Test: Crithidia luciliae
- Detection of antibodies against dsDNA.
- Indication: lupus erythematosus disseminatus.
- Initial dilution: 1:10, conjugate class anti-human IgG, FITC-labelled.
- Animal pathogenic haemoflagellates of Crithidia luciliae are used for the detection of autoantibodies against double-stranded, native DNA (dsDNA, nDNA) with indirect immunofluorescence. These protozoa possess a giant mitochondrion containing dsDNA (kinetoplast) that, in general, does not show any of the other antigens present in the cell nuclei. Antibodies reacting with the kinetoplast are therefore only directed against dsDNA.
- Alongside the conventional CLIFT, which shows a particularly high disease specificity, EUROIMMUN has developed an Anti-Crithidia luciliae sensitive IFT (order no. FA 1572-####-1), which is comparable in sensitivity to the Anti-dsDNA-NcX ELISA and the Farr assay. However, despite comparable sensitivities, the assays identify different patients. To increase the serological hit rate different test systems are often combined.

Microplate ELISA: Anti-dsDNA-NcX
- Monospecific detection of antibodies against dsDNA.
- Indication: lupus erythematosus disseminatus.
- Serum dilution: 1:200, conjugate class anti-human IgG, POD-labelled.
- Antibodies against dsDNA can be determined quantitatively in IU/ml.
- 3-point calibration, quantitative.
- Antigen: double-stranded DNA, complexed with nucleosomes (NcX).
- Due to good sensitivity and specificity, the Anti-dsDNA-NcX ELISA stands out by high diagnostic efficiency. High concentrations of autoantibodies against dsDNA in the ELISA are considered to be a reliable marker for the diagnosis or prognosis of SLE. Individual changes in the dsDNA antibody concentration correlate with the activity of the disease and can be used for monitoring the development of the disease in SLE patients. In cases of immunosuppressive therapy or clinical remission dsDNA antibodies cannot be detected with the ELISA anymore.

Anti-dsDNA RIA by Farr
- Monospecific detection of antibodies against dsDNA.
- Indication: lupus erythematosus disseminatus.
- Use of undiluted samples.
- Antigen: 125I-labelled dsDNA from plasmid DNA.
- The Farr radiolmmunoassay has always been of great importance. On the whole, it has the same specificity as the immunofluorescence test and the same sensitivity as the ELISA. Apparently, its well-known high diagnostic efficiency is based on the fact that only the fraction of the anti-dsDNA antibodies which is able to form bigger precipitating immune complexes with circulating DNA in liquid phase contributes to the measuring signal. The principle of the Farr test reflects, so to speak, the significant step in the pathomechanism of SLE: the formation of appropriate immune complexes, deposits of which build up in the joints, kidneys, liver and other organs.
Autoantibodies against CCP and Sa

Microplate ELISA: Anti-CCP, Anti-Sa

- Screening test for the specific determination of autoantibodies against cyclic citrullinated peptides (CCP) and citrullinated Sa.
- Indication: rheumatoid arthritis (RA).
- Serum dilution 1:100; conjugate class anti-human IgG, POD-labelled.
- Antibodies against CCP and Sa can be determined quantitatively in RU/ml.
- Optional reference control for the determination of ratio values.
- Antigen: synthetic cyclic citrullinated peptides (CCP, second generation), citrullinated Sa.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Order No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCP</td>
<td>EA 1505-9601 G</td>
</tr>
<tr>
<td>Sa</td>
<td>EA 151a-4802 G</td>
</tr>
</tbody>
</table>

In recent years it has been shown that the rare amino acid citrulline, which is present in filaggrin, is a substantial component of the antigenic epitope. Enzyme immunoassays which use synthetic citrullinated peptide as the target antigen offer a useful alternative to indirect immunofluorescence. A direct comparison study demonstrated that the sensitivity can be increased from 45% to 68% by using cyclic citrullinated peptide instead of linear citrullinated peptide as an ELISA substrate. Antibodies against cyclic citrullinated peptides (CCP) are a new and highly specific marker for RA.

Antibodies against CCP are predominantly of class IgG and have a specificity of 88% for RA. They are observed very early in the disease course and have a high predictive value: patients with anti-CCP antibodies develop significantly more radiologically detectable joint damage than anti-CCP-negative patients. Antibodies against CCP possess a much higher specificity than RF (anti-CCP: 89%; RF: 62%) with the same sensitivity (anti-CCP: 79%; RF: 78%). They can be detected in early stages of the disease in more than 75% of patients.

EUROIMMUN offers an innovative microplate ELISA for quantitative determination of autoantibodies against CCP. Diluted patient sera are incubated in wells coated with synthetic cyclic citrullinated peptides (second generation). Specific antibodies in the serum bind to the immobilised antigen and cause a photometric colour reaction by means of an enzyme-coupled secondary antibody. Five calibration sera ensure reliable measurement of antibody concentrations. The EUROIMMUN Anti-CCP ELISA is a highly specific and sensitive serological test system for the diagnosis of RA.
Autoantibodies against Mitochondria (AMA)

Indirect Immunofluorescence Test: EUROPLUS™ Rat Kidney and M2-3E BIOCHIPs
- Screening test for the detection of antibodies against mitochondria (AMA) including simultaneous confirmation of the subtype AMA M2.
- Indication: primary biliary cirrhosis (PBC).
- Initial dilution 1:100; polyvalent conjugate anti-human IgAGM, FITC-labelled.
- Rat kidney is the standard substrate for detecting anti-mitochondrial antibodies. Nine AMA types (M1 to M9) can be differentiated.
- The BIOCHIP coated with M2-3E permits monospecific confirmation of antibodies against the native pyruvate dehydrogenase complex and the recombinant M2 fusion protein (BPO) in one single test procedure, thus a PBC can be diagnosed serologically with confidence.
- This BIOCHIP Mosaic™ can be supplemented as required using additional substrates, e.g. HEp-2 cells (ANA, nuclear dots), rat liver (liver-kidney microsomes, LKM) or rat stomach (ASMA).

EUROASSAY: AMA Profile M2, M4, M9
- Differentiation of mitochondrial antibodies (AMA).
- Indication: primary biliary cirrhosis (PBC).
- Serum dilution 1:100; conjugate class anti-human IgGM, AP-labelled.
- 3 relevant mitochondrial antibodies can be detected simultaneously and monospecifically: antibodies against M2, M4, M9.
- Native antigens: pyruvate dehydrogenase complex (M2), sulfite oxidase (M4), glycogen phosphorylase (M9).

Associated diseases

<table>
<thead>
<tr>
<th>Anti-</th>
<th>Associated diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>M2</td>
<td>Primary biliary cirrhosis (high titers), other chronic liver diseases</td>
</tr>
<tr>
<td>M4</td>
<td>Primary biliary cirrhosis</td>
</tr>
<tr>
<td>M9</td>
<td>Early phase of primary biliary cirrhosis</td>
</tr>
</tbody>
</table>

Incubated EUROASSAY AMA Profile M2, M4, M9.

Microplate ELISA: Anti-M2-3E
- Differentiation of mitochondrial antibodies (AMA).
- Indication: primary biliary cirrhosis (PBC).
- Serum dilution 1:100; conjugate class anti-human IgG, POD-labelled.
- Antibodies against M2 can be determined quantitatively in RU/ml.
- Antigen: native pyruvate dehydrogenase complex plus recombinant M2 fusion protein (BPO) containing the immunogenic domains of the E2 subunits of PDH, BCOADH and OGDH.

Incubated ELISA anti-M2-3E.
Autoantibodies against Liver Antigens

Indirect Immunofluorescence Test: Liver Mosaic 8

- Screening and differentiation test for the detection of liver-specific antibodies, antibodies against mitochondria (AMA), antibodies against cell nuclei (ANA), antibodies against smooth muscles (ASMA), F-actin and other autoantibodies.
- Indications: autoimmune hepatitis, primary biliary cirrhosis, rheumatic diseases.
- Initial dilution 1:100; polyvalent conjugate anti-human IgAGM, FITC-labelled.
- The BIOCHIP Mosaic™ consists of 6 substrates: human epithelial cells (HEp-2), primate liver, rat kidney, rat liver, rat stomach, VSM47. Thus, a broad spectrum of antigens is present, allowing not only targeted serological diagnoses, but also frequently yielding additional results with clinical relevance.
- Antibodies against cell nuclei (ANA) can be particularly well demonstrated using HEp-2 cells and primate liver, and are identified according to their fluorescence patterns. However, they also stain the cell nuclei of the other tissues more or less intensely. Clinical significance: rheumatic diseases, primary biliary cirrhosis (antibodies against nuclear dots).
- With primate liver, several liver-specific autoantibodies can be investigated e.g. antibodies against liver cell membrane (anti-LMA) and liver-specific protein (anti-LSP). Clinical significance: autoimmune hepatitis.
- Antibodies against mitochondria (AMA) show a granular cytoplasmic fluorescence on all 6 substrates. With the standard substrate rat kidney, the proximal and distal tubule cells fluoresce equally. Clinical significance: primary biliary cirrhosis.
- Autoantibodies against liver-kidney microsomes (anti-LKM) react with rat liver and rat kidney (see below). The other substrates are essentially negative.
- In the case of autoantibodies against smooth muscles (ASMA), the tunica muscularis, the lamina muscularis mucosa as well as the interglandular contractile fibrils fluoresce on the rat stomach. ASMA directed against the target antigen F-actin furthermore react with the cytoskeleton of HEp-2 cells and the bile canaliculi of primate liver. The substrate VSM47 reacts very specifically, showing a filamentous, needle-like fluorescence. Clinical significance: autoimmune (lupoid) chronic active hepatitis.
- The BIOCHIP Mosaic™ can be supplemented as required with additional substrates, e.g. Crithidia luciliae (antibodies against dsDNA), musculus iliopsoas (antibodies against skeletal muscles), heart (antibodies against striated muscles, antibodies against intercalated disks, AMA M7), different EUROPLUS™ substrates (AMA-M2-3E, Sp100, gp210, PML, SLA/LP, LC-1, LKM).

Rat liver and rat kidney: antibodies against liver-kidney microsomes (anti-LKM).

Indirect Immunofluorescence Test: BIOCHIP Mosaic™ Rat Liver/Rat Kidney (Liver Mosaic 1)

- Specific detection of antibodies against liver-kidney microsomes (anti-LKM).
- Indications: autoimmune hepatitis, often associated with extrahepatic syndromes such as arthralgias, glomerulonephritis, vitiligo and chronic inflammatory bowel diseases.
- Initial dilution 1:100; polyvalent conjugate anti-human IgAGM, FITC-labelled.
- Autoantibodies against liver-kidney microsomes react with rat liver and generate a smooth staining in the cytoplasm of the hepatocytes.
- In rat kidney, particularly in the cortex area, a fine granular fluorescence of the proximal tubules – recognizable by the luminal brush border – is visible. The distal tubules are negative. The fluorescence intensity of the liver cells is normally stronger than that of the proximal renal tubules.
- The differentiation between autoimmune hepatitis and virus-induced hepatitis can additionally be accomplished by investigating the appropriate viral parameters.
Autoantibodies against Liver Antigens

EUROLINE: Profile Autoimmune Liver Diseases
- Differentiation of antibodies in autoimmune liver diseases.
- Indications: autoimmune hepatitis, primary biliary cirrhosis, overlap syndromes.
- Serum dilution 1:100, conjugate class anti-human IgG, AP-labelled.
- With the EUROLINE Profile Autoimmune Liver Diseases, nine autoantibodies can be determined: antibodies against AMA M2, M2-3E (BPO), Sp100, PML, gp210, LKM-1, LC-1, SLA/LP and Ro-52.
- Test strips can be automatically incubated and evaluated using the systems EUROBlotMaster und EUROLineScan (see page 27).
- Further antigen combinations on page 80.

