We Apply Science

D-tek s.a. is a middle-sized private Belgian biotechnology company created in 1995 in Mons 40 km from Brussels and less than 1 h 20 from Paris. The company is highly specialized in the development and manufacturing of diagnostic kits for autoimmune diseases.

Thanks to its long experience in autoimmunity, its strong skills in Research and Development and its understanding of the customer's needs, *D-tek* has developed an innovative range of diagnostic products. The know-how of *D-tek* has permitted the launching of more than 50 different kits on the market, many were a world première like the first kit for the detection of anti-Nucleosome antibody and some of them still stay unique such as the APSGD-24, the only Immunodot kit which allows the simultaneous detection of the main autoimmune markers for the Antiphospholipid Syndrome.

At the same time, all the diagnostic kits produced by *D-tek* have been CE marked and the company has been certified ISO 9001 since 2003. The same engagement for quality has motivated the company *D-tek* to participate in some external quality assessment programs such as the UKNEQAS.

Besides diagnostic kits, R&D experience accumulated over the years has also conducted *D-tek* to propose some of its intermediate manufacturing products such as liquid substrates and stabilizers.

The impressive range of products, their quality and the commitment to the customer's satisfaction explain why *D-tek*'s products are now sold worldwide through a network of experienced distributors.

Autoimmune diseases

Autoimmune diseases are due to inappropriate immune response against the organism's own antigens (autoantigens), leading to chronic inflammation, tissue destruction and/or dysfunction. Generally unknown by the public and perceived as uncommon, they represent nevertheless the third cause of morbidity in industrialized countries.

Doctors generally distinguish systemic and organ-specific autoimmune diseases, depending if they affect multiple organs or only one organ specifically.

The etiology of the autoimmune diseases is clearly multifactorial. Both intrinsic factors (e.g. genetics, hormones, age) and environmental factors (e.g. infections, diet, drugs, environmental chemicals) may contribute to the induction, development and progression of autoimmune diseases.

According to the estimation of the World Health Organization, they affect up to 5% of the global population. Scientists and doctors agree that more than 60 diseases have a proven or strongly suspected autoimmune cause. Moreover, there are epidemiological evidences of increasing prevalence of certain autoimmune diseases, which cannot be attributed to focus on or improvement of the diagnosis alone.

As the autoimmune diseases are generally incurable and require a lifelong treatment they have a strong impact on public health cost. Their accurate diagnosis has therefore become of major importance these last decades. All the diagnosis techniques available on the market are based on the ability to detect the presence of autoantibodies in the patient's serum.

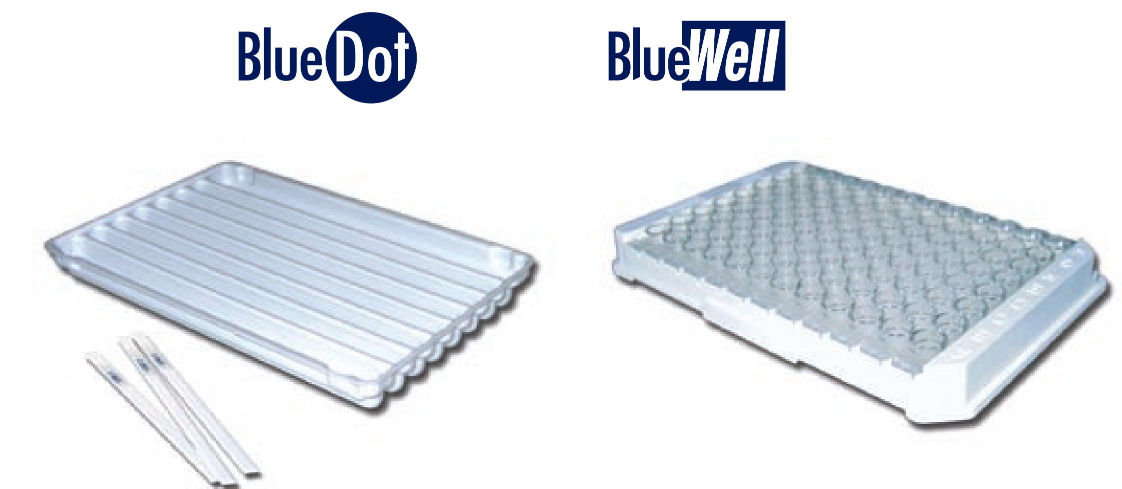
Trends in autoimmunity

Many autoantibodies are discovered and described every year. Only a few of them appear to be diagnostically relevant and have to be included in the general testing.

This increase of parameters comes concurrently with the need to obtain the most complete autoantibodies profile: Scientific evolution of knowledge shows in fact that the simultaneous presence of different autoantibodies – more than the presence of one specific antibody alone – informs the clinician about the prognosis, the follow-up and the treatment of the autoimmune diseases.

DIAGNOSTIC KITS

The core business of the company *D-tek* is the development and the production of diagnostic tests in autoimmunity. The constant increase of new diagnostic parameters for autoimmune diseases and the evolution of the kit users' demands have led *D-tek* to develop a complete range of two complementary product brands: *BlueWell* and *BlueDot*, based on ELISA and Immunodot techniques respectively.



BlueWell and BlueDot share the following advantages

→ Ease of use:

- *BlueWell* and *BlueDot* products are based on a classical and well-known EIA (Enzyme Immuno Assay) procedure. No specific training or teaching is needed for professional users.
- In both product lines, the reagents for the test are ready-to-use (except the Wash buffer). This allows to save on time and reduces the risk of inappropriate manipulations. Moreover, all these reagents are color-coded for easy recognition.

→ Efficiency:

- Only 10 µl of serum or plasma is needed to perform an analysis either on *BlueDot* or *BlueWell*.
- The results are obtained in only 1h30.

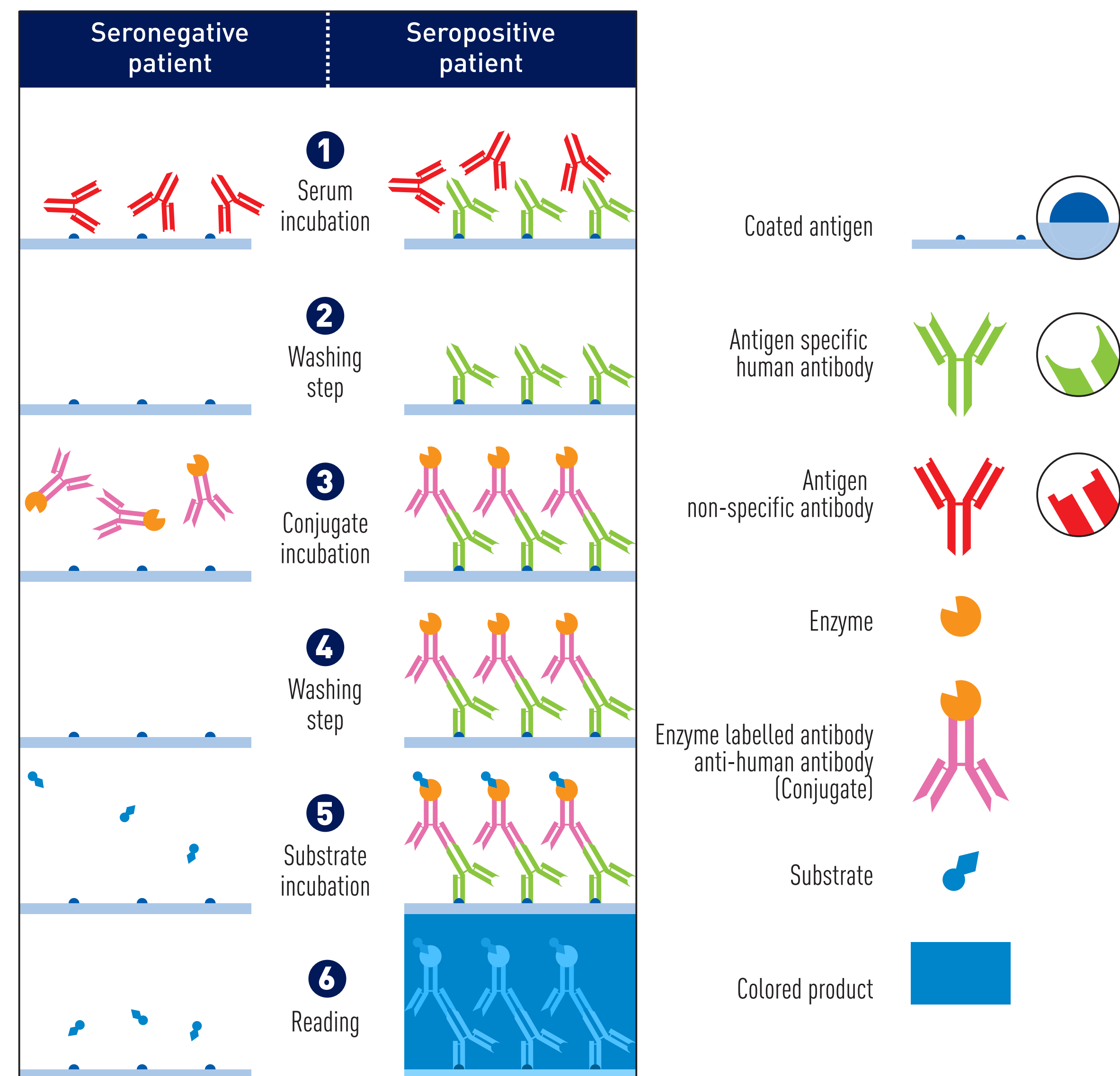
→ Cost effectiveness:

