EliA™

Celikey™ (anti-tTG) Gliadin

EliA™

Excellence in Autoimmunity

The EliA™ System

Time for the Essentials

- completely automated (true walk-away, overnight runs)
- easy instrument management by custom-made software
- barcode-reader (optional for ImmunoCAP™ 100€)
- protocols, QC and raw data easily accessible
- optional host link
- detailed QC management
- integrated stock management system

Cost efficient and flexible

- autoimmunity and allergy on the same instrument
- different autoimmune tests in the same run (puzzle-kit-approach)
- no batching of samples necessary - small runs can be handled cost-effectively
- once-monthly calibration
- several ImmunoCAP® instruments can be linked

A boost in service for your laboratory and your clinicians

- sample – result turnaround the same day
- STAT function on ImmunoCAP™ 250 for immediate testing of emergency samples
- overnight runs possible
- detailed documentation of results (patient or requester specific)
- ImmunoCAP™ 100€ – up to 46 determinations in less than 2.5 hours
- ImmunoCAP™ 250 – fully automated random access – up to 350 determinations per shift
- multiple methods in one run
- positive identification and traceability of samples and reagents on ImmunoCAP™ 250

The EliA™ Celikey™ and EliA™ Gliadin Assays – surer Diagnosis of Celiac Disease

Quantitative determination of IgA or IgG antibodies to tTG and gliadin, the most clinically relevant antibodies in the differential diagnosis of celiac disease

High clinical relevance

- high specificity of the EliA™ Celikey™ assays avoids unnecessary intestinal biopsies
- high sensitivity of the EliA™ Celikey™ assays supports diagnosis
- use of recombinant tTG antigen for EliA™ Celikey™ reduces false positive results
- EliA™ Celikey™ is equivalent to the endomysium antibody test (EMA)

High technical performance

- low variances and high reproducibility for consistent results
- high lot-to-lot consistency due to validated production procedures
- quantitative results expressed as U/ml based on a six-point standard curve
- large lot sizes - fewer revalidations necessary

State-of-the-art antigens

- human recombinant tTG antigen produced in the eucaryotic baculovirus/insect cell system
- especially processed wheat gliadin antigen containing all antigenic polypeptides

Proven Quality

- EliA™ Celikey™ performs identically to the well-established Celikey™ test in the Varelisa™ format
- many important clinical studies rely on Celikey™
- Celikey™ is regarded as the standard among tTG antibody tests

Automation

- fully automated tTG and gliadin antibody testing
- two ImmunoCAP instruments available for low to high troughput
- tTG and gliadin, both IgA and IgG in one run
- calibration for IgA and IgG only every four weeks
- even very few samples can be run cost-efficiently

Celiac Disease

Celiac disease (CD) is characterised by a life-long intolerance to gluten from wheat, barley or rye. Although it is incurable, an effective treatment, in the form of a gluten-free diet, is available. The classical presentation of the disease includes gastrointestinal symptoms such as diarrhoea, abdominal discomfort or bloating. However, a large proportion of CD patients present with atypical symptoms such as general weakness, bad temper, anaemia, menstruation disturbance or even depression. All these symptoms are the consequence of pathogenic alterations of the small intestinal mucosa (villous atrophy) leading to malabsorption of nutrients. Even though CD usually occurs in early childhood, only a few patients are diagnosed correctly during this time while the vast majority of CD patients are diagnosed much later or not at all. Thus, the already diagnosed CD patients represent only the tip of the iceberg. The reasons for this situation are that about half of the CD patients do not show the typical gastrointestinal symptoms and have so-called latent or silent CD and, also, that the awareness for CD among pediatricians or general practitioners is still not very high. As a result, the prevalence of celiac disease has been underestimated for a long time. Recent screening studies show that it is a very common disease affecting about 1% of the population.

Early diagnosis of CD in children is very important, because the disease may lead to growth and development retardation. However, CD is not just a disease of childhood but also affects adult patients, who may experience a substantially decreased quality of life and have an increased risk for osteoporosis, autoimmune diseases, or even malignancy. Although population screening for CD using serological tests is still a controversial topic, screening of high risk groups, such as first degree relatives of CD patients, patients suffering from osteoporosis, anemia, type I diabetes, thyroiditis, IgA deficiency, or other autoimmune diseases is strongly recommended.

The diagnosis of celiac disease consists of three parts: serology, jejunal biopsy and remission of the disease following adherence to a gluten-free diet. The serological tests available detect antibodies to gliadin, issue transglutaminase (tTG) or endomysium and are usually followed by intestinal biopsy if positive.

The Antigens

Human recombinant tTG for outstanding performance

The first antigen which was found to be associated with CD is gliadin, the alcohol soluble fraction of gluten. The immune reaction towards gliadin is crucial for the development of CD but is not of autoimmune character. Only in a second step, antibodies to the body's own tissue transglutaminase (tTG) are produced thus defining CD as an autoimmune disease. Tissue transglutaminase is a Ca^{2+}-dependent enzyme which has multiple functions including catalysing protein crosslinking or incorporation of amines into proteins. In 1997 tTG was identified as the antigen for endomysium antibodies (EMA) which are detected by indirect immunofluorescence assays. Therefore EMA and tTG can be regarded as equivalent, if the correct anti-tTG test is chosen.