<table>
<thead>
<tr>
<th>Anti</th>
<th>Associated Diseases</th>
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<tbody>
<tr>
<td>M2, Sp100, PML, gp210</td>
<td>Primary biliary cirrhosis</td>
</tr>
<tr>
<td>LKM-1, SLA/LP, LC-1</td>
<td>Autoimmune hepatitis</td>
</tr>
<tr>
<td>Ro-52</td>
<td>Autoimmune hepatitis, rheumatic diseases</td>
</tr>
</tbody>
</table>

EUROASSAY: Liver Profile (Anti-M2, -LKM-1, LC-1, -SLA/LP)
- Determination of mitochondrial antibodies AMA M2, antibodies against liver-kidney microsomes type 1 (LKM-1), antibodies against liver cytosolic antigen type 1 (LC-1), as well as of antibodies against soluble liver antigen/liver-pancreas antigen (SLA/LP).
- Indication: autoimmune liver diseases.
- Serum dilution 1:100, conjugate class anti-human IgG, AP-labelled.
- Antibodies against M2, LKM-1, LC-1 and SLA/LP can be detected simultaneously and monospecifically.
- Antigens: pyruvate dehydrogenase complex (M2, native), cytochrome P450 IID6 (LKM-1, recombinant), formiminotransferase-cyclodeaminase (LC-1, recombinant) and soluble liver antigen/liver-pancreas antigen (SLA/LP, recombinant).

Microplate ELISA: Anti-SLA/LP, Anti-LC-1, Anti-LKM-1
- Monospecific determination of antibodies against soluble liver antigen/liver-pancreas antigen (SLA/LP), cytosolic liver antigen type 1 (LC-1) and liver-kidney microsomes type 1 (LKM-1).
- Indication: autoimmune hepatitis.
- Serum dilution 1:100, conjugate class anti-human IgG, POD-labelled.
- 3-point calibration, quantitative (exception: Anti-LC-1, semi-quantitative).
- Identical incubation conditions and times: all tests can be combined without difficulty on one and the same microplate.
- Recombinant antigens: soluble liver antigen/liver-pancreas antigen (SLA/LP), formiminotransferase-cyclodeaminase (LC-1) and cytochrome P450 IID6 (LKM-1). The corresponding human cDNA was expressed in E. coli (SLA/LP) or insect cells (LC-1, LKM-1).
Autoantibodies against Thyroid Gland Antigens / Antigen Detections

Indirect Immunofluorescence Test: EUROPLUS™ Thyroid Gland (unfixed) and Thyroglobulin
- Detection of antibodies against thyroid gland antigens.
- Indications: Basedow’s disease, Hashimoto autoimmune thyroiditis.
- Initial dilution 1:10; conjugate class anti-human IgG, FITC-labelled.
- Using unfixed thyroid tissue, two important thyroid-specific antibodies can be found: Autoantibodies against thyroid microsomes (MAb) give a granular staining in the cytoplasm of the follicle epithelium (target antigen: thyroid peroxidase, TPO). Autoantibodies against thyroglobulin (TAb) react with the colloid of all follicles of the thyroid tissue and cause a reticular fluorescence pattern.
- With the thyroglobulin-coated BIOCHIP, autoantibodies against thyroglobulin (TG) can be confirmed monospecifically in one and the same test procedure.
- This BIOCHIP Mosaic™ can be supplemented as required with further substrates, e.g. rat kidney, to achieve a differentiation of antibodies against thyroid microsomes from mitochondrial antibodies (AMA). For a serological diagnosis of autoimmune polyendocrinopathies, BIOCHIPS with frozen sections of pancreas, adrenal gland, ovary, testis and stomach can be added.

Radioimmunoassays (RIA/IRMA): Thyroid Specific Autoantibodies, Antigens and Hormones
- Monospecific detection of autoantibodies against thyroglobulin (TG), thyroid peroxidase (TPO) and thyrotropin receptor (TSH-R).
- Specific detection of the thyroid antigen thyrotropin and the hormones free triiodothyronine (FT3), free thyroxine (FT4), thyrotropin (TSH), calcitonin.
- Indications: Basedow’s disease, Hashimoto autoimmune thyroiditis, medullary thyroid carcinoma, thyroidal C-cell hyperplasia, therapy controls in hyper- and hypothyrosis.
- Serum dilutions: 1:50 for anti-TG and anti-TPO (magnetic), 1:20 for anti-TG and anti-TPO (precipitation), undiluted for all remaining test kits.
- 5-point to 8-point calibration (quantitative).

Microplate ELISA: Anti-Thyroglobulin, Anti-Thyroid Peroxidase, Anti-TSH receptor
- Monospecific determination of antibodies against thyroglobulin (TG), thyroid peroxidase (TPO), and thyrotropin receptor (TSH-R).
- Indications: Basedow’s disease, Hashimoto autoimmune thyroiditis.
- Serum dilution 1:200 (exception: anti-TSH-R undiluted); conjugate class anti-human IgG, POD-labelled (anti-TSH-R: avidin-labelled).
- 3-point calibration (exception: anti-TSH-R, 5-point calibration).
- The quantification is carried out according to international reference preparations (anti-TG: NIBSC 65/93; anti-TPO: NIBSC 66/387; anti-TSH-R: NIBSC 90/672).
- Thyroglobulin/TSH-R: native antigen; thyroid peroxidase: recombinant antigen.
**Indirect Immunofluorescence test: Dermatology Mosaic 7**

- Screening and differentiation test for detection of skin-specific antibodies.
- Indication: autoimmune bullous dermatoses.
- Initial dilution 1:10; conjugate class anti-human IgG, FITC-labelled.
- The BIOCHIP Mosaic consists of 6 substrates: primate oesophagus, salt-split skin, desmoglein-1-expressing cells, desmoglein-3-expressing cells, BP230-expressing cells (gc) and BP180 (EUROPLUS). Thus a comprehensive antigen spectrum is available in a single analysis, allowing targeted serological diagnosis.
- Antibodies against prickle cell desmosomes react with surface antigens of keratinocytes. Tissue sections of oesophagus and tongue show a granular fluorescence of the intercellular matter in the whole stratum spinosum, but differentiation between desmoglein 1 and desmoglein 3 is difficult. When specific transfected cells are employed in addition, a targeted diagnosis in a single test run is possible.
- Antibodies against basal membrane structures react with salt-split skin. Anti-BP180, anti-BP230 and anti-LAD97 cause staining of the epidermal side, and antibodies against laminin 5, collagen VII and other antigens staining of the dermal side of salt-split skin.
- When autoantibodies against BP180 or BP230 are present, the epidermal basal membrane in the oesophagus or tongue is visible as a fine linear colouring between the stratum basale and the connective tissue. These antibodies can be differentiated by means of BP180-NC16A-4X coated BIOCHIPS and cells specifically transfected with BP230 (globular C-terminal domain (gC)).
- This BIOCHIP Mosaic can be customised with further substrates if required, e.g. tongue (antibodies against prickle cell desmosomes, epidermal basal membrane), bladder (antibodies against plakins).

<table>
<thead>
<tr>
<th>Substrate</th>
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<tbody>
<tr>
<td>Desmoglein 1 (transfected/non-transfected cells)</td>
<td>FA 1495-####-50</td>
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<tr>
<td>Desmoglein 3 (transfected/non-transfected cells)</td>
<td>FA 1496-####-50</td>
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<tr>
<td>Oesophagus</td>
<td>FA 1501-####</td>
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<td>Oesophagus/Tongue</td>
<td>FA 1501-####-1</td>
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<tr>
<td>Tongue</td>
<td>FA 1502-####</td>
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<tr>
<td>Bladder mucosa</td>
<td>FA 1507-####</td>
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<tr>
<td>Salt split skin</td>
<td>FA 150b-####</td>
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<tr>
<td>BP180-NC16A-4X/BP230gC</td>
<td>FA 1502-####-1</td>
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<tr>
<td>Envoiplakin</td>
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<tr>
<td>Collagen type VII NC1</td>
<td>FA 1947-####-50</td>
</tr>
</tbody>
</table>

**Microplate ELISA: Anti-Desmoglein 1, Anti-Desmoglein 3, Anti-Envoiplakin, Anti-BP180-NC16A-4X, Anti-BP230-CF**

- Monospecific detection of antibodies against desmoglein 1, desmoglein 3, envoiplakin, BP180 und BP230.
- Indication: autoimmune bullous dermatoses.
- Serum dilution 1:100; conjugate class anti-human IgG, POD-labelled.
- 3-point calibration, quantitative (exception: anti-enviplakin, semiquantitative).
- Identical incubation conditions and times: all tests can be combined on one microplate.
- Recombinant antigens: extracellular domain of desmoglein 1 or 3, envoiplakin, tetramer of NC16A domain of BP180 protein, C-terminal segment of BP230 protein. The corresponding human cDNA is produced in E. coli (BP180-NC16A-4X, BP230-CF, envoiplakin) or in mammalian cells (desmoglein 1, desmoglein 3).
Autoantibodies against Neuronal Antigens

Indirect Immunofluorescence Test: BIOCHIP Mosaic™ Cerebellum/Peripheral Nerves/Intestinal Tissue

- Screening test for the detection of antibodies against neuronal antigens.
- Indications: neurological diseases.
- Initial dilution 1:10; conjugate class anti-human IgAGM, FITC-labelled.
- Primate cerebellum and primate nerves are the standard substrates for the determination of various neuronal antibodies. The parallel use of primate intestine permits the reliable differentiation from other autoantibodies (e.g. ANA) and makes it possible to distinguish between anti-Ri and anti-Hu.
- Antibodies against grey matter react intensively with the stratum granulosum and in a weaker form with the stratum moleculare of the cerebellum. Target antigen: glutamic acid decarboxylase (GAD). Clinical significance: stiff person syndrome, diabetes mellitus type I.
- Antibodies against Yo stain exclusively the cytoplasm of the Purkinje cells in the cerebellum. Clinical significance: paraneoplastic neurological syndromes (PNS), indication of a malignoma.
- In the case of antibodies against Hu and Ri all neurone nuclei in the grey matter show a granular fluorescence. Hu antibodies react in the intestine with cell nuclei of the plexus myentericus, whereas Ri antibodies do not. Clinical significance: paraneoplastic neurological syndromes (PNS), indication of a malignoma.
- The white matter of the cerebellum is stained by antibodies against myelin, which present as hyaline cylinders in tissue sections of peripheral nerves. A “droplike” ring-shaped fluorescence is observed in cross sections of nerves.
- The fluorescence of the Virchow-Robin space (cerebellum, optic nerve) and the pia is caused by autoantibodies developed in neuromyelitis optica (NMO-IgG).
- Antibodies against myelin-associated glycoprotein (MAG), on the other hand, show a streaky fluorescence pattern on nerve tissue and a mostly fine-granular ring-shaped fluorescence on cross sections of peripheral nerves. Clinical significance: paraproteinemic neuropathy.
- The BIOCHIP Mosaic™ can be supplemented as required with further substrates, e.g. cerebrum (antibodies against astrocytes), optic nerve, primate liver plus HEp-2 cells (to rule out ANA), Crithidia luciliae (anti-dsDNA), primate stomach (parietal cell antibodies), Borrelia (neuroborreliosis-associated). Aquaporin-4(AQP4) transfected HEK cells allow a monospecific antibody determination in suspected cases of neuromyelitis optica (NMO).