- Both lines, *BlueWell* and *BlueDot*, can be fully automated from the test itself to the final interpretation step.
- Thanks to their formats and their standardized protocol, *BlueWell* and *BlueDot* kits allow the laboratory technicians to customize their specific analysis.

Principle of the EIA test

BlueWell and *BlueDot* are based on the principle of an Enzyme ImmunoAssay (EIA).

1. The reaction support (ELISA plate for *BlueWell* or nitrocellulose strip for *BlueDot*) is incubated with diluted patient serum. Specific human antibodies, if present, bind to the corresponding antigen(s) coated on the support.
2. Unbound or excess antibodies are removed thanks to the first washing step.
3. The conjugate (which is an enzyme labelled antibody anti-human antibody) is added to the support and binds to the antigen-antibody complexes if present.
4. The second washing step removes excess conjugate.
5. The substrate solution is added and reacts to antigen-antibody-conjugate complexes if present.
6. The enzyme activity leads to the development of a coloration which is directly proportional to the amount of antibodies present in the patient serum.



Coated antigen

Antigen specific human antibody

Antigen non-specific antibody

Enzyme

Enzyme labelled antibody anti-human antibody (Conjugate)

Substrate

Colored product

BlueWell CHARACTERISTICS

BlueWell

Key characteristics

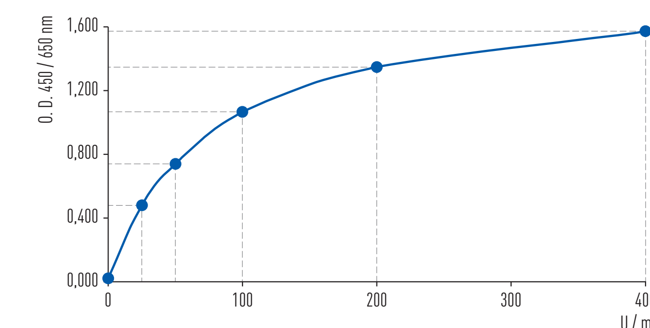
- Well adapted for important laboratories or hospitals screening important patient populations
- Standardized incubation times and test procedures
- Customized testings
- Easily adaptable for automation

BlueWell kits are indirect ELISA (Enzyme-Linked Immuno Sorbent Assay) tests. The support is polystyrene plate of 96 microwells which allows in theory the testing of 96 patients in one run. Each plate is coated with one antigen and allows the screening of one unique autoantibody.

Thanks to the *BlueWell*'s plate structure (easily break apart wells) the user can freely adapt the number of wells to the number of patients he has to test.

The incubation times and test procedures are standardized. Different tests can be combined and performed simultaneously on the same plate which allows to save on time and money.

The *BlueWell* kits can be performed manually but they are also easily adaptable to most automated open ELISA instruments.



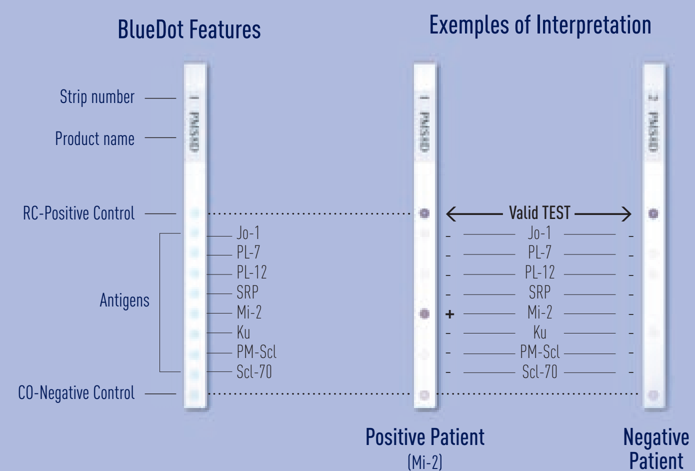
Example of 6 points calibration curve obtains with a *BlueWell* kit.

The colorimetric results are either qualitative (results are expressed in Binding Index, the ratio between the sample and the cut off's Optical Density) or quantitative (the 6 points calibration curve provides the user with the statically most accurate results).

BlueDot CHARACTERISTICS

Key characteristics

- If performed manually, *BlueDot* tests are adapted for small laboratories or hospitals. However, with automated systems, *BlueDot* tests can be used for high throughputs.
- Standardized incubation times and test procedures.
- Customized testings, several antigen combinations can be processed in the same run.
- No need for external controls, the one patient/one strip concept allows small series, even 1 single patient, to be run without increase in material costs.
- Wide range of antigen combinations.



BlueDot kits are composed of 24 nitrocellulose strips on which the antigens have been coated in a dot shape format. One *BlueDot* strip allows the testing of one patient for many different autoantibodies in the same run.

In addition to the antigens, each strip possesses two built-in reactive controls.

The first one, the **R**eaction **C**ontrol (**RC**), controls the validity of the test: its coloration proves the correct functioning of the test.

The second one, the **C**ut-**O**ff Control (**CO**), allows the interpretation of the test: any color intensity equal to or below that of the cut-off is considered negative, any color intensity superior to that of the cut-off is considered positive.

The interpretation can be done by sight or by the use of the *Dr DOT* software which allows a semi-quantitative interpretation. Moreover, the *Dr DOT* software allows to print, store the results, send them by e-mail or even export them in CSV (Comma-Separated Values) format.

There are different automated instruments available on the market to perform the *BlueDot* test. Do not hesitate to contact *D-tek* to obtain further information: info@d-tek.be.

The *BlueDot* results can be easily interpreted by sight but for more convenience, *D-tek* has developed the *Dr DOT* software. This multilingual software allows a quick, easy and objective semi-quantification of the *BlueDot* test results. It only requests a computer and a flat bed scanner.

The *Dr DOT* software contains a database that keeps record of all saved results (numeric values and scanned images). One specific result can be found thanks to a multi-criteria research engine.

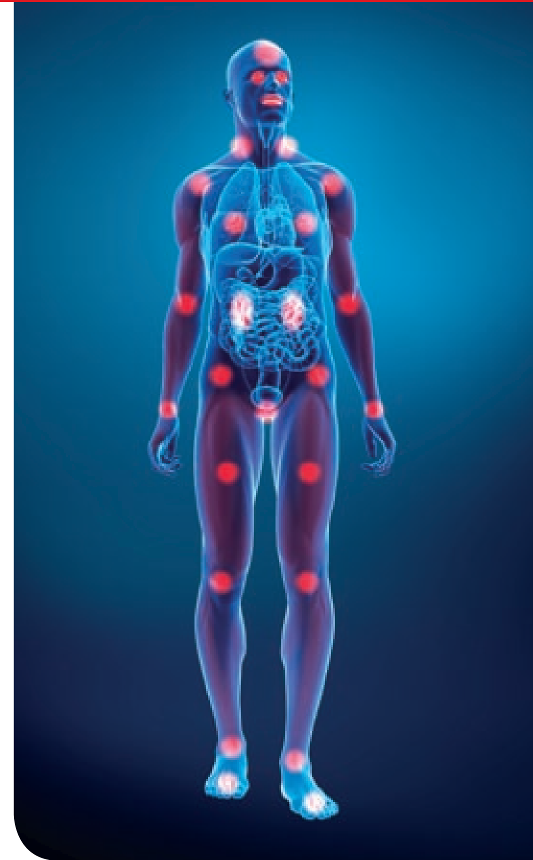
All results can easily be sent by e-mail (Outlook or Outlook Express), printed or even exported in CSV format for further analysis and use.