In order to obtain a highly sensitive and specific immunoassay which gives identical results to the EMA test, the tTG antigen has to fulfil certain requirements. It must be of high purity and it must present the same correct three-dimensional structure as the EMA antigen. We achieve this by producing the human recombinant tTG used in our EliA™ Celikey™ assays in the eucaryotic baculovirus/insect cell system which guarantees a very pure antigen of correct structure and results in assays of the highest clinical sensitivity and specificity possible. Many independent publications show the outstanding clinical performance of the Celikey™ assays as well as their excellent agreement with the EMA test.

Since gliadin represents a group of proteins from cereals the antigen used in our EliA™ Gliadin assays is purified from wheat and provides a comprehensive mixture of all antigenic targets required. Antibodies of both immunoglobulin classes IgA and IgG can be seen in CD patients. In contrast to gliadin antibodies where both IgA and IgG are important, only tTG IgA antibodies are useful for detecting CD in the general population. IgG antibodies to tTG, however, provide valuable help in the diagnosis of CD in patients suffering from IgA deficiency, which is more frequent in CD patients than in the normal population.

High Clinical Relevance

EliA™ Celikey™ – performs identically to Celikey™ on Varelisa™

In contrast to other autoimmune diseases, celiac disease has the advantage of having very sensitive and specific serological markers. A simple blood test can virtually rule out or confirm CD with almost 100 % certainty. Therefore these tests can also be used for screening children and groups at high risk for CD, such as first degree relatives of CD patients, patients with osteoporosis, anemia, type I diabetes, thyroiditis, IgA deficiency or other autoimmune diseases.

EliA™ Celikey™ performs virtually identically to the well-established Celikey™ tests in the Varelisa™ format. This has been shown in an internal study using sera from 93 CD patients and 101 disease controls (Table 1).

	Patients	Elia™ Celikey™ IgA		
		positive	equivocal	negative
Celikey™ on Varelisa™	positive	85	0	0
	equivocal	4	3	0
	negative	0	0	1

	Disease Controls	Elia™ Celikey™ IgA		
		positive	equivocal	negative
Celikey™ on Varelisa™	positive	3	0	0
	equivocal	2	0	1
	negative	0	0	95

Table 1: *Correlation of EliA™ Celikey™ and Celikey™ in the Varelisa™ format using 9-field analysis.*

The concordance of both Celikey™ tests has recently been confirmed by Alessio et al. (2004). Thus, the data obtained with the Celikey™ tests in the Varelisa™ format can be easily transferred to the respective EliA™ tests.

Celikey™ – the standard in anti-tTG IgA tests

Because of its increasing relevance for diagnosis of CD, both clinical sensitivity and specificity are of great importance for an anti-tTG IgA assay. An external clinical study including 208 patients with celiac disease and 157 control patients diagnosed as free from celiac disease, but suffering from other gastrointestinal symptoms (Bürgin-Wolff et al., 2002) resulted in excellent values for Celikey™ (Table 2).

No. of subjects: 208 CD patients, 157 disease controls	
	Celikey™
Sensitivity	96.2 %
Specificity	99.4 %
Positive predictive value	99.5 %
Negative predictive value	95.1 %
Efficiency	97.5 %

Table 2:
Clinical sensitivity, specificity, PPV, NPV and efficiency of Celikey™ (taken from Bürgin-Wolff et al., 2002).

Celikey™ avoids many unnecessary biopsies

Because a positive screen test result usually leads to a confirmatory biopsy, high specificity of the tests for CD is particularly important. Due to the prevalence for CD of about 1 % in a screening population, even a slight decrease in specificity will result in a dramatic increase in unnecessary intestinal biopsies.
Two examples using a screening population of 1000 subjects demonstrate this (Fig. 1):

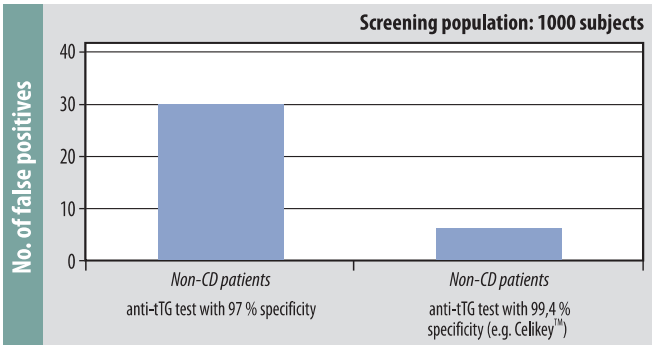

Figure 1:
Effect of specificity on the number of false positives in a screening population of 1000 patients.

The less specific assay would result in 5 times more biopsies of non-CD patients in a screening population, most of which could be avoided by using the assay with the higher specificity.

Celikey™ is equivalent to the EMA test

From an excellent anti-tTG test you should expect a good correlation to the endomysium antibody test (EMA) based on indirect immunofluorescence, which is still regarded as the gold standard among serological CD tests. The labour intensive and costly immunofluorescence technique can be cost-effectively replaced by Celikey™ without any loss of diagnostic accuracy. Figure 2 summarises that Celikey™ shows an excellent agreement of 99 % to EMA in terms of clinical performance (Bürgin-Wolff et