EUROLINE: Neuronal Antigens Profile 2

- Differentiation of antibodies against neuronal antigens.
- Indication: paraneoplastic neurologic syndromes (PNS).
- Serum dilution 1:100; conjugate class anti-human IgG, AP-labelled.
- With the EUROLINE Neuronal Antigens Profile 2, six autoantibodies can be determined: antibodies against amphiphysin, CV2.1*, PNMA2 (Ma/Ta), Ri, Yo and Hu.
- Test strips can be automatically incubated and evaluated using the systems EUROBlotMaster und EUROLineScan (see page 27).
- Additionally, EUROIMMUN offers a Westernblot for the detection of antibodies against neuronal antigens: DW 1111-1601 G.

*) CV2 partial protein, which only contains the N-terminally localised epitopes of the antigen.
Autoantibodies against Neuronal Antigens

Indirect Immunofluorescence Test: Autoimmune Encephalitis Mosaic 1

- Screening test for detection of neuropil antibodies (glutamate receptors type NMDA and type AMPA, Lgi1, Caspr2, GABA\(_B\) receptors).
- Indication: autoimmune encephalitis.
- Initial dilution 1:10; conjugate class anti-human IgG, FITC-labelled.
- Immunohistochemistry with tissue sections of rat hippocampus and rat cerebellum allows identification of antibodies against glutamate receptors (type NMDA, type AMPA) and other antibodies (e.g. VGKC-associated proteins Lgi1 and Caspr2). The parallel use of transfected HEK293 cells enables sensitive and monospecific antibody detection and differentiation of various neuropil antibodies.
- Neuropil antibodies show a flat, smooth to fine-granular fluorescence in the cytoplasm of transfected HEK293 cells. Clinical significance: autoimmune encephalitis.

EUROLINE-WB: Anti-Neuronal Antigens

- Determination of human autoantibodies against neuronal antigens.
- Indication: paraneoplastic neurological syndromes (PNS).
- Serum dilution 1:51; conjugate class anti-human IgG, AP-labelled.
- EUROLINE-WB is a combination of westernblot and line blot techniques. Proteins of a primate cerebellum extract are electrophoretically separated according to molecular mass and transferred onto a nitrocellulose membrane (westernblot). A membrane chip coated with highly purified recombinant Hu, recombinant Ri and recombinant Yo is subsequently applied to the westernblot strip.
- Test strips can be automatically incubated using the system EUROBlotMaster (Seite 27).

Transfected cells: antibodies against NMDR and GABA\(_B\)-R (top), AMPA1 and Lgi1 (middle), AMPA2 and Caspr2 (bottom).

<table>
<thead>
<tr>
<th>Order No. FA112d-####-1G</th>
<th>Formats page 119</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order No. DW1111-####-2G</td>
<td>Formats page 83</td>
</tr>
</tbody>
</table>

Transfected cells: antibodies against NMDR and GABA\(_B\)-R (top), AMPA1 and Lgi1 (middle), AMPA2 and Caspr2 (bottom).
Autoantibodies against Islet Cell Antigens

Indirect Immunofluorescence Test: Primate Pancreas
- Detection of antibodies against islet cells.
- Indications: Differentiation between a late manifestation of diabetes type 1 (latent autoimmune diabetes in adulthood, LADA) and diabetes type 2.
- For a reliable determination of antibodies against islet cells an extended incubation time of 18 hours for the patient serum must be observed. The incubation time may be reduced to 2 hours but this will lead to a decrease in the sensitivity of the antibody detection test.
- Standardised control with JDF units available (order no. CA 1021-0101-1).
- With indirect immunofluorescence autoantibodies against pancreas islets (ICA) can be detected in 80% of patients with new-onset diabetes type 1. Two target antigens of ICA have been identified so far: the enzymes glutamic acid decarboxylase (GAD) and tyrosine phosphatase (IA2).
- This BIOCHIP may be supplemented with further substrates, e.g. primate cerebellum for the detection of antibodies against GAD.
- The microscopic evaluation can be significantly simplified by using small BIOCHIPs (1 x 1 mm). The BIOCHIPs appear almost completely in the field of view and facilitate finding the islet cells, thus rendering a time-consuming search unnecessary, especially in negative samples.

Microplate ELISA: Anti-GAD, Anti-IA2, Anti-GAD/IA2 Pool
- Monospecific detection of antibodies against glutamic acid decarboxylase (GAD), tyrosine phosphatase (IA2) or bispecific detection of both antibodies in a single reagent well.
- Indications: early diagnosis of diabetes mellitus type 1, risk prediction in first grade relatives, prognosis of the clinical progression of diabetes type 1 for prediction of insulin dependence, differential diagnosis in gestational diabetes, differentiation between a late manifestation of diabetes type 1 (latent autoimmune diabetes in adulthood, LADA) and diabetes type 2.
- Use of undiluted samples. Similar incubation conditions and times. Manual or automated test performance.
- Multipoint calibration. The quantitation is based on an international reference preparation (NIBSC 97/550).
- GAD and IA2: human, recombinant antigens.

Antigen | Order no.
---|---
Glutamic acid decarboxylase (GAD) | EA 1022-9601 G
Tyrosine phosphatase (IA2) | EA 1023-9601 G
GAD/IA2 Pool | EA 1022-9601-1 G

RIA: Anti-GAD, Anti-IA2, Anti-Insulin
- Monospecific detection of antibodies against glutamic acid decarboxylase (GAD), tyrosine phosphatase (IA2) and insulin.
- Indications: Early diagnosis of diabetes mellitus type 1, risk prediction in first grade relatives, prognosis of the clinical progression of diabetes type 1 for prediction of insulin dependence, differential diagnosis in gestational diabetes, differentiation between a late manifestation of diabetes type 1 (latent autoimmune diabetes in adulthood, LADA) and diabetes type 2.
- Use of undiluted samples. Similar incubation conditions and times. Manual or automated test performance.
- Test kit formats for 50 or 100 determinations.
- GAD and IA2: human, recombinant, 125I-labelled antigens, insulin: human, synthetic, 125I-labelled antigen.

Antigen | Order no.
---|---
Glutamat-Decarboxylase (GAD) | RA 1022-####
Tyrosin-Phosphatase (IA2) | RA 1023-####
Insulin | RA 1024-####
Autoantibodies against Parietal Cells (PCA)

Indirect Immunofluorescence Test: Primate Stomach with Urea Pretreatment

- Screening test for detection of antibodies against parietal cells.
- Indications: forms of chronic atrophic gastritis, pernicious anemia, funicular myelosis, various autoimmune endocrinopathies such as Basedow’s and Addison’s diseases.
- Initial dilution 1:10; polyvalent conjugate anti-human IgAGM, FITC-labelled.
- Primate stomach is the standard substrate for detection of parietal cell antibodies. For titration, stomach tissue from rat or mouse is sufficient.
- With positive results the parietal cells show a coarse granular to clumpy fluorescence, and the surrounding areas are usually dark.
- Parietal cell antibodies (PCA) are often mixed up with antibodies against mitochondria (AMA) in microscopic analysis. The latter give an even fine granular fluorescence of the parietal cell cytoplasm, with the surrounding region showing a (weaker) reaction.
- For reliable differentiation of both types of antibody, a 30-minute pretreatment of the tissue sections with urea-glycine buffer (order no. ZF1140-0101, see page 151) is recommended.
- The cytoplasmic fluorescence of parietal cells resulting from PCA occurs with the same intensity with or without urea pretreatment. The typical pattern of mitochondrial antibodies is almost completely suppressed by urea pretreatment, greatly facilitating PCA diagnostics.
- In some AMA-positive samples it is possible to detect PCA that are obscured by the AMA pattern in conventional tissue sections.
- The urea-pretreated tissue shows a significantly darker background, enabling specific fluorescence to be more easily and reliably identified.
- This BIOCHIP can be supplemented with additional substrates, for example, thyroid (thyroid peroxidase, thyroglobulin), pancreas (pancreas islets), adrenal gland (adrenal cortex), ovary (ovary antigens), testis (Leydig cells), and intrinsic factor.

Microplate ELISA: Anti-Parietal Cells

- Monospecific detection of antibodies against parietal cells (PCA).
- Indications: forms of chronic atrophic gastritis, pernicious anemia, funicular myelosis, various autoimmune endocrinopathies such as Basedow’s and Addison’s diseases
- Serum dilution 1:200; conjugate class anti-human IgG, POD-labelled.
- 3-point calibration, quantitative.
- Native antigen: H+/K+-ATPase, purified by affinity chromatography.
Autoantibodies against Granulocyte Cytoplasm (cANCA/pANCA)

Indirect Immunofluorescence Test: EUROPLUS™ Granulocyte Mosaic 25
- Screening test for the detection of antibodies against granulocyte cytoplasm (ANCA).
- Indications: Wegener’s granulomatosis, various forms of glomerulonephritis, primary sclerosing cholangitis, ulcerative colitis, Crohn’s disease.
- Initial dilution: serum 1:10; conjugate anti-human IgG, FITC-labelled.
- Using ethanol-fixed granulocytes, antibodies against granulocyte cytoplasm can be detected. In this case, two fluorescence patterns have to be differentiated: a granular fluorescence which is distributed evenly over the entire cytoplasm, leaving the cell nuclei free (cytoplasmic type, cANCA) or a smooth fluorescence wrapped ribbon-like around the cell nuclei (perinuclear type, pANCA).
- Antibodies against all relevant granulocyte antigens as well as against further, as yet unknown antigens are detected simultaneously:

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Target antigen</th>
<th>Associated diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>cANCA</td>
<td>Proteinase 3</td>
<td>Wegener’s granulomatosis</td>
</tr>
<tr>
<td>pANCA</td>
<td>Myeloperoxidase</td>
<td>Microscopic arteritis, Churg-Strauss syndrome, polyarteritis nodosa</td>
</tr>
<tr>
<td>pANCA</td>
<td>Elastase</td>
<td>Ulcerative colitis, Crohn’s disease, primary sclerosing cholangitis, systemic lupus erythematosus</td>
</tr>
<tr>
<td>pANCA</td>
<td>Cathepsin G</td>
<td>Ulcerative colitis, primary sclerosing cholangitis, Crohn’s disease</td>
</tr>
<tr>
<td>pANCA</td>
<td>Lysozyme</td>
<td>Ulcerative colitis, primary sclerosing cholangitis, Crohn’s disease</td>
</tr>
<tr>
<td>pANCA</td>
<td>Lactoferrin</td>
<td>Ulcerative colitis, systemic lupus erythematosus, rheumatoid arthritis</td>
</tr>
<tr>
<td>pANCA</td>
<td>unknown</td>
<td>Ulcerative colitis, Crohn’s disease</td>
</tr>
</tbody>
</table>

- The HEp-2 cells + granulocytes enable one to differentiate between pANCA and anti-nuclear antibodies (ANA) which can easily be confused when using ethanol-fixed granulocytes: In the case of a positive ANA result all nuclei of the HEp-2 cells fluoresce, whereas in the case of pANCA (as well as cANCA) only the granulocytes fluoresce.
- The EUROPLUS substrates PR3 and MPO as monospecific tests can confirm results from conventional granulocyte screening tests. Recombinant GBM EUROPLUS substrate also provides additional reliability for diagnosis. When fluorescence patterns are unclear (e.g., unspecific fluorescence caused by other cytoplasmic antibodies) these substrates facilitate evaluation.