Once installed on a computer, the software works free of charge for a 30 days evaluation period. After that, the user has to register for a license which is granted for life and includes free of charge updates.

D-tek has developed its product lines on the basis of 3 different approaches so that the user can select, through an extensive range of parameters and combinations, the most appropriate products for specific purposes.

- **Disease-orientated approach:** *D-tek* focuses on the different autoimmune diseases. The products allow the detection of one or several antibodies typically found in a given or a group of autoimmune diseases.
- **Technique-orientated approach:** *D-tek* focuses on practical difficulties faced by clinicians. The products allow the differential identification of antibodies whose distinction may be difficult, confusing or ambiguous by other techniques.
- **Research-orientated approach:** *D-tek* benefits from its accumulated knowledge in order to propose innovative products. The products allow the detection of particular antibodies or antibody subgroups whose identification may represent valuable information for clinical research.

Connective Tissue Diseases



Connective Tissue Diseases are a group of systemic autoimmune diseases. Connectivitis include **S**ystemic **L**upus **E**rythematosus (**SLE**), Sharp Syndrome, Scleroderma, Polymyositis, Dermatomyositis, Sjögren's Syndrome, Antiphospholipid Syndrome.

Systemic Lupus Erythematosus (SLE)

The prevalence of SLE in the general population is estimated to 40-50 cases per 100.000 persons. Its clinical manifestations are so diverse and variable that the **A**merican **C**ollege of **R**heumatology (**ACR**) has established a list of 11 criteria. SLE is defined if at least 4 of them are present.

- Malar rash
- Discoid rash
- Photosensitivity
- Oral or nasopharyngeal ulceration
- Nonerosive Arthritis, involving two or more joints
- Serositis: Pleuritis or Pericarditis
- Nephritis: persistent Proteinuria or cellular casts
- Neurologic disorder: seizure or psychosis
- Hematologic disorder: Hemolytic Anemia or Leukopenia
- Immunologic findings: presence of dsDNA, Sm or Phospholipid antibodies
- Abnormal titer of Antinuclear antibody

Among these 11 criteria, the last two are directly linked to the abnormal presence of autoantibodies and notably **A**nti**N**uclear **A**utoantibodies (**ANA**). They are directed against nuclear antigens that are generally classified into subgroups based on their differential solubility in saline buffers:

Extractable **N**uclear **A**ntigens (**ENA**) which are soluble in physiological buffers and are considered as major autoimmune markers. The most common anti-ENA antibodies include anti-Sm, RNP, SSA (Ro), SSB (La), Jo-1, Scl-70 and PM-Scl.

Non-extractable nuclear antigens are defined as being non-soluble in physiological conditions. As shown by Immunofluorescence patterns, most of them are closely linked to the chromatin structure and their isolation generally requires laborious purification techniques. Both Immunodiffusion and CIE are unsuitable methods for detecting the corresponding antibodies and only the molecular cloning allowed the commercialization of specific immunoassays based on recombinant antigens (e.g. the centromere antigen CENP-B).

dsDNA antibodies are considered to be diagnostic markers (ACR criterion) for SLE. They are among the most frequently detected autoantibodies associated with SLE. Moreover the dsDNA antibody titer usually correlates with disease activity and dsDNA antibodies are therefore classified as markers of SLE activity.

Histone proteins (H1, H2A, H2B, H3 and H4) are in close contact with dsDNA. Although anti-Histone antibodies (AHA) are generally present in patients with anti-dsDNA antibodies, they are not specific for SLE but can be detected in a number of autoimmune, infectious or drug-induced diseases. Interestingly, high titers of anti-Histone antibodies are found almost exclusively in patients with SLE and Drug-Induced Lupus (DIL). The detection of high AHA titers in the absence of SLE marker antibodies is characteristic of DIL.

Nucleosome antibodies are also important markers for SLE. They recognize restricted nucleosome antigens expressed on the intact Nucleosome. Nucleosome consists of a sequence of DNA wrapped around a Histone octamer. They are responsible of the compactness of DNA in the nucleus. These autoantibodies have an important predictive value as they appear at an earlier stage than anti-dsDNA. They are also a significant marker of Lupus Nephritis, anti-Nucleosome complexes forming deposits on the Glomerular Basement Membrane. As such, anti-Nucleosome antibodies appear to be more specific markers of Lupus Nephritis than anti-dsDNA antibodies, explaining the discrepancies for cases in which anti-dsDNA antibodies do not correlate with a renal involvement.

Sm antibodies target spliceosome components designated **U-snRNP** (**U**ridin-rich **s**mall **n**uclear **R**ibo**N**ucleo **P**roteins) that play an important role in mRNA processing. The main Sm antigen is the so-called D1 protein contained in U_1, U_2, U_4, U_5 and U_6-snRNP. Sm antibodies are diagnostic markers (ACR criterion) for SLE, with very high specificity (99 %) but low sensitivity for the disease.

Ribosome **P** **P**rotein (**RPP**) antibodies target the phosphoproteins P0 (38 kD), P1 (19 kD) and P2 (17 kD) of the 60S subunit of the ribosomal complex. RPP antibodies are detected in 10-35 % of SLE patients and have high specificity (99 %) and positive predictive value. Their detection in patients suffering from other autoimmune diseases may be a sign of SLE overlap. Moreover RPP antibodies were shown to be associated with disease activity and particular organ involvements (Nephritis, Liver manifestations).

Proliferating **C**ell **N**uclear **A**ntigen (**PCNA**) is a homopolymer complex (trimer) of 34 kD. This auxiliary protein of DNA polymerase δ plays a key role in the regulation of DNA replication and repair. PCNA is also important in cell cycle regulation. The presence of PCNA antibodies is highly specific of SLE but rarely found as it represents only 3 % of patients overall. These autoantibodies are generally associated with renal involvement, Central Nervous System (CNS) manifestations and Thrombocytopenia.

Centromere antibodies, also called **Anti-Centromere Antibodies** (**ACA**), are directed against **Cen**tromere-associated **P**roteins (**CENP**), mainly two polypeptides designated CENP-A (19 kD) and CENP-B (80 kD). They are diagnostic and prognosis markers of Scleroderma and appear also in 57-82 % of patients with **CREST** Syndrome which is characterized by **C**alcinosis cutis, **R**aynaud's phenomenon, **E**sophageal dysfunction, **S**clerodactyly and **T**eleangiectasia.

Sharp Syndrome

Also known as **M**ixed **C**onnective **T**issue **D**isease (**MCTD**), Sharp Syndrome is characterized by manifestations that overlap SLE, Scleroderma, Polymyositis and Rheumatoid Arthritis. Symptoms are Raynaud's phenomenon (inadequate blood supply to the fingers), swollen fingers or diffusely swollen hands, dysmotility (abnormal muscle contractions in the gut or gastrointestinal tract), mild Myositis and pulmonary involvement.

RNP antibodies target a complex containing a series of snRNP including three polypeptides (68 kD, A and C) that are linked non-covalently with U1-RNA as part of the spliceosome complex. These autoantibodies have a specificity of 100 % for Sharp Syndrome.

Sm/RNP antibodies are directed against the U_1 snRNP complex which is composed of Sm and the three polypeptides of the RNP antigen. Anti-Sm/RNP antibodies are very sensitive markers for Sharp Syndrome. Their sensitivity is 100 %.

Polymyositis / Dermatomyositis and Scleroderma

Poly**M**yositis and **D**ermato**M**yositis (**PM / DM**) are autoimmune diseases which primarily affect the muscles and/or the skin, although other organs may also be involved (lung and heart). The illnesses severely affect the quality of life and may, if not diagnosed and treated rapidly, progress over time to a life-threatening state. The diagnosis of PM/DM however is difficult since overlap syndromes with features of other diseases such as Scleroderma are frequent.

PM/DM are connective tissue diseases which are of an uncertain etiology. Probable causes include a genetic predisposition and exposure to external factors like drugs or strong radiations. Hormonal status may also be of relevance since women are more affected than men. The age of predilection is from age 5-15 (juvenile form) and between ages 35-55. The course of the PM/DM can vary from mild to severe forms which may lead to various complications with poor prognosis, like the development of an interstitial pulmonary disease.

Diagnosis of PM/DM is based on a combination of medical history, physical examination, electromyography, biopsy and serological findings. A striking feature of PM/DM, including overlap syndromes, is the occurrence of specific antibodies to different antigens.

The most important autoantibodies for the serological diagnosis of PM/DM are anti-aminoacyl-tRNA synthetases which are highly specific for Idiopathic Myositis and Myositis in overlap syndromes.

Jo-1 antibodies (Histidyl-tRNA-synthetase) are found in about 60 % of patients with a combination of Myositis and Fibrosing Alveolitis. Furthermore Jo-1 is considered as a useful prognostic marker for more severe clinical course, frequent active episodes and a poor prognosis.

PL-7 and **PL-12** antibodies (respectively Threonyl-tRNA synthetase and Alanyl-tRNA synthetase), although less frequently detected in Idiopathic Myositis (around 2-3 %), are important markers for the differential diagnosis and therapy of Myositis of unclear origin. Indeed PL-7 or PL-12 associated Myositis appears to be more difficult to treat than Myositis associated with other antibodies (e.g. PM-Scl).

SRP antibodies are highly specific for Polymyositis. About 5 % of Myositis patients are positive for anti-SRP antibodies, rising to 18 % in the subgroup of Jo-1 negative patients. In contrast to Myositis patients with aminoacyl-tRNA synthetase antibodies, SRP positive patients do not show involvement of the joints, lungs or skin. The classic « anti-SRP syndrome » is a severe form of Polymyositis with acute Myositic inflammation and frequent cardiac involvement. Patients generally respond poorly to immunosuppressive therapy. They have the poorest prognosis of all patients with Myositis.

Mi-2 antibodies are detected almost exclusively in patients with Dermatomyositis. Compared to Myositis patients who test positive for anti-aminoacyl-tRNA synthetase antibodies, those positive for Mi-2 generally have a relatively mild clinical course, respond well to glucocorticosteroids and therefore tend to have a good prognosis.

PM-Scl antibodies are found almost exclusively in patients with Polymyositis/Scleroderma overlap syndrome (20-25 %), PM/DM (8-12 %) and Scleroderma (1-16 %). Interestingly PM-Scl is a reliable marker for Juvenile Scleromyositis (overlap syndrome, mild Scleroderma and Myositis in children) which appears to be the most common Scleroderma-like disease of childhood. The clinical course is relatively benign compared to that of Juvenile Dermatomyositis or Scleroderma.

Ku antibodies are detectable in 5-25 % of patients with Polymyositis/Scleroderma overlap syndrome. However their specificity is low and the determination of Ku antibodies in case of suspicion of Polymyositis/Scleroderma overlap is diagnostically relevant only after the possibility of another connective tissue disease (e.g. SLE) has been ruled out.

Scl-70 antibodies are highly specific for Systemic Sclerosis (Scleroderma). They are essentially prevalent in the diffuse forms and are associated with a severe systemic course and a poor prognosis.

Sjögren's Syndrome

Sjögren's Syndrome is an autoimmune exocrinopathy characterized by both organ-specific autoimmunity (preferentially affecting the salivary and/or lacrimal glands) and systemic manifestations. Concretely, the diminution of gland secretions causes notably Xerostomia (dry mouth) and Xerophthalmia (dry eyes). The prevalence of this autoimmune disease is estimated to be 15 per 100.000 and presents a female preponderance.

Primary Sjögren's Syndrome is diagnosed if no other autoimmune disease is present. The Secondary Sjögren's Syndrome is associated with another connective tissue disorder.

The anti-**SSA** antibodies are directed against SSA (Ro), a protein of 60 kD which belongs to small cytoplasmic ribonucleoprotein complexes, the hY-RNP complexes also known as Ro/SSA hY-RNA. These autoantibodies are diagnostic markers and classification criteria for Sjögren's Syndrome. They are also associated with SLE and **S**ubacute **C**utaneous **L**upus **E**rythematosus (**SCLE**) and present in 90 % of patients with **N**eonatal **L**upus **E**rythematosus (**NLE**).

Trim21, also called SSA 52, is an E3 ubiquitin ligase of 52 kD. Many scientific reports and articles have mentioned the possible implication of anti-Trim21 antibodies in different pathologies such as Myositis, congenital heart block and Cutaneous Lupus.

SSB antibodies target a multifunctional phosphoprotein SSB (La) which is involved in the termination of RNA polymerase III transcription. It forms a macromolecular complex with Ro/SSA antigens and with small uridine-rich RNA molecules. Anti-SSB antibodies are a diagnostic marker of Sjögren's Syndrome. They are also associated to SLE (19-30 %).

AntiPhospholipid Syndrome

Anti**P**hospholipid **S**yndrome (**APS**) is a systemic autoimmune disease characterized by vascular thrombosis (venous, arterial, or small vessel) and/or obstetrical complications (fetal loss, premature birth, or recurrent embryonic losses) occurring in patients with persistent autoantibodies directed against phospholipid-binding plasma proteins.

Up to 15 % of SLE patients will have an APS and 50 % of the patients that suffer from an APS, have a SLE. In this situation, the APS is called Secondary APS, otherwise it is called Primary APS.

APS may conduct to venous and/or arterial thrombosis. In the first case, the most common manifestation is deep venous thrombosis of the legs, associated or not with pulmonary embolism. Arterial thrombosis may lead to strokes, myocardial infarction or **C**erebro**V**ascular **A**ccident (**CVA**).

In general, treatment of APS must be individualized according to the patient's clinical status and history of thrombotic events. Therapeutic agents are based on anticoagulant properties and benefits are weighed carefully against their significant risk. An international consensus statement on the APS classification was published in 1999 after a workshop in Sapporo (Japan). These criteria have been updated in 2006 and are composed of clinical and laboratory criteria. The laboratory criteria highlight the importance of two autoantibodies: anti-**Cardiolipin** and anti-**ß2-GPI**.

Cardiolipin antibodies (aCL), cofactor independent, are detectable in most patients with typical symptoms of APS but are not specifically associated with the clinical picture of the syndrome as they are also present in various infectious diseases (e.g. Syphilis). In the course of numerous clinical trials, investigators found that the detection of anti-CL antibodies was enhanced by ß2-Glycoprotein I (ß2-GPI), a cofactor that has binding affinities for phospholipids. The complex CL / ß2-GPI induces a conformational change in the ß2-GPI and the expression of a cryptic epitope responsible for aPL binding. These Anti-CL / ß2-GPI antibodies are associated with the well-known

thrombotic event in APS and are specific for the syndrome.

Anti-ß2-GPI antibodies exclusively directed against ß2-GPI in the absence of CL have also been reported. It is believed that, by binding on the test support (polystyrene plates or membrane), the ß2-GPI undergoes a similar conformational change that leads to the expression of a neo-epitope. However, it has been demonstrated that the antibodies directed against the ß2-GPI / Cardiolipin complex are not identical to ß2-GPI antibodies.