Microplate ELISA: ANCA Profile
- Differentiation of antibodies against granulocyte cytoplasm (ANCA).
- Indications: Wegener’s granulomatosis, various forms of glomerulonephritis, primary sclerosing cholangitis, ulcerative colitis, Crohn’s disease.
- Serum dilution 1:100; conjugate class anti-human IgG, POD-labelled.
- 6 relevant anti-granulocyte antibodies can be detected simultaneously and monospecifically: autoantibodies against proteinase 3, lactoferrin, myeloperoxidase, elastase, cathepsin G, BPI.
- 1-point calibration, semi-quantitative. Calibrator pool and serum sample on the same microplate strip.
- Native antigens, purified by affinity chromatography.
- Available individual ELISA (3-point calibration, quantitative):

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Order No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinase 3 (Capture ELISA)</td>
<td>EA 1201-9601-1 G</td>
</tr>
<tr>
<td>Proteinase 3 (PR3-hn-hr: native/recombinant)</td>
<td>EA 1201-9601-2 G</td>
</tr>
<tr>
<td>Myeloperoxidase</td>
<td>EA 1211-9601 G</td>
</tr>
</tbody>
</table>
Cost-effective Strategy for the Detection of Autoantibodies against Granulocyte Cytoplasm (ANCA)

Screening test indirect immunofluorescence: BIOCHIP sextet granulocytes (EOH), granulocytes (HCHO), EUROLUS™ microdots PR3, EUROLUS™ microdots MPO, human epithelial cells (HEp-2), and primer liver

- A Granulocytes (EOH-fixed)
- B Granulocytes (HCHO-fixed)
- C EUROLUS™ PR3 microdots
- D EUROLUS™ MPO microdots
- E HEp-2 cells
- F Primate liver

Perinuclear fluorescence of granulocytes (A, F)
Fluorescence of all cell nuclei (A, E, F)
Fluorescence of all cell nuclei (A, E, F), granuloc. accent. (F)
Cytoplasmic fluorescence of granulocytes (B)
Nuclei of granuloc. brighter than nuclei of hepatocytes (F), B neg.
ANA reaction at A, ANA reaction at E & F

- pANCA, formalin-resistant
- pANCA, formalin-sensitive
- only ANA
- ANA and pANCA
- pANCA, formalin-res. & ANA
- pANCA, formalin-sens. & ANA
- pANCA, formalin-sens.7 & ANA

ELISA: anti-MPO
AAb against MPO
MPA 53 %

ELISA: ANCA Profile
AAb against PR3, MPO, elastase, cathespin G, BPI, lactoferrin
WG 5 % (BPI)
MPA 53 % (MPO)
MPA 3 % (LF)
RA vasculitidis 45 % (LF)
SLE 6 % (EL)

Qualified ANA diagnostics: screening test using HEp-2/primate liver (E, F), differentiation using ELISA, EUROSSAYS, EUROLUNE, WestembloT
AAb against dsDNA, ssDNA, nucleosomes, histones, nuclear membrane, nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Ku, cyclin I (PCNA), mitosan (CENP-F, cyclin II), nuclear dots, centromeres (CENP F), spindle fibres, midbody, centrioles, ScI-70, PM-Scl, fibrillarin, RNA polymerase I, N0R, ribosomal P-proteins, Jo-1, PL-7, PL-12, Mi-2, mitochondria (AMA), lysosomes, Golgi apparatus, vimentin, tropomyosin, actin.

See EUROIMMUN poster: “Strategy for Determination of Autoantibodies against Cell Nuclei (ANA) and Cytoplasm Components”

The highest diagnostic sensitivity in the determination of autoantibodies against neutrophil granulocytes (ANCA) is achieved by using indirect immunofluorescence and assays with defined target antigens (particularly PR3 and MPO) simultaneously at the start. However, under the pressure of cost optimisation, an immunofluorescence test may be performed on its own and then followed up by specific ELISA tests only if the result is positive. Ethanol-fixed human granulocytes are the standard substrate for indirect immunofluorescence. With this substrate two relevant fluorescence patterns can be differentiated: the cytoplasmic type (cANCA) associated with Wegener’s granulomatosis and the perinuclear type (pANCA), which indicates a range of various diseases. The differentiation of pANCA from antibodies against cell nuclei (ANA) is often difficult. Therefore, HEp-2 cells (possibly with sedimented granulocytes) or primate liver are used as an additional substrate. If ANA and pANCA occur together, the granulocytes show a much brighter fluorescence than the cell nuclei. Thanks to EUROIMMUN BIOCHIPs it is not necessary to incubate human epithelial cells on a second slide in parallel for the exclusion of cell nuclei antibodies, since all substrates are present in one and the same test field. A fourth BIOCHIP with formalin-fixed human granulocytes detects a large proportion of the diagnostically relevant antibodies against myeloperoxidase, whereas other pANCA (which are particularly important in gastroenterology) and almost all antibodies against cell nuclei (whose differentiation is a separate chapter in autoantibody diagnostics) are generally completely suppressed. The EUROLUS™ substrates PR3 and MPO help to confirm diagnosis and allow a quick and reliable interpretation of results even in problematic cases.

ANA: anti-nuclear antibodies
BPI: bacterial permeability increasing protein
cANCA: anti-neutrophil cytoplasmic antibodies
Cytoplasmic type CD: Crohn’s disease
CSS: Churg-Strauss syndrome
EL: elastase
EOH: ethanol
HCHO: formalin
HEp-2: human epithelial cells
IFT: immunofluorescence test
LF: lactoferrin
MPA: microscopical polynucleoid
MPO: myeloperoxidase
PAN: polyarteritis nodosa
pANCA: anti-neutrophil cytoplasmic antibodies
PR3: proteinase 3
PSC: primary sclerosing cholangitis
RA: rheumatoid arthritis
SLE: systemic lupus erythematosus
UC: ulcerative colitis
WG: Wegener’s granulomatosis
Antibodies against Endomysium and Gliadin

Indirect Immunofluorescence Test: EUROPLUS™ Primate Liver and Gliadin (GAF-3X) BIOCHIPs
- Detection of antibodies against endomysium and gliadin.
- Indications: gluten-sensitive enteropathy (celiac disease, non-tropical sprue), Duhring’s herpetiform dermatitis.
- Initial dilution 1:10; conjugate class anti-human IgA or IgG, FITC-labelled.
- Autoantibodies against endomysium react with many types of tissue, e.g. primate oesophagus. The most suitable substrate, however, is primate liver: in the case of a positive sample, filamentous linings of the intralobular sinusoids react.
- With the gliadin (GAF-3X)-coated BIOCHIP, antibodies against gliadin can be analyzed in one and the same test procedure.
- Both anti-endomysium antibodies and antibodies against gliadin (class IgA) are reliable serological markers for an active gluten-sensitive enteropathy. Therefore, their determination can in many cases replace endoscopy and biopsy.

Microplate ELISA: Anti-Gliadin (GAF-3X)
- Monospecific detection of antibodies against gliadin.
- Indications: gluten-sensitive enteropathy (celiac disease, non-tropical sprue), Duhring’s herpetiform dermatitis.
- Serum dilution 1:200; conjugate class anti-human IgA or IgG, POD-labelled.
- 3-point calibration. Identical incubation conditions and times: the investigation of IgA and IgG antibodies can be combined without difficulty on one and the same microplate.
- Antigen: Gliadin-analogue fusion peptide (GAF-3X).
- The quantitative determination of antibodies against gliadin is very suitable for monitoring the progress of the disease, compliance with a gluten-free diet, or a gluten tolerance test.

Microplate ELISA: Anti-Tissue Transglutaminase (Endomysium)
- Monospecific detection of antibodies against tissue transglutaminase.
- Indications: gluten-sensitive enteropathy (celiac disease, non-tropical sprue), Duhring’s herpetiform dermatitis.
- Serum dilution 1:200; conjugate class anti-human IgA or IgG, POD-labelled.
- 3-point calibration, quantitative.
- Antigen: recombinant, expression with the baculovirus vector in insect cells.
Antibodies against Borrelia

Indirect Immunofluorescence Test: EUROPLUS™ Anti-Borrelia afzelii, Borrelia burgdorferi, VlsE and OspC Antigen

- Sensitive screening test for the detection of anti-Borrelia antibodies.
- Indications: erythema chronicum migrans, lymphadenosis cutis benigna, lymphocytic meningoradiculitis, carditis, arthritis, acrodermatitis chronica atrophicans, neuroborreliosis.
- Initial dilution 1:10 (IgM), 1:100 (IgG).
- If antibodies against Borrelia afzelii or Borrelia burgdorferi are present, a distinct fluorescence of the bacteria in the smear is obtained.
- With the VlsE or OspC coated BIOCHIP's antibodies against the highly specific and highly sensitive marker antigens VlsE (IgG) or OspC (IgM) can be determined monospecifically in one and the same test procedure. If these antigens fluoresce, the antibody result is positive even if the bacteria smears show a negative reaction. Thus, the BIOCHIP Mosaic helps to increase specificity and sensitivity in Borrelia diagnostics.
- The BIOCHIP can be supplemented as required using further substrates, e.g. Borrelia burgdorferi sensu stricto (strains CH or USA) and TBE virus infected cells.