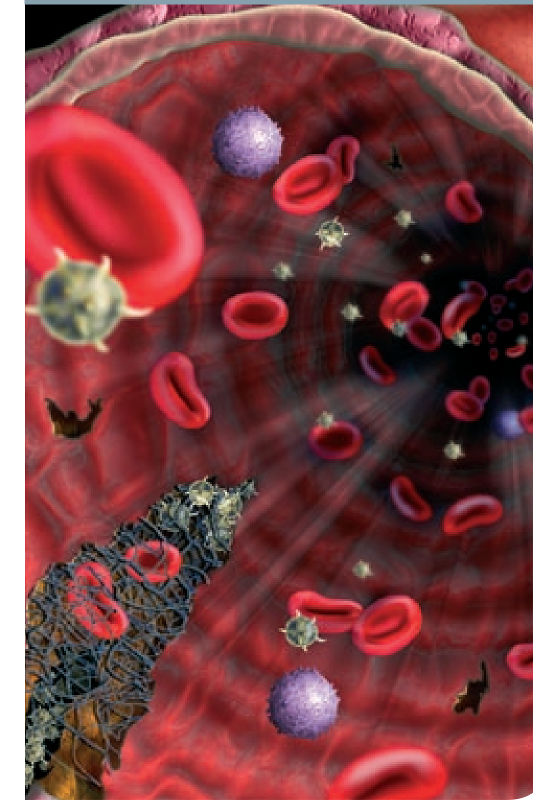
BlueDot / Connective Tissue Diseases

Kits	Class of Antibodies detected	Parameters detected
ANA12D-24	IgG	Sm • RNP (68kD/A/C) • Sm/RNP • SSA (Ro) • SSB (La) • Jo-1 • Scl-70 • PM-Scl • Ku • CENP-A/B • PCNA Ribosome P0
ANA12SD-24	IgG	Sm • RNP (68kD/A/C) • Sm/RNP • SSA (Ro) • SSB (La) • Jo-1 • Scl-70 • PM-Scl • Ku • CENP-A/B • PCNA • Mi-2
ANA10D-24	IgG	Sm • Sm/RNP • SSA (Ro) • SSB (La) • Jo-1 • Scl-70 • PM-Scl • Ku • CENP-A/B • PCNA
ANA8D-24	IgG	Sm • Sm/RNP • SSA (Ro) • SSB (La) • Jo-1 • Scl-70 • PM-Scl • CENP-A/B
APSGD-24	IgG	Cardiolipin/ß2-GPI complex • ß2-GPI
CHRD-24	IgG	Nucleosome • dsDNA • Histones
CT10D-24	IgG	Nucleosome • dsDNA • Histones • Sm • Sm/RNP • SSA (Ro) • TRIM21 • SSB (La) • Jo-1 • Scl-70
CTD-24	IgG	Nucleosome • Histones • Sm • Sm/RNP • SSA (Ro) • SSB (La) • Jo-1 • Scl-70
DNAD-24	IgG	dsDNA
ENAD-24	IgG	Sm • Sm/RNP • SSA (Ro) • SSB (La) • Jo-1 • Scl-70
LU6D-24	IgG	Nucleosome • dsDNA • Histones • Sm • Ribosome P0 • Cardiolipin/ß2-GPI complex
LUD-24	IgG	Nucleosome • Histones • Sm • Ribosome P0
NUD-24	IgG	Nucleosome
NUENAD-24	IgG	Nucleosome • Sm • Sm/RNP • SSA (Ro) • SSB (La) • Jo-1 • Scl-70
NUHISD-24	IgG	Nucleosome • Histones
PMS8D-24	IgG	Jo-1 • PL-7 • PL-12 • SRP • Mi-2 • Ku • PM-Scl • Scl-70

BlueWell / Connective Tissue Diseases

Kits	Class of Antibodies detected	Parameters detected
CLG02-96	IgG	Cardiolipin / ß2-GPI complex
CLM02-96	IgM	Cardiolipin / ß2-GPI complex
CLS01-96	IgA / IgG / IgM	Cardiolipin / ß2-GPI complex
DNA02-96	IgG	dsDNA
GPG02-96	IgG	ß2-GPI
GPM02-96	IgM	ß2-GPI
NU02-96	IgG	Nucleosome

Vasculitis and Goodpasture Syndrome



Vasculitis

The **A**nti-**N**eutrophil **C**ytoplasmic **A**ntibodies (**ANCA**) are a group of autoantibodies directed against cytoplasmic components of the neutrophilic granulocytes. They have been classified into cytoplasmic (c-ANCA) and perinuclear (p-ANCA) according to the indirect immunofluorescence pattern they give on ethanol-fixed human neutrophils and are useful serological markers for vasculitides.

Most c-ANCA and p-ANCA antibodies recognize two major target autoantigens, Proteinase 3 (PR3) and Myeloperoxidase (MPO) respectively.

Whereas anti-PR3 antibodies have a high specificity for **W**egener´s **G**ranulomatosis (**WG**), anti-MPO antibodies have been reported in a variety of vasculitides (e.g. Microscopic Polyangiitis, Churg-Strauss Syndrome, Polyarteritis Nodosa) and Glomerulonephritides (e.g. Rapidly Progressive Glomerulonephritis).

Goodpasture Syndrome

The Goodpasture Syndrome is a medical emergency with a high fatality rate if not treated. The syndrome is essentially characterized by Glomerulonephritis, pulmonary bleeding and antibodies targeted to a component of the Glomerular Basement Membrane (GBM). In fact, the anti-GBM antibodies are targeted to collagen IV, a component of the GBM and specifically to the NC1 domain of the α3 chain (Goodpasture antigen). They bind along the Glomerular Basement Membrane as a characteristic continuous layer which is demonstrable by Immunofluorescence on kidney sections. Anti-GBM antibodies are the primary pathogenic autoantibodies, inducing Glomerulonephritis, in all patients with Goodpasture Syndrome. Their determination allows differentiation of the Syndrome from other causes of Glomerular Nephritis and Pulmonary Bleeding.

BlueDot / Vasculitis and Goodpasture Syndrome

Kits	Class of Antibodies detected	Parameters detected
ANCAD-24	IgG	MPO • PR3
ANCAGD-24	IgG	MPO • PR3 • GBM
GBD-24	IgG	GBM

Autoimmune Liver Diseases

Autoimmune Liver Diseases are defined as immune mediated chronic liver diseases of unknown etiology, including namely **P**rimary **B**iliary **C**irrhosis (**PBC**) and **A**uto**I**mmune **H**epatitis types **1** and **2** (**AIH-1** and **2**). PBC and AIH have long been considered rare diseases. Their incidence however has recently increased, notably because of a greater awareness of the diseases and the development of new diagnostic techniques.

Diagnosis of AIH and PBC is based on a combination of clinical, biochemical, histological and serological findings. Apart from testing for markers of viral hepatitis (to rule out this cause), serology is mainly based upon screening for marker autoantibodies that are crucial for the correct diagnosis and classification of the diseases.

Primary Biliary Cirrhosis (PBC)

The **M2** antibodies belong to the group of **A**nti**M**itochondrial **A**ntibodies (**AMA**) and are strongly associated with PBC. At least nine distinct AMA have been identified, which have been classified M1-M9 according to their antigen specificity and disease association. Of these, only the M2 subtype seems to approach absolute specificity for PBC. Indeed, about 95 % of PBC patients have M2 autoantibodies and, conversely, about 90 % of asymptomatic individuals who are found to be M2-positive on routine screening can be shown to have underlying PBC on further investigation. The target antigens of M2 antibodies have been identified as components of the 2-Oxo-Acid Dehydrogenase Complex, the immunodominant epitopes being located on the E2 subunits of **P**yruvate **D**ehydrogenase **C**omplex (**PDC-E2**), **B**ranched-**C**hain **O**xo-**A**cid **D**ehydrogenase **C**omplex (**BCOADC-E2**) and **O**xo-**G**lutarate **D**ehydrogenase **C**omplex (**OGDC-E2**).

PBC patients usually display a variety of AntiNuclear Antibodies (ANA), some of which overlap with other systemic autoimmune diseases. In immunofluorescence assays, two labelling patterns however are typical for PBC: nuclear rim and multiple nuclear dots. Antibodies that are responsible for these patterns most often recognize the nuclear envelope glycoprotein **gp210** and the nuclear body protein **sp100**, respectively. Although the clinical significance of these antibodies remains to be determined precisely, they are of particular utility in assessing PBC patients without antimitochondrial antibodies or with other atypical presentations. Moreover recent data indicate that, unlike M2 antibodies, PBC-specific ANAs correlate with disease severity and therefore may represent important markers of poor prognosis.

AutoImmune Hepatitis (AIH)

LKM1 (**L**iver **K**idney **M**icrosome) antibodies react with the microsomal cytochrome isoform P450 IID6 and are the most prevalent markers for AIH type 2. They are considered specific for this type, although low titers of antibodies have been reported in about 5 % of patients with Chronic Hepatitis C.

LC1 (**L**iver **C**ytosol) antibodies react with a soluble cytosol enzyme (Formiminotransferase Cyclodeaminase) and occur in about 30 % of patients with AIH type 2, though frequently associated with LKM1 antibodies, they are the sole liver-related circulating autoantibodies in about 10 % of cases. Moreover anti-LC1 antibodies are clinically associated in most cases with greater disease activity and younger age.

SLA (**S**oluble **L**iver **A**ntigen) antibodies seem to have a high positive predictive value for AIH type 1, though relatively rare. However, they occur in a significant number of patients who are seronegative at presentation for other autoantibodies and therefore may be useful diagnostically.

F-actin antibodies are the main component of the broad family of **S**mooth **M**uscle **A**ntibodies (**SMA**). They bind to the F-actin component, a globular protein of 34 kD (polymerized into filaments), in the cytoskeleton of the cells. F-actin in its biologically active polymerized form is the most specific and sensitive autoantigen of AIH type 1. A correct interpreted F-actin pattern on SMA is reported to have a sensitivity of ~90 % and a specificity of ~100 % for patients with acute phase AIH type 1.

BlueDot / Autoimmune Liver Diseases

Kits	Class of Antibodies detected	Parameters detected
LI7D-24	IgG	M2 (OGDC-E2, BCOADC-E2, PDC-E2) • gp210 • sp100 • LKM1 • LC1 • SLA • F-actin
LID-24	IgG	M2 (OGDC-E2, BCOADC-E2, PDC-E2) • LKM1 • LC1
LISD-24	IgG	M2 (OGDC-E2, BCOADC-E2, PDC-E2) • LKM1 • LC1 • SLA • F-actin
MI3D-24	IgG/IgM	OGDC-E2 • BCOADC-E2 • PDC-E2
MID-24	IgG/IgM	M2 (OGDC-E2, BCOADC-E2, PDC-E2)

Celiac Disease

Celiac **D**isease (**CD**) is a permanent gluten-sensitive enteropathy characterized by an abnormal flattening of the intestinal mucosa, resulting in abnormal intestinal absorption. CD is triggered in predisposed patients by the ingestion of **gliadin**, a component of wheat gluten. Although gliadin was very early recognized to be an environmental etiologic factor of CD, the precise mechanism for the gluten-induced damage to the intestine has for a long time remained unclear.

In 1996, the **t**issue **T**rans**G**lutaminase (**tTG**), an enzyme already supposed to play a key role in the pathogenesis of CD, was confirmed as a preferred, highly sensitive and specific endomysial target antigen for autoantibodies in CD patients. A body of evidence strongly suggests that the digested gliadin acts as a substrate for tTG and causes, in genetically predisposed patients, its immunological recognition through the expression of cryptic epitopes which are otherwise hidden in the passive molecule. These neo-epitopes are considered responsible for self-recognition impairing and play a central role in the auto-

aggressive immune mechanisms of CD. In this context, anti-gliadin antibodies appear as an epiphenomenon with only a marginal role in the pathogenic events leading to CD and despite their initial usefulness these antibodies have lost diagnostic importance due to their lower sensitivity and specificity as CD markers.

Recently it has been shown that tTG selectively converts glutamine residues into glutamic acid in the gliadin protein (deamidation process) and gliadin antibodies from sera of patients with active CD preferentially recognize the **deamidated gliadin**. These antibodies represent a new serological marker different from conventional gliadin antibodies as well as from tTG autoantibodies. New immunoassays applying deamidated gliadin have improved the usefulness of gliadin antibodies in the diagnosis of CD and will likely provide new information on the pathophysiological mechanisms in the disease.

The class A autoantibodies are recognized as the most specific for CD. Unfortunately, selective IgA deficiency is quite common in Caucasian populations. That is the reason for the diagnosis of class G autoantibodies.

BlueDot / Celiac Disease

Kits	Class of Antibodies detected	Parameters detected
ENDA-24	IgA	Deamidated gliadin • tTG
ENDG-24	IgG	Deamidated gliadin • tTG
TTAD-24	IgA	tTG
TTGD-24	IgG	tTG

BlueWell / Celiac Disease

Kits	Class of Antibodies detected	Parameters detected
DGLA02-96	IgA	Deamidated gliadin
DGLG02-96	IgG	Deamidated gliadin
ENE02-96	IgA	tTG
GLA02-96	IgA	Gliadin (crude)
GLG02-96	IgG	Gliadin (crude)
TTA02-96	IgA	tTG
TTC02-96	IgA/IgG	tTG
TTG02-96	IgG	tTG

Crohn's Disease

Crohn's Disease is one of the two major **I**nflammatory **B**owel **D**iseases (**IBD**). IBD is an umbrella term covering both primary chronic disorders that cause inflammation or ulceration in the small and large intestine, Crohn's Disease and Colitis Ulcerosa.

In contrast to Colitis Ulcerosa, Crohn's Disease affects both the small bowel and the colon. Crohn's is a chronic, recurrent disease affecting more women than men, with an incidence of up to 1/100.000, a peak onset between 15 and 25 years of age and a familial aggregation. The etiology is still unrevealed, although a genetic and an infectious background for this disease is in discussion.

Diagnosis is currently established by Colonoscopy and Ileoscopy. Though Crohn's Disease and Colitis Ulcerosa share a number of symptoms, the course of the diseases, the complications and the management are different, especially when it comes down to surgery. Thus the differential diagnosis of both diseases is crucial prior to treatment. Unfortunately, there are 5-10 % of the patients that cannot be distinguished clearly by the existing available diagnostic methodologies and are consequently referred to as Indeterminate Colitis.

Anti-**S**accharomyces **C**erevisiae **A**ntibodies (**ASCA**) have been reported in patients with Crohn's Disease by Main et al. already in 1988. However the methodologies available at the time, Immunofluorescence mainly, and their lack of sensitivity and specificity limited the use of this marker to research. It was the identification of the antigen **mannan** (an outer cell wall component of yeast) which enabled the set-up of tests in standardized Enzyme Immunoassay format.

The availability of a serological test for Crohn's Disease will become an important tool for clinicians in their difficult task of diagnosing IBD, especially for cases of Indeterminate Colitis. Furthermore it could be the only tool in patient groups that are reluctant to endoscopies such as pediatric patients. The high positive predictive value of ASCA determination could additionally offer a possibility for a convenient and reliable screening and monitoring of risk groups. The value of ASCA for therapy monitoring is subject of future studies.

BlueDot / Crohn's Disease

Kits	Class of Antibodies detected	Parameters detected
ASCCD-24	IgA/IgG	Mannan from Saccharomyces cerevisiae

BlueWell / Crohn's Disease

Kits	Class of Antibodies detected	Parameters detected
ASCA02-96	IgA	Mannan from Saccharomyces cerevisiae
ASCC02-96	IgA/IgG	Mannan from Saccharomyces cerevisiae
ASCG02-96	IgG	Mannan from Saccharomyces cerevisiae

Milk Intolerance

The permeability of the intestinal mucosa to some specific dietary proteins (cow's milk proteins namely) is increased during neonatal period, as the gastrointestinal tract of new-borns is still immature. **C**ow's **M**ilk **P**rotein **I**ntolerance (**CMPI**) is thought to involve a malfunction of immunological tolerance mechanisms at this stage and often appears as the primary cause of a number of disorders frequently observed in young children.

Hypersensitivity reactions usually occur when switching from mother's breast feeding to cow's milk diet. The symptoms affect the gastrointestinal tract as well as the skin, the respiratory tract and may have unsuspected physiological consequences too, such as severe insomnia or hyper-agitation. Endoscopy and biopsy examination demonstrate typical intestinal mucosa alteration.

It is important to distinguish Intolerance from Allergy. Indeed Milk Intolerance is an IgG-mediated immunological process with long-term pathological consequences on the structure of the intestinal mucosa, whereas Milk Allergy is an IgE mediated, short-term hypersensitivity reaction without intestinal involvement.

Anti-**ß-Lactoglobulin** IgG antibodies are considered the most specific markers for the diagnosis of CMPI in young children. Testing for Anti-**Soya** IgG antibodies is important after a CMPI has been diagnosed and a soya-formulated diet has been set up. Cow's milk proteins sensitized children very often develop a hypersensitivity to soya proteins.

BlueDot / Milk Intolerance

Kits	Class of Antibodies detected	Parameters detected
BSD-24	IgG	ß-lactoglobulin (cow's milk) • Soya

BlueWell / Milk Intolerance

Kits	Class of Antibodies detected	Parameters detected
BL02-96	IgG	ß-lactoglobulin (cow's milk)
SO02-96	IgG	Soya

Autoimmune Gastritis

Autoimmune Gastritis is an organ-specific autoimmune disease characterized by Type A Chronic Atrophic Gastritis. The end-stage of the disease is Pernicious Anemia (Biermer's Anemia), which affects predominantly Caucasians of Northern European origin and is considered to be the most common cause of vitamin B12 deficiency in Western countries. Beside typical histological features (e.g. gastric mucosal atrophy), Type A Chronic Atrophic Gastritis is characterized by circulating antibodies to parietal cells and **I**ntrinsic **F**actor (**IF**).

Intrinsic Factor (IF) antibodies are strictly restricted to Pernicious Anemia and are much more specific markers for this severe outcome. Two types of autoantibodies to IF have been described. The first one are the blocking antibodies (type I) which are directed against the binding site of vitamin B12 to IF and prevent the formation of B12-IF complexes. The second one, type II antibodies, recognize a non-catalytic epitope and may bind indifferently to free Intrinsic Factor molecules or B12-IF complexes.