Microplate ELISA: Anti-Borrelia plus VlsE

- Sensitive screening test for the detection of anti-Borrelia antibodies.
- Indications: erythema chronicum migrans, lymphadenosis cutis benigna, lymphocytic meningoradiculitis, carditis, arthritis, acrodermatitis chronica atrophicans, neuroborreliosis.
- Serum dilution 1:100; conjugate class anti-IgG, anti-IgM or anti-IgAGM (VlsE: -IgG only), POD-labelled.
- 3-point calibration (IgG and IgM). Identical incubation conditions and times: all tests can be combined without difficulty on one and the same microplate.
- Antigens: extract of Borrelia burgdorferi sensu stricto, Borrelia garinii and Borrelia afzelii (whole antigen) / recombinant VlsE from Borrelia burgdorferi sensu stricto. VlsE (variable major protein-like sequence, expressed) is a newly characterized surface protein of Borrelia which is expressed exclusively in vivo and which contains conserved and highly immunogenic epitopes.
- IgM test kit (Anti-Borrelia) includes IgG/RF absorbent in sample buffer for IgG absorption in preparation for the determination of specific IgM class antibodies.

Microplate ELISA: Anti-Borrelia plus VlsE, Antibody Determination in Serum and Cerebrospinal Fluid for Detection of Intrathecal Synthesis of Specific Antibodies against Borrelia

- Antibody determination in serum and cerebrospinal fluid (CSF).
- Indication: Neuroborreliosis.
- CSF dilution 1:2, serum dilution 1:404; conjugate class anti-IgG, POD-labelled.
- 4-point calibration, quantitative.
- Microplate ELISA for the detection of Borrelia antibodies of class IgM in serum and CSF are also available.
Anti-Borrelia EUROLINE-RN-AT

- Specific confirmatory test for the detection of antibodies against Borrelia.
- Indications: erythema chronicum migrans, lymphadenosis cutis benigna, lymphocytic meningoradiculitis, carditis, arthritis, acrodermatitis chronica atrophicans, neuroborreliosis.
- The Anti-Borrelia EUROLINE is coated with both native and recombinant proteins and provides a unique mixture of Borrelia specific antigens: The classical antigens OspC, p83 and p39, which show the highest specificity in their native form, were accurately cut from a Westernblot and applied onto the membrane of the line blot. Five new, recombinant designer antigens (p18, p19, p20, p21, p58) having a very high specificity (IgG) were identified using bioinformatic analysis of the Borrelia genome. For the first time, lipids which have been proven to be immunoreactive and were extracted from the Borrelia membranes, three native OspC antigens (IgM) from B. afzelii, B. burgdorferi and B. garinii and three different VlsE antigens (IgG) from B. afzelii, B. burgdorferi and B. garinii are available on the line blot.
- The serological hit rate is increased by 10% by using three OspC variants in the IgM test.
- Serum dilution 1:50; conjugate class anti-IgG or anti-IgM, AP-labelled.

EUROLINE-WB: Anti-Borrelia (Whole Antigen plus VlsE)

- Specific confirmatory test for the detection of antibodies against Borrelia.
- EUROLINE-WB is a combination of westernblot and line blot techniques. An SDS extract of a Borrelia afzelii strain is electrophoretically separated according to molecular mass and transferred onto a nitrocellulose membrane. A membrane chip coated with highly purified recombinant VlsE-Antigen is then added to the westernblot strips.
- By additionally determining antibodies against VlsE the serological hit rate can be increased by 20% compared to whole extract Westernblots and by 30% compared to recombinant antigen Westernblots. Of all recombinant antigens, VlsE possesses the highest sensitivity for the detection of a Borrelia infection. Over 85% of IgG-positive sera could be identified at a glance by assessing the VlsE band. VlsE allows detection of antibodies against all Borrelia species, and the risk of a false negative reaction due to species differences is ten times lower.
- Serum dilution 1:50; conjugate class anti-IgG or anti-IgM, AP-labelled.

Automated Evaluation of Incubated Membrane Strips with the System EUROLineScan

- The program EUROLineScan from EUROIMMUN has been developed to enable quantitative evaluation of membrane based test systems, facilitate management of data, and provide detailed documentation of results — tasks which have until now required considerable time.
- First, the incubated test strips are scanned using a flatbed scanner or camera system.
- EUROLineScan recognizes the position of the strips, even if they have been placed inexacty, identifies the bands, and measures their intensity. The automated evaluation can be monitored, and it is possible to supplement the data manually.
- The results are then saved together with the image data. It is no longer necessary to archive (potentially infectious) incubated test strips. A separate results sheet can be produced for each patient.
Antibodies against Epstein-Barr Virus (EBV)

Indirect Immunofluorescence Test: BIOCHIP Sequence EBV

- Gold standard for the determination of antibodies against the EBV-CA antigens (Epstein-Barr virus capsid antigen), EBV-EA (Epstein-Barr early antigen) and EBNA (Epstein-Barr nuclear antigen).
- Indication: infectious mononucleosis, Burkitt’s lymphoma, nasopharyngeal carcinoma (NPC).
- IgG antibodies against EBV-CA indicate an EBV infection. An at least twofold increase in titer and the absence of antibodies against EBNA at the same time is characteristic for the early phase of the infection. IgM antibodies against EBV-CA and antibodies against EBV-EA can also be found in acute infections, but they do not necessarily always occur. The presence of antibodies against EBNA generally indicates the late phase of an EBNA infection.
- In cases of an acute EBV infection which cannot be reliably discriminated from a relapse or reinfection, the determination of the antibody avidity using a modified immunofluorescence test as an additional parameter is useful. This requires an additional incubation with urea solution (ZF 1130-0801). The determination of low-avidity antibodies against EBV-CA indicates an acute infection.
- For the monospecific confirmation of EBV-CA antibodies in the same test procedure the BIOCHIP containing ECV-CA is supplemented with the antigen substrates gp125 antigen (native) and p19 antigen (recombinant) (EUROPLUS FI 2791-####-20 G or M).
- For highly differentiated diagnostics the BIOCHIP Sequence EBV (FI 2799-####-1 X) can be supplemented by using the antigens gp125 and p19 (EUROPLUS FI 2799-####-21 X).
- Due to the high prevalence of anti-EBV-CA IgA in NPC patients, the parameter is well suited for screening. Confirmation of the result by determination of IgA antibodies against EBV-EA is recommended. Further anti-EBV test kits for indirect immunofluorescence:

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Order No.</th>
<th>Substrate</th>
<th>Order No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV-CA</td>
<td>FI 2791-#### A, G or M</td>
<td>EBV-EA</td>
<td>FI 2795-#### A or G</td>
</tr>
<tr>
<td>EBNA</td>
<td>FI 2793-#### G</td>
<td>EBV-CA &amp; EBV-EA</td>
<td>FI 2791-####-2 A or G</td>
</tr>
</tbody>
</table>

Microplate ELISA: Anti-EBV-CA, Anti-EBNA-1, Anti-EBV-EA

- Specific confirmatory test for antibodies against EBV-CA, EBNA-1 or EBV-EA.
- Indications: infectious mononucleosis, Burkitt’s lymphoma, nasopharyngeal carcinoma.
- Serum dilution 1:100; conjugate class anti-IgA, -IgG or IgM, POD-labelled.
- 1-point calibration, semi-quantitative (IgA, IgM) or 3-point calibration, quantitative (IgG). Identical incubation conditions and times: all tests can be combined without difficulty on one and the same microplate.
- EBV-CA: native antigen, purified by affinity chromatography; EBV-NA-1 and EBV-EA: recombinant antigen.
- IgM test kit includes IgG/RF absorbent in sample buffer for IgG absorption in preparation for the determination of specific IgM class antibodies.
- Available individual ELISA:

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Order No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV-CA</td>
<td>EI 2791-9601 A, G or M</td>
</tr>
<tr>
<td>EBNA-1</td>
<td>EI 2793-9601 G</td>
</tr>
<tr>
<td>EBV-EA</td>
<td>EI 2795-9601 A, G or M</td>
</tr>
</tbody>
</table>
Antibodies against Epstein-Barr Virus (EBV)

Determination of Low-Avidity Antibodies against EBV-CA

- An alternative principle for the serological diagnosis of fresh infections with EBV has been established by investigating the antibody avidity.
- The first reaction of the immune system following an infection is the formation of low-avidity antibodies. As the infection proceeds, increasingly antigen-adapted IgG is formed, and avidity grows. As long as high-avidity IgG is not yet detected in the serum, it can be assumed that the infection is still in an early stage.
- To identify low-avidity antibodies against EBV-CA in a patient’s serum, two microplate ELISA or immunofluorescence tests are performed in parallel: one test is carried out in the conventional way, the other one includes urea treatment between incubations with patient’s serum and peroxidase-labelled anti-human IgG, resulting in the detachment of low-avidity antibodies from the antigens.
- Low-avidity antibodies against EBV-CA are present if the ELISA extinction is significantly reduced by urea treatment. For an objective interpretation, the relative avidity index (RAI) can be calculated out of the measured values with and without urea incubation.
- With the immunofluorescence test the presence of low-avidity antibodies has been proved if the test using urea treatment gives a far weaker fluorescence than the two-step test.

EUROLINE: EBV Profile 2

- Differentiation of antibodies against Epstein-Barr virus antigens.
- Indications: infectious mononucleosis, Burkitt’s lymphoma, nasopharyngeal carcinoma.
- Serum dilution 1:100; conjugate class anti-human IgG or IgM, AP-labelled.
- With the EUROLINE EBV Profile 2, five different antibodies can be determined: antibodies against VCA gp125, VCA p19, EBNA-1, p22, EA-D.
- Recombinant antigens (exception: VCA gp125, native, purified by affinity chromatography).
- EBNA-1 (IgG) negative und VCA (IgG and IgM) positive: acute (fresh) infection.
- EBNA-1 (IgG) and VCA (IgG) positive and VCA (IgM) negative: late phase of infection.
- EBNA-1 (IgG) negative, but band p22 (IgG) and VCA (IgG) positive: late phase of infection with loss of anti-EBNA-1.
- Test strips can be automatically incubated and evaluated using the systems EUROBlotMaster und EUROLineScan (see page 27).

Westernblot: Anti-EBV

- Specific confirmatory test for the detection of antibodies against Epstein-Barr virus antigens, differentiation of antibodies against Epstein-Barr virus antigens.
- Indications: infectious mononucleosis, Burkitt’s lymphoma, nasopharyngeal carcinoma.
- Serum dilution 1:50; conjugate class anti-IgG or anti-IgM, AP-labelled.
- Antigens: whole antigen, SDS extract.
- Bands from all specific antigens are included and clearly separated.
- EBNA-1 (IgG) negative und VCA (IgG and IgM) positive: acute (fresh) infection.
- EBNA-1 (IgG) and VCA (IgG) positive and VCA (IgM) negative: late phase of infection.
- EBNA-1 (IgG) negative, but band p22 (IgG) and VCA (IgG) positive: late phase of infection with loss of anti-EBNA-1.
- Test strips can be automatically incubated and evaluated using the systems EUROBlotMaster und EUROLineScan (see page 27).
Antibodies against Helicobacter pylori

Indirect Immunofluorescence Test: Anti-Helicobacter pylori
- Sensitive screening test for the detection of antibodies against Helicobacter pylori.
- Indications: gastritis, ulcer ventriculi et duodeni. Late consequences: MALT lymphomas and adenocarcinomas.
- Initial dilution 1:10 (IgM), 1:100 (IgG), 1:32 (IgA).
- If antibodies against Helicobacter pylori are present, a distinct fluorescence of the bacteria in the smear is obtained.
- A positive IgA result correlates well with the activity of a gastritis. An increased IgG antibody titer is considered to be a marker for chronic infections. A significant drop in the IgG antibody titer about 6 months after therapy is a sign of success.
- The BIOCHIP can be supplemented as required with further substrates, e.g. other infectious agents or tissue sections of primate stomach.