Radio**I**mmuno**A**ssays (**RIA**) has been for a long time the reference technique for the detection of anti-IF antibodies, but the **E**nzyme **I**mmuno**A**ssays (**EIA**), such as the *BlueDot* and *BlueWell* products, are now recognized as more sensitive and specific. In fact RIA uses radio-labelled vitamin B12 in the test as a competitor for the binding of autoantibodies to IF and therefore is only able to detect Type I (blocking) antibodies. Moreover RIA results may be seriously impaired by the interfering presence in the sample of high levels of unlabelled vitamin B12 (e.g. upon treatment of the patient) or B12-transport proteins (e.g. Transcobalamin II) which lead to false positive results. Contrarily EIA methods, which are based on a classical non-competitive detection of IF-bound autoantibodies, are able to detect both type I and type II antibodies (increased sensitivity) and are not influenced by the presence in the serum of unlabelled vitamin B12 or B12-absorbing proteins (increased specificity).

The Parietal Cell Antibodies (PCA) target the H^+/K^+ ATPase. This membrane enzyme catalyzes the counter transport of H^+ and K^+ and is responsible for the acidification of the stomach. PCA can be detected in 80-90% of Type A Chronic Atrophic Gastritis patients, from early stages to Pernicious Anaemia, by indirect immunofluorescence; they are also detected in 2-5% of the adult healthy population.

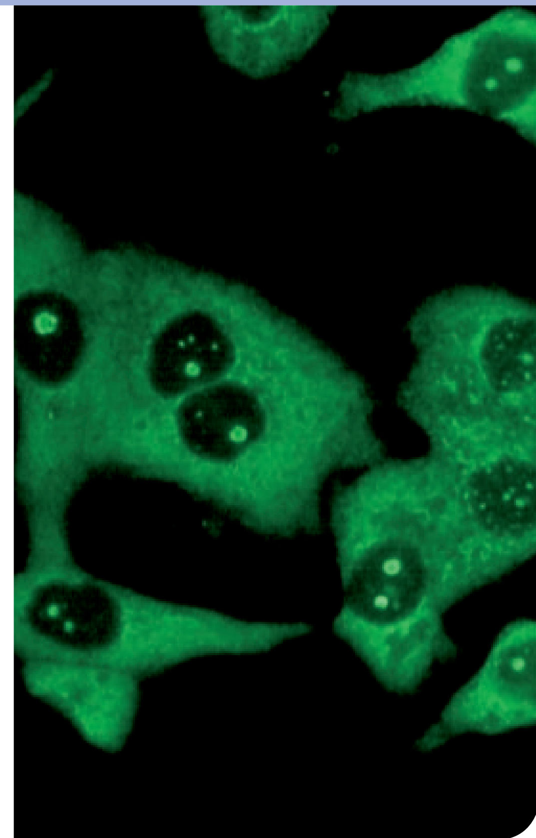
BlueDot / Autoimmune Gastritis

Kits	Class of Antibodies detected	Parameters detected
IFD-24	IgG	IF
IFPCAD-24	IgG	IF • PCA

BlueWell / Autoimmune Gastritis

Kits	Class of Antibodies detected	Parameters detected
IF01-96	IgG	IF

Confusing Immunofluorescence patterns



Immunofluorescence is widely considered as the gold standard in autoimmunity. However, it appears that some patterns are quite confusing and notably for the less experimented clinician. A secondary technique is generally needed to confirm the result. The best example is without any doubt the Immunofluorescence's homogenous staining which can be associated with **M2** (PBC), **Jo-1**, **PL-7**, **PL-12** or **SRP** (PM/DM) or **Ribosome P0** (SLE).

With this in mind, *D-tek* has imagined a comprehensive *BlueDot* product to help the clinicians to confirm these confusing patterns.

BlueDot / Confusing Immunofluorescence patterns

Kits	Class of Antibodies detected	Parameters detected
CY6D-24	IgG	M2 (OGDC-E2, BCOADC-E2, PDC-E2) • Jo-1 • PL-7 • PL-12 • SRP • Ribosome P0

Research

True to its culture, *D-tek* is always ready to propose innovative products for the diagnosis but also to help clinicians in their investigations.

With this in mind *D-tek* was the first diagnostic company to propose anti-Nucleosome detection.

D-tek has now developed the unique kit allowing the simultaneous detection of the three components of the 2-Oxo-Acid Dehydrogenase Complex: **P**yruvate **D**ehydrogenase **C**omplex (**PDC-E2**), **B**ranched-**C**hain **O**xo**A**cid **D**ehydrogenase **C**omplex (**BCOADC-E2**) and **O**xo**G**lutarate **D**ehydrogenase **C**omplex (**OGDC-E2**).

BlueDot / Research products

Kits	Class of Antibodies detected	Parameters detected
MI3D-24	IgG / IgM	OGDC-E2 • BCOADC-E2 • PDC-E2

LIQUID SUBSTRATES AND STABILIZERS

Liquid substrates and stabilizers are essential solutions that are widely used in today's IVD industry and research laboratories. They allow to prepare ready-to-use stable reagents that retain their initial activity upon storage, ensuring convenience of use and reproducibility over extended periods of time.

Taking advantage of its long R&D experience in stabilizing technologies, D-tek has developed a comprehensive range of products, all characterized by **single component, ready-to-use format** and **long shelf life**.

LIQUID SUBSTRATES

Horseradish peroxidase and Alkaline phosphatase are the most widely used enzymes to yield colorimetric results in EIA tests. Whatever the enzyme used and the end-product needed (soluble or insoluble), *D-tek* has the right reagent for your specific application.

		ENZYME USED	
		Phosphatase	**Peroxydase**
EXPECTED RESULTS	**Soluble end-product** (Microwell application)	p-NPP	TMB-Elisa
	Precipitate end-product (Membrane application)	NBT/BCIP	TMB-Membrane

NBT/BCIP

NBT/BCIP, the combination of 5-**B**romo-4-**C**hloro-3'-**I**ndolyl-**P**hosphate (**BCIP**) and **N**itro-**B**lue **T**etrazolium (**NBT**), is a highly active and stable formulation intended for measuring Alkaline Phosphatase (AP) probe activity.

The reducing components which are formed during phosphate hydrolysis of BCIP by AP, convert NBT to the insoluble purple NBT formazan which deposits on the membrane at the reaction sites.

The NBT/BCIP substrate is thus particularly useful for colorimetric detection on membrane immune-assays. Moreover, the black-purple precipitate presents a minimal fading so the coloration is stable and allows permanent record of the results

D-tek's NBT/BCIP single component liquid substrate is stable for at least 4 years from manufacture date onwards if stored at 2-8 °C and protected from light.

p-NPP

para-**N**itro**P**henyl **P**hosphate (**p-NPP**) is a ready-to-use solution that yields after reaction with Phosphatase (such as Phosphatase-labelled antibodies) a soluble yellow end-produc. This end-product can be quantified at 405 nm.

p-NPP substrate is thus recommended for Microwell application but it is not suitable for applications that require precipitating reaction.

D-tek's p-NPP single component liquid substrate is stable at least 2 years from manufacture date onwards if stored at 2-8 °C and protected from light.

TMB ELISA

TMB ELISA (**TMBE**) is a highly active and stable 3,3',5,5'-tetramethylbenzidine / H_2O_2 formulation intended for measuring **H**orse**R**adish **P**eroxidase (**HRP**) probe activity, specifically in Microwell application.

In presence of peroxydase (such as peroxydase-labelled antibodies), the TMBE is oxidized during the enzymatic degradation of H_2O_2. The soluble end-product is blue and can be quantified at 650 nm. The use of a stop solution enhances sensitivity 2-4 fold and produces a yellow end product that can be read at 450 nm. The stability of the yellow end-product is at least 1 hour.

TMBE substrate is thus recommended for Microwell application but it is not suitable for applications that require precipitating reaction.

The stability is at least 3 years from manufacture date onwards if stored at 2-8 °C and protected from light.

TMB Membrane

TMB Membrane (**TMBM**) contains 3,3',5,5'-tetramethylbenzidine optimized for use in membrane assays and dot blots. In presence of peroxydase (such as peroxidase-labelled antibodies), a deep blue color appears at the reaction site. TMBM substrate is thus recommended for Membrane application.

The stability is at least 5 years from the date of manufacture onwards if stored at 2-8 °C and protected from light.