Microplate ELISA: Anti-Helicobacter pylori
- Sensitive screening test for the detection of antibodies against Helicobacter pylori.
- Indications: gastritis, ulcer ventriculi et duodeni. Late consequences: MALT lymphomas and adenocarcinomas.
- Serum dilution 1:100; conjugate class anti-IgA or anti-IgG, POD-labelled.
- Antibodies against Helicobacter pylori antigens can be determined quantitatively in RU/ml.
- 1-point calibration, semi-quantitative (IgA) or 3-point calibration, quantitative (IgG). Identical incubation conditions and times: both tests can be combined without difficulty on one and the same microplate.
- Native antigens.

EUROLINE-WB: Anti-Helicobacter pylori
- Specific confirmatory test for the detection of antibodies against Helicobacter pylori.
- Indications: gastritis, ulcer ventriculi et duodeni. Late consequences: MALT lymphomas and adenocarcinomas.
- Serum dilution 1:50; conjugate class anti-IgA or anti-IgG, AP-labelled.
- EUROLINE-WB is a combination of westernblot and line blot techniques. An SDS extract of a Helicobacter pylori strain is electrophoretically separated according to molecular mass and transferred onto a nitrocellulose membrane (westernblot). Two membrane chips coated with highly purified recombinant CagA and VacA are subsequently applied to the westernblot strips.
- Bands from all specific antigens are included and clearly separated.
- Test strips can be automatically incubated and evaluated using the systems EUROBlotMaster und EUROLineScan (see page 27).
Antibodies against Herpes Simplex Virus (HSV)

Indirect Immunofluorescence Test: BIOCHIP Mosaic™ HSV-1/HSV-2

- Sensitive screening test for the detection of antibodies against herpes simplex viruses.
- Indication: herpes simplex.
- Initial dilution 1:10 (IgM), 1:100 (IgG).
- If antibodies against herpes simplex-2 virus are present in the sample, a typical fluorescence of the infected cells is obtained – mainly in the outspread cytoplasm, less in the cell nuclei.
- As HSV-1 and HSV-2 are morphologically and immunologically closely related, cross-reactions can occur. Differentiation may be attempted by testing a serum against both antigen substrates and comparing the titers.
- The BIOCHIP Mosaic™ can be supplemented as required with further substrates, e.g. other infectious agents.
- Anti-HSV individual tests for indirect immunofluorescence:

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Order No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1</td>
<td>FI 2531-#### G or M</td>
</tr>
<tr>
<td>HSV-2</td>
<td>FI 2532-#### G or M</td>
</tr>
<tr>
<td>HSV-1 and HSV-2</td>
<td>FI 2531-####-1 G or M</td>
</tr>
</tbody>
</table>

Microplate ELISA: Anti-HSV-1, Anti-HSV-2

- Specific confirmatory tests for antibodies against HSV-1 or HSV-2.
- Indication: herpes simplex.
- Serum dilution 1:100; conjugate class anti-IgG or anti-IgM, POD-labelled.
- 1-point calibration, semiquantitative (IgM) or 3-point calibration, quantitative (IgG). Identical incubation conditions and times: all tests can be combined without difficulty on one and the same microplate.
- Antigens: type-specific glycoproteins C1 or G, purified by affinity chromatography. Cross-reactions do not occur.
- IgM test kit includes IgG/RF absorbent in sample buffer for IgG absorption in preparation for the determination of specific IgM class antibodies.
- Available individual ELISA:

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Order No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1</td>
<td>EI 2531-9601-2 G or M</td>
</tr>
<tr>
<td>HSV-2</td>
<td>EI 2532-9601-2 G or M</td>
</tr>
<tr>
<td>HSV-1/2-Pool</td>
<td>EI 2531-9601-1 G or M</td>
</tr>
</tbody>
</table>

EUROLEINE-WB: Anti-HSV

- Specific confirmatory test for the differentiation of antibodies against HSV-1 and HSV-2.
- Indication: herpes simplex.
- Serum dilution 1:50; conjugate class anti-IgG or anti-IgM, AP-labelled.
- EUROLEINE-WB is a combination of westernblot and line blot techniques. Proteins from an SDS extract of HSV-1 are electrophoretically separated according to molecular mass and transferred onto a nitrocellulose membrane. A membrane chip coated with HSV-2 type-specific glycoprotein G2 (gG2), purified by affinity chromatography, is then added to the westernblot strips.
- Bands from all specific antigens are included and clearly separated.
- The gG2 band allows the simple differentiation between HSV-1 and HSV-2 infections.
- Test strips can be automatically incubated and evaluated using the systems EUROBlotMaster und EUROLEineScan (see page 27).

<table>
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<tr>
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<tbody>
<tr>
<td>DY 531-1601-1 G or M</td>
<td>page 85</td>
</tr>
</tbody>
</table>

Antibodies against HSV-1 and HSV-2.
Antibodies against Chlamydia

Indirect Immunofluorescence test: Anti-Chlamydia MIF (Micro-Immunofluorescence test)
- Serological gold standard for the determination of antibodies against Chlamydia.
- Indication: trachoma, urogenital infections, lymphogranuloma venereum, laryngitis, sinusitis, bronchitis, pneumonia, psittacosis.
- Initial dilution 1:10 (IgA), 1:100 (IgG), 1:10 (IgM).
- The micro-immunofluorescence test uses purified elementary bodies of the species C. trachomatis, C. pneumoniae and C. psittaci as the antigen. The mutual lipopolysaccharide (LPS) antigen is inactivated to minimise cross reactivity.
- The evaluation of the MIF could be significantly facilitated compared to conventional test systems by using a cell-based substrate.
- The fourth BIOCHIP with non-infected cells allows a reliable differentiation between unspecific and specific fluorescence.

Microplate ELISA: Anti-Chlamydia trachomatis
- Monospecific detection of antibodies against Chlamydia trachomatis.
- Indication: trachoma, conjunctivitis, urogenital infections, pneumonia in infants, lymphogranuloma venereum.
- Serum dilution 1:100, conjugate class anti-human IgA, IgG or IgM, POD-labelled.
- 1-point calibration, semiquantitative (IgA and IgM) or 3-point calibration, quantitative (IgG).
- Antigen: native MOMP antigen (major outer membrane protein). MOMP is a transmembrane protein and represents the main part of the outer membrane of the elementary bodies. Protein purification starts with BGM cells infected with Chlamydia trachomatis of the serotype K.
- IgM test kit includes IgG/RF absorbent in sample buffer for IgG absorption in preparation for the determination of specific IgM class antibodies.

Microplate ELISA: Anti-Chlamydia pneumoniae
- Monospecific detection of antibodies against Chlamydia pneumoniae.
- Indication: laryngitis, sinusitis, bronchitis, pneumonia.
- Serum dilution 1:100, conjugate class anti-human IgA, IgG or IgM, POD-labelled.
- 1-point calibration, semiquantitative (IgA and IgM) or 3-point calibration, quantitative (IgG).
- Antigen: cell lysate from HL cells, strain CDC/CWL-029.
- IgM test kit includes IgG/RF absorbent in sample buffer for IgG absorption in preparation for the determination of specific IgM class antibodies.
Antibodies against Emerging Viruses

Indirect Immunofluorescence Test

- Over the past years it has been observed that a number of new viruses ("emerging" or "re-emerging viruses") and other pathogens has spread worldwide, introducing since then unknown diseases into previously unaffected regions.
- EUROIMMUN offers a broad spectrum of indirect immunofluorescence tests for the detection of specific antibodies against:

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Disease, syndromes</th>
<th>see page</th>
</tr>
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<tbody>
<tr>
<td>Corona virus</td>
<td>SARS corona virus (SARS-CoV)</td>
<td>Severe acute respiratory syndrome (SARS)</td>
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<tr>
<td>Flaviviruses</td>
<td>TBE virus (TBEV)</td>
<td>Tick-borne encephalitis</td>
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<tr>
<td></td>
<td>West Nile virus (WNV)</td>
<td>West Nile fever, encephalitis</td>
</tr>
<tr>
<td></td>
<td>Japanese encephalitis virus (JEV)</td>
<td>Japanese encephalitis</td>
</tr>
<tr>
<td></td>
<td>Yellow fever virus (YFV)</td>
<td>Yellow fever, hepatitis, haemorrhagic fever, arthritis</td>
</tr>
<tr>
<td></td>
<td>Dengue virus (DENV, types 1-4)</td>
<td>Dengue fever, haemorrhagic fever</td>
</tr>
<tr>
<td>Bunyaviruses</td>
<td>Hantavirus (types Hantaan, Puurula, Seoul, Saaremaa, Dobrava, Sin Nombre and Andes)</td>
<td>HFRS (haemorrhagic fever with renal syndrome); HCPS (Hantaviral cardiopulmonary syndrome)</td>
</tr>
<tr>
<td></td>
<td>Sandfly fever virus (types Sicilian, Naples, Toscana and Cyprus)</td>
<td>Pappacaci fever, meningitis, encephalitis</td>
</tr>
<tr>
<td></td>
<td>Rift valley fever virus (RVFV)</td>
<td>Rift valley fever, haemorrhagic fever, hepatitis</td>
</tr>
<tr>
<td></td>
<td>Crimean-Congo fever virus (CCHFV-GPC and -N)</td>
<td>Crimean-Congo haemorrhagic fever</td>
</tr>
<tr>
<td>Togaviruses</td>
<td>Chikungunya virus (CHIKV)</td>
<td>Chikungunya fever, arthritis</td>
</tr>
<tr>
<td></td>
<td>Sindbis virus (SINV)</td>
<td>Sindbis fever</td>
</tr>
<tr>
<td>Kinotoplastida</td>
<td>Leishmania donovani</td>
<td>Visceral leishmaniasis</td>
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<td>Haemosporina</td>
<td>Plasmodium falciparum</td>
<td>Malaria tropica</td>
</tr>
<tr>
<td></td>
<td>Plasmodium vivax</td>
<td>Malaria tertiana</td>
</tr>
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</table>

- Many of these substrates are available as single substrate with non-infected cells or as useful combinations (syndrome or geographically orientated) for the investigation of serum samples.
- IgG absorption as preparatory step for the determination of specific antibodies of class IgM: page 60.
- Cross reactions within the virus family, especially with Flaviviruses, should be taken into consideration since they may cause false-positive results. The infectious agent can be determined by titration of the sample and comparison of

Microplate ELISA: Anti-TBE Virus, Anti-West Nile Virus, Anti-Dengue Virus

- Monospecific determination of antibodies against TBE, West Nile and Dengue virus.
- Serum dilution 1: 100, conjugate class anti-human IgG or IgM, POD-labelled.
- 1-point calibration, semiquantitative (IgM) or 3-point calibration, quantitative (IgG). Similar incubation conditions and times: All tests can be combined on one and the same microplate.
- IgM test kit with IgG/RF absorbent in the sample buffer for IgG absorption as preparatory step for the determination of specific IgM antibodies.
- Available single ELISA:

<table>
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<td>TBE</td>
<td>EI 2661-9601 G or M, avidity, Ab determination in CSF</td>
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<tr>
<td>TBE „Vienna“</td>
<td>EI 2661-9601-9 G</td>
</tr>
<tr>
<td>WNV</td>
<td>EI 2662-9601 G or M, avidity</td>
</tr>
<tr>
<td>Dengue</td>
<td>EI 266b-9601 G or M</td>
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Order No. (Anti-TBE) | EI 2661-9601 G or M |
Formats | page 94
**BIOCHIP Mosaics™ for Infectious Serology**

(For formats see pages 147-148)

**FI 2821-1001-1 G**

**RESPIRATORY TRACT PROFILE 1**

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<tr>
<td><strong>B</strong></td>
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<tr>
<td>1. Measles virus</td>
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<td>9. Parainfluenza virus type 4</td>
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<td>11. Bordetella parapertussis**</td>
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<td>12. Mycoplasma pneumoniae</td>
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<td>15. Echo virus type 7</td>
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<td>16. Chlamydia pneumoniae</td>
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<td><strong>E</strong></td>
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</table>
| 17. Haemophilus influenzae*  
18. Klebsiella pneumoniae*  
19. Legionella pneumophila serotype 1**  
20. Legionella pneumophila serotype 12** |

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**FI 2822-1001-1 G**

**EXANTHEMA PROFILE 1**

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<td>1. HHV-6</td>
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<td>4. Mumps virus</td>
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<tr>
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<tr>
<td>14. Borrelia afzelii</td>
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<td>15. Borrelia burgdorferi sensu stricto (CH)</td>
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<td>16. Borrelia garinii</td>
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**FI 2823-1001-1 G**

**LYMPHADENITIS PROFILE 1**

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<tbody>
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<tr>
<td>1. Measles virus</td>
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<tr>
<td>14. HSV-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. CMV**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16. Coxsackie virus type B5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Field</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E</strong></td>
<td>1:100</td>
<td>1:100</td>
</tr>
<tr>
<td>17. Coxsackie virus type A9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18. Bartonella henselae**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19. Chlamydia trachomatis**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20. Chlamydia pneumoniae</td>
<td></td>
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</tbody>
</table>

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**FI 2824-1001-1 G**

**CENTRAL NERVOUS SYSTEM PROFILE 1**

<table>
<thead>
<tr>
<th>Field</th>
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<tbody>
<tr>
<td><strong>A</strong></td>
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<td>1:10</td>
</tr>
<tr>
<td>V</td>
<td>Verification BIOCHIP***</td>
<td></td>
</tr>
<tr>
<td>1. Rubella virus*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Measles virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Mumps virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. VZV</td>
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<table>
<thead>
<tr>
<th>Field</th>
<th>IgG</th>
<th>IgM</th>
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</thead>
<tbody>
<tr>
<td><strong>B</strong></td>
<td>1:10</td>
<td>1:10</td>
</tr>
<tr>
<td>1. Adenovirus type 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. EBV-CA**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Treponema pallidum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Toxoplasma gondii**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Field</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C</strong></td>
<td>1:100</td>
<td>1:10</td>
</tr>
<tr>
<td>9. HSV-1</td>
<td></td>
<td></td>
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<tr>
<td>10. HSV-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Coxsackie virus type B1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Coxsackie virus type A7</td>
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</table>

<table>
<thead>
<tr>
<th>Field</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D</strong></td>
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<td>1:10</td>
</tr>
<tr>
<td>13. Echo virus type 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14. Borrelia afzelii</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. Borrelia burgdorferi sensu stricto (CH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16. Borrelia garinii</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Field</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E</strong></td>
<td>1:100</td>
<td>1:100</td>
</tr>
<tr>
<td>17. CMV</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| 18. Haemophilus influenzae*  
19. Listeria monocytogenes 1/2a*  
20. Listeria monocytogenes 4b* |

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* For clinical evaluation the results must be confirmed with a CE approved test.
** For practical reasons the recommended incubation differs from the standard incubation for this substrate.
*** Field A contains a verification BIOCHIP. The incubation was performed correctly if the dots show a visible colour reaction.
BIOCHIP Mosaics™ containing fewer substrates can be produced upon request.
BIOCHIP Mosaics™ for Infectious Serology

(For formats see pages 147-148)

**FI 2825-1001-1 G**

**MYOCARDITIS PROFILE 1**

<table>
<thead>
<tr>
<th>Field A</th>
<th>IgG</th>
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</thead>
<tbody>
<tr>
<td>V</td>
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</tr>
</tbody>
</table>

**Field A**
- V, Verification BIOCHIP***
- 1. Mumps virus
- 2. Adenovirus type 3
- 3. Influenza virus type A (H1N1)
- 4. Influenza virus type A (H3N2)

**Field B**
- 1. Mumps virus
- 2. Influenza virus type B
- 3. Adenovirus type 3
- 4. Toxoplasma gondii

**Field C**
- 1. Coxsackie virus type B1
- 2. Coxsackie virus type A16
- 3. Echo virus type 7
- 4. Mycoplasma pneumoniae

**Field D**
- 5. Influenza virus type B
- 6. Parainfluenza virus type 1
- 7. Parainfluenza virus type 2
- 8. Mycoplasma pneumoniae

**FI 2826-1001-1 G**

**INFECTION ARTHRITIS PROFILE 1**

<table>
<thead>
<tr>
<th>Field A</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>1:10</td>
<td>1:10</td>
</tr>
</tbody>
</table>

**Field A**
- V, Verification BIOCHIP***
- 1. VZV
- 2. Influenza virus type A (H1N1)
- 3. Influenza virus type A (H3N2)
- 4. Influenza virus type B

**Field B**
- 5. Yersinia enterocolitica O:3**
- 6. Yersinia enterocolitica O:6**
- 7. Yersinia enterocolitica O:9**
- 8. Toxoplasma gondii**

**Field C**
- 9. Borrelia afzelii
- 10. Borrelia burgdorferi sensu stricto (CH)
- 11. Borrelia garinii
- 12. Chlamydia trachomatis**

**FI 2827-1001-1 G**

**GASTROINTESTINAL TRACT PROFILE 1**

<table>
<thead>
<tr>
<th>Field A</th>
<th>IgG</th>
<th>IgM</th>
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</thead>
<tbody>
<tr>
<td>V</td>
<td>1:10</td>
<td>1:10</td>
</tr>
</tbody>
</table>

**Field A**
- V, Verification BIOCHIP***
- 1. Adenovirus type 3
- 2. Yersinia enterocolitica O:3**
- 3. Yersinia enterocolitica O:6**
- 4. Yersinia enterocolitica O:9**

**Field B**
- 1. Adenovirus type 3
- 2. Yersinia enterocolitica O:3**
- 3. EBV-CA**
- 4. Yersinia enterocolitica O:6**
- 5. Coxsackie virus type B3
- 6. Coxsackie virus type A7
- 7. Echo virus type 7
- 8. Campylobacter jejuni**

**Field C**
- 9. Campylobacter coli**
- 10. Helicobacter pylori**
- 11. Klebsiella pneumoniae*
- 12. Candida albicans**

**FI 2828-1001-1 G**

**ACCOMPANYING HEPATITIS PROFILE 1**

<table>
<thead>
<tr>
<th>Field A</th>
<th>IgG</th>
<th>IgM</th>
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</thead>
<tbody>
<tr>
<td>V</td>
<td>1:10</td>
<td>1:10</td>
</tr>
</tbody>
</table>

**Field A**
- V, Verification BIOCHIP***
- 1. Adenovirus type 3
- 2. EBV-CA**
- 3. EBV-EA
- 4. Toxoplasma gondii**

**Field B**
- 5. HSV-1
- 6. HSV-2
- 7. CMV**
- 8. Coxsackie virus type B5

**Field C**
- 9. Coxsackie virus type A9
- 10. Echo virus Typ 7
- 11. Echinococcus granulosus**

**FI 2829-1001-1 G**

**OPHTHALMOLOGY PROFILE 1**

<table>
<thead>
<tr>
<th>Field A</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>1:10</td>
<td>1:10</td>
</tr>
</tbody>
</table>

**Field A**
- V, Verification BIOCHIP***
- 1. Measles virus
- 2. VZV
- 3. Adenovirus type 3
- 4. Toxoplasma gondii**

**Field B**
- 5. HSV-1
- 6. HSV-2
- 7. Coxsackie virus type A24
- 8. Chlamydia trachomatis**

**FI 2831-1001-1 G**

**PREGNANCY PROFILE 1**

<table>
<thead>
<tr>
<th>Field A</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>1:10</td>
<td>1:10</td>
</tr>
</tbody>
</table>

**Field A**
- V, Verification BIOCHIP***
- 1. Rubella virus*
- 2. Toxoplasma gondii**
- 3. HIV-1*
- 4. HIV-2*

**Field B**
- 5. Chlamydia trachomatis**
- 6. CMV**
- 7. HSV-2
- 8. VZV**

* For clinical evaluation the results must be confirmed with a CE approved test.
** For practical reasons the recommended incubation differs from the standard incubation for this substrate.
*** Field A contains a verification BIOCHIP. The incubation was performed correctly if the dots show a visible colour reaction.
**** BIOCHIP Mosaics™ containing fewer substrates can be produced upon request.
Additional Reagents for the Determination of Acute Infections

IgG Absorption
- Before a patient’s serum is tested for specific antibodies of the IgM class, antibodies of class IgG must be removed by ultracentrifugation, chromatography or immunoabsorption.
- Specifically bound IgG would displace IgM from the antigen leading to false IgM negative test results.
- Moreover, the absorption prevents any IgM class rheumatoid factors present from reacting with specifically bound IgG and thus leading to false IgM positive test results.
- Indication: an IgG absorption of serum samples should always be performed for all IgM antibody determinations in infectious serology before incubating the sera.

EUROSORB IgG/RF Absorbent for Indirect Immunofluorescence
- Functional principle: the EUROSORB reagent contains an anti-human IgG antibody preparation from goat. Immunoglobulin G of a serum or plasma sample is bound with high specificity by these antibodies and precipitated. If the sample also contains rheumatoid factors, these will be absorbed by the anti-human IgG/IgG complex.
- Incubation time of the sample with the reagent is 15 minutes. A centrifugation step for a subsequent investigation by immunofluorescence is recommended.
- All IgG subclasses are bound and precipitated by the anti-human IgG antibodies.
- Human serum IgG in concentrations of up to 15 mg/ml and rheumatoid factors are completely removed by the absorbent (average serum IgG concentration in adults: 12 mg/ml).
- The recovery rate of the IgM fraction is almost 100%.
- One unit contains 4.5 ml absorbent, sufficient for the absorption of 100 serum

Urea Solutions and Avidity Buffers for the Determination of Low-Avidity Antibodies in Infectious Serology
- To identify low-avidity antibodies in a patient’s serum, two immunofluorescence tests are performed in parallel: one test is carried out in the conventional way, the other one includes urea treatment between incubations with patient’s serum and peroxidase-labelled anti-human IgG, resulting in the detachment of low-avidity antibodies from the antigens.
- Low-avidity antibodies are present if the fluorescence intensity is significantly reduced (two intensity levels or more) by urea treatment.
- The following reagents for avidity determination are available:

<table>
<thead>
<tr>
<th>IFT, Ab against</th>
<th>Order No.</th>
<th>Avidity Solution</th>
<th>Incubation Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubella</td>
<td>ZF 1130-0501</td>
<td>urea solution, 5 M</td>
<td>10 min</td>
</tr>
<tr>
<td>WNV</td>
<td>ZF 1130-0601</td>
<td>urea solution, 6 M</td>
<td>10 min</td>
</tr>
<tr>
<td>Toxoplasma gondii</td>
<td>ZF 1130-0801</td>
<td>urea solution, 8 M</td>
<td>10 min</td>
</tr>
<tr>
<td>EBV-EA, EBV-CA</td>
<td>ZF 1130-0801</td>
<td>urea solution, 8 M</td>
<td>30 min</td>
</tr>
<tr>
<td>CMV</td>
<td>ZF 1131-0101-1</td>
<td>avidity buffer 1</td>
<td>10 min</td>
</tr>
<tr>
<td>VZV</td>
<td>ZF 1131-0101-2</td>
<td>avidity buffer 2</td>
<td>30 min</td>
</tr>
</tbody>
</table>

Reagents for determination of low-avidity antibodies in infectious serology.
EUROIMMUN PRODUCTS FOR ALLERGOLOGY
Antibodies of Class IgE against Allergens

EUROLINE: Specific IgE
- Determination of specific IgE in serum.
- Indication: Clarification of allergic reactions to inhalation allergens, food allergens and cross-reactive allergens (pollen-associated food allergies).
- Serum dilution: undiluted (or 1 : 10); conjugate class anti-IgE (monoclonal), AP-labelled.
- Antibodies against up to 36 allergens per strip can be simultaneously monospecifically detected.
- EUROLINE profiles with different allergen compositions are available for various test requirements: atopy, inhalation, food and cross reactions.
- The programme EUROLineScan from EUROIMMUN has been developed to enable semi-quantitative evaluation of membrane-based test systems, facilitate management of data, and provide detailed documentation of results.
- The incubated EUROLINE test strips are scanned using a flatbed scanner. EUROLineScan recognises the position of the strips, identifies the bands, and measures their intensity.

EUROIMMUN Microplate ELISA: Total IgE
- Determination of the total IgE concentration in serum.
- Indications: Differentiation between allergic and intrinsic asthma, between allergic and vasomotor rhinitis, and between atopic and seborrhoic dermatitis.
- Serum dilution 1 : 10; conjugate class anti-IgE (monoclonal), POD-labelled.
- One microplate well incubated per patient.
- 4-point calibration, quantitative.
- Coating: anti-human IgE, polyclonal.
- The Total IgE ELISA serves as a screening test for allergy diagnostics and provides an indication for the presence of an allergic reaction.

Allercoat™ 6 Microplate ELISA: Specific IgE
- Determination of allergen-specific IgE concentrations in serum.
- Indication: identification of allergic reactions to more than 600 different allergens and allergen mixtures.
- Serum dilution: undiluted, conjugate class anti-human IgE, AP-labelled.
- One microplate well per allergen/allergen mixture is incubated for each patient.
- Calibration: 6-point calibration, quantitative; using reference preparation 2 IRP 75/502 from the WHO.
- The allergens are coupled to paper rings and can be flexibly configured for each sample according to the analysis.
- Customized pre-assembled microplates with individual allergy parameters are available on request (Order no.: EP 9901-0101). Orders can be made online with the provided software. Please contact us!
- A light table and special evaluation software are available to supplement the simple manual Allercoat™ 6 ELISA procedure.
- Allercoat™ 6 ELISA are automatable using the EUROIMMUN Analyzer I and the EUROIMMUN Allercoat software.
Antibodies of Class IgE against Allergens

Customized Laboratory Software for Flexible Automation of Allercoat™ System

- Convenient online ordering of individual pre-prepared Allercoat™ microtiter plates.
- Direct control of photometer, automated evaluation via calibration curve and compilation of results.
- Fully automated incubation of samples and evaluation of results via connection to the EUROIMMUN Analyzer I.
- Fully automated administration, documentation and archiving of all data.
- Simple operation using graphic user commands and clear presentation of the most important information at a glance.
- Connection to commonly used laboratory systems for convenient transfer of requests and results.
- Additional security through user administration with individual access rights and confirmation step before results editing.

<table>
<thead>
<tr>
<th>Individual equipment with allergen rings</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Pipette: 50 µl per well</td>
<td>undiluted samples</td>
</tr>
<tr>
<td>Incubate: 60 min, 37 °C</td>
<td></td>
</tr>
<tr>
<td>Wash: 300 µl wash buffer per well</td>
<td>enzyme conjugate</td>
</tr>
<tr>
<td>Pipette: 50 µl per well</td>
<td></td>
</tr>
<tr>
<td>Incubate: 60 min, 37 °C</td>
<td></td>
</tr>
<tr>
<td>Wash: 300 µl wash buffer per well</td>
<td>chromogen-/substrate sol.</td>
</tr>
<tr>
<td>Pipette: 100 µl per well</td>
<td>stop solution</td>
</tr>
<tr>
<td>Incubate: 30 min, 37 °C</td>
<td></td>
</tr>
<tr>
<td>Pipette: 100 µl per well</td>
<td>photometric measurement</td>
</tr>
<tr>
<td>Evaluate: photometric measurement at a wavelength of 405 nm</td>
<td></td>
</tr>
</tbody>
</table>
General Delivery Conditions

Products from EUROIMMUN AG will be delivered according to the following conditions, as long as no other conditions have been agreed in writing. On issuing an order, the buyer acknowledges the delivery conditions. EUROIMMUN does not accept the sales conditions of the buyer, even when EUROIMMUN has not expressly contradicted them. Contract terms are valid for all future business connection even if they have not been expressly agreed upon again.

Orders: Orders to EUROIMMUN can be made in writing (including electronic communication) or verbally. Verbally issued orders first become legally binding for EUROIMMUN with a written confirmation from the orderer or with the delivery of goods.

Delivery: EUROIMMUN generally delivers to end users within 7 days of order receipt and to distributors within 14 days, but is not tied to any definite delivery period. If a delivery is not possible through unforeseeable circumstances (e.g., factory disruptions, raw material delivery delays, transport difficulties, strikes), the delivery obligation does not apply. EUROIMMUN reserves the right to deliver in part. The delivery obligation of EUROIMMUN AG ceases as long as the customer is in arrears. EUROIMMUN chooses the packing and dispatching method at its own discretion, according to the respective requirements.

Product characteristics: The delivered products comply with specifications given in the product catalogue, on the product itself, or in the supplied information sheets. If specifications are inconsistent, the labels on the product itself and the details in the information sheet provided with the product apply. After the given expiration date has passed, the test reagents must not be used.

EUROIMMUN products must only be used for the intended purpose. It is the buyer’s responsibility to check the functional capability immediately on receipt of the product as well as on every day of usage. In particular, the functioning of test reagents can be perturbed through causes outside the direct influence of the manufacturer, for example, through suboptimal transportation, incorrect storage, or incorrect usage. When test reagents delivered from EUROIMMUN are used, the user must supervise the analysis with appropriate proficiency.

Warranty and liability: If nothing else has been agreed upon in writing, all risks pass to the buyer as soon as the goods have been delivered to the carrier or has left EUROIMMUN AG premises for dispatch. With notification of dispatch by EUROIMMUN the risk passes to the buyer if the delivery is postponed or delayed by request of the buyer. On receipt of the goods, the buyer has one working day to check if they are in accordance with the nature and quantity of the agreement and if the goods are free from visible defects. If any complaints arise from this inspection, they should be communicated to EUROIMMUN AG in writing within 2 working days. Hidden defects or functional faults that were not identifiable with the initial inspection and are later discovered should be communicated to EUROIMMUN AG in writing within 14 days of receipt of the goods. If no complaints are received within the stipulated period, it is assumed that the goods are of appropriate quality and quantity for the customer. Complaints do not release the buyer from payment obligation.

With prompt and reasonable complaints on the grounds of product defects or the delivery of something other than the ordered goods, EUROIMMUN is obliged to exchange or amend the goods within 14 days or to withdraw or reimburse the payment, as it chooses. If the defect is not corrected in spite of delivery of a replacement or an amended product, the buyer can demand that the sale is cancelled.

If defects are promptly reclaimed, EUROIMMUN has the choice between a further delivery or an appropriate credit note. Further compensatory claims from the buyer are excluded, as long as EUROIMMUN has not violated its contractual or legal obligations through outright negligence or by intention. In such cases EUROIMMUN provides compensation of up to a maximum of 20 times the price of one packet (test kit) of the defective product. EUROIMMUN takes no responsibility for any damage resulting from faulty goods.

Prices: Prices from the official EUROIMMUN pricelist in the country of the buyer are applicable. Invoices are provided in the agreed currency. Prices are “ex works”. Expenses for packing and freight as well as for cooling during transport are added on, including costs for the disposal of packaging material by the customer. Statutory value added tax is not included in the list prices.

Payment terms: Payment obligations in countries of export must be settled within 30 days after receiving the goods. No cash discounts will be given unless agreed in writing. Payments by cash transfer or check are valid from the timepoint that the invoice amount is credited to a EUROIMMUN AG bank account. In cases of payment arrears, EUROIMMUN reserves the right to charge compensatory interest of 10% p.a., applicable from the settlement date, without any further notice. In special cases EUROIMMUN can arrange alternative payment periods or demand advance payments. EUROIMMUN’s demands resulting from an order cannot be offset by the customer through counter demands.

Ownership rights: The delivered goods remain the property of EUROIMMUN AG until full payment. Selling on of EUROIMMUN products is only permitted for companies who are authorized in writing from EUROIMMUN to do so. Software received from EUROIMMUN should not be passed on to third parties without written permission from EUROIMMUN AG.

Consultation: Advice from EUROIMMUN AG, although provided to the best knowledge, is nevertheless not binding. No liability claims can ensue from erroneous advice.

Applicable law, place of jurisdiction, ineffective regulations: The contract conditions are subject to the laws of the Federal Republic of Germany. The United Nations Conventions on Contracts for the International Sale of Goods does not apply. The place of jurisdiction and fulfilment is Luebeck. If any provision of these general delivery conditions will be held invalid or unenforceable, this general delivery condition will not be rendered invalid as a whole, and the provisions will be changed and interpreted so as best to accomplish the objective.
EUROIMMUN Medizinische Labordiagnostika AG

EUROLabOffice