LIQUID STABILIZERS

In the diagnostic field, the key of success lies without any doubt in the ability to preserve the biological product from the effect of time and to keep its diagnostic potentiality intact either in solution or coated on a support.

D-tek's long and valuable experience in the autoimmune diagnostic field has led to the development of an efficient range of stabilizers.

Antibody Stabilizing matrix

The Antibody Stabilizing matrix (AB-Stab) is dedicated to stabilize antibodies in solution. The reagent prevents the loss of AB activity at ready-to-use concentrations for easy, rapid and consistent tests.

D-tek's AB-Stab is stable for at least 2 years from manufacture date onwards if stored at 2-8°C.

AP-Conjugate Stabilizer.

The AP-Conjugate Stabilizer (AP-Stab) allows the stabilizing of **A**lkaline **P**hosphatase (**AP**) conjugates. The product is a ready-to-use solution in which AP conjugates can be directly diluted at working concentration.

D-tek's AP-Stab is stable for at least 2 years from manufacture date onwards if stored at 2-8°C.

The stability of the conjugate solution itself is not limited by the expiry date on the reagent label, it may vary depending on the conjugate used and should be determined internally for each specific application.

HRP-Conjugate Stabilizer.

The HRP-Conjugate Stabilizer (HRP-Stab) allows the stabilizing of **H**orse**R**adish **P**eroxidase (**HRP**) conjugates. The product is a ready-to-use solution in which HRP conjugates can be directly diluted at working concentrations.

D-tek's HRP-Stab is stable for at least 2 years from the manufacture date onwards if stored at 2-8°C.

The stability of the conjugate solution itself is not limited by the expiry date on the reagent label, it may vary depending on the conjugate used and should be determined internally for each specific application.

Dotting Solutions

The valuable experience of *D-tek* in membrane application for diagnostic purpose has led to the development of 2 different dotting solutions: **DOTSE** and **DOTST**.

These ready-to-use solutions allow antigen coating by maximizing their binding to the solid phase. They improve homogeneity and signal reproducibility on spots/lines assays production.

DOTSE is specifically designed to improve sensibility; it ensures a highly efficient and strong binding of most ligands on the support, for overall superior signal intensities.

DOTST specifically increases stability; it prevents fragile ligands from deterioration upon storage and optimizes reproducible signal intensities over time.

Plate Stabilizing Solution

The *D-tek*'s **P**late **S**tabilizing **S**olution (**PLS**) is a blocking buffer intended for an instantaneous, complete and stable blocking of the remaining free binding sites after microplate coating in Elisa production. It allows to reduce the background without reducing sensitivity and to save time in the manufacturing process.

PLS is recommended for solid phase ELISA assays and is particularly interesting when traditional blocking solutions (e.g. Non Fat Dry Milk, BSA or casein) do not succeed.

PLS is a protein-free formulation which eliminates the risk of false positive cross reactions with the analyte (e.g. antibody) or the tracer (e.g. conjugate) in the test, while avoiding the safety hazards related to the use of bovine biological material (BSA, Milk proteins...).

The stability is 1 year from the manufacture date onwards if stored at 2-8°C.

	AntiPhospholipid Syndrome	Autoimmune Hepatitis type 1	Autoimmune Hepatitis type 2	Celiac Disease	Churg Strauss' Syndrome	Congenital Heart Block	CREST Syndrome	Crohn's Disease	Drug Induced Lupus	Goodpasture Syndrome	Microscopic Polyangiitis	Milk Intolerance	Neonatal Lupus Erythematosus	Pernicious Anemia	Polymyositis/Dermatomyositis	Polymyositis/Scleroderma overlap syndrome	Primary Biliary Cirrhosis	Scleroderma	Sharp Syndrome	Sjogren's Syndrome	Subacute Cutaneous Lupus Erythematosus	Systemic Lupus Erythematosus	Vasculitis	Wegener's Granulomatosis	BlueDot Kit	BlueWell Kit
BCOADC-E2																	■								MI3D-24	
ß-lactoglobulin												■													BSD-24	BL02-96
ß2-GPI	■																					■			APSGD-24	GPG02-96 ; GPM02-96
Cardiolipin/ß2-GPI	■																					■			APSGD-24 ; LU6D-24	CLG02-96 ; CLM02-96 ; CLS01-96
CENP-A/B							■																		ANA12D-24 ; ANA12SD ; ANA10D-24 ; ANA8D-24	
dsDNA																						■			CHRD-24 ; CT10D-24 ; DNAD-24 ; LU6D-24	DNA02-96
F-actin		■																							LISD-24 ; LI7D-24	
GBM										■															ANCAGD-24 ; GBD-24	
Gliadin				■																					ENDA-24 ; ENDG-24	DGLA02-96 ; DGLG02-96 ; GLA02-96 ; GLG02-96
gp210																	■								LI7D-24	
Histones									■													■			CHRD-24 ; CT10D-24 ; CTD-24 ; LU6D-24 ; LUD-24 ; NUHISD-24	
IF														■											IFD-24 ; IFPCAD-24	IF01-96
Jo-1															■										ANA12D-24 ; ANA12SD ; ANA10D-24 ; ANA8D-24 ; CT10D-24 ; CTD-24 ; CY6D-24 ; ENAD-24 ; NUENAD-24 ; PMS8D-24	
Ku																■									ANA12D-24 ; ANA12SD ; ANA10D-24 ; PMS8D-24	
LC1			■																						LI7D-24 ; LID-24 ; LISD-24	
LKM1			■																						LI7D-24 ; LID-24 ; LISD-24	
M2																	■								CY6D-24 ; LI7D-24 ; LID-24 ; LISD-24 ; MID-24	
Mannan								■																	ASCCD-24	ASCA02-96 ; ASCC02-96 ; ASCG02-96
Mi-2															■										ANA12SD-24 ; PMS8D-24	
MPO					■						■														ANCAD-24 ; ANCAGD-24	
Nucleosome																						■			CHRD-24 ; CT10D-24 ; CTD-24 ; LU6D-24 ; LUD-24 ; NUD-24 ; NUENAD-24 ; NUHISD-24	NU02-96
OGDC-E2																	■								MI3D-24	
PCA														■											IFPCAD-24	
PCNA																						■			ANA12D-24 ; ANA12SD-24 ; ANA10D-24	
PDC-E2																	■								MI3D-24	
PL-12															■										CY6D-24 ; PMS8D-24	
PL-7															■										CY6D-24 ; PMS8D-24	
PM/Scl																■									ANA12D-24 ; ANA12SD-24 ; ANA10D-24 ; ANA8D-24 ; PMS8D-24	
PR3																								■	ANCAD-24 ; ANCAGD-24	
Ribosome P																						■			ANA12D-24 ; LU6D-24 ; LUD-24 ; CY6D-24	
RNP (68K/A/C)																			■						ANA12D-24 ; ANA12SD-24	
Scl-70																		■							ANA12D-24 ; ANA12SD-24 ; ANA10D-24 ; ANA8D-24 ; CT10D-24 ; CTD-24 ; ENAD-24 ; NUENAD-24 ; PMS8D-24	
SLA		■																							LI7D-24 ; LISD-24	
Sm																						■			ANA12D-24 ; ANA12SD-24 ; ANA10D-24 ; ANA8D-24 ; CT10D-24 ; CTD-24 ; ENAD-24 ; NUENAD-24 ; LU6D-24 ; LUD-24	
Sm/RNP																			■						ANA12D-24 ; ANA12SD-24 ; ANA10D-24 ; ANA8D-24 ; CT10D-24 ; CTD-24 ; ENAD-24 ; NUENAD-24	
Soya												■													BSD-24	SO02-96
sp100																	■								LI7D-24	
SRP															■										CY6D-24 ; PMS8D-24	
SSA (Ro)						■							■							■	■				ANA12D-24 ; ANA12SD-24 ; ANA10D-24 ; ANA8D-24 ; CT10D-24 ; CTD-24 ; ENAD-24 ; NUENAD-24	
SSB (La)						■							■							■	■				ANA12D-24 ; ANA12SD-24 ; ANA10D-24 ; ANA8D-24 ; CT10D-24 ; CTD-24 ; ENAD-24 ; NUENAD-24	
TRIM 21						■																			CT10D-24	
tTG				■																					ENDA-24 ; ENDG-24 ; TTAD-24 ; TTGD-24	ENE02-96 ; TTA02-96 ; TTC02-96 ; TTG02-96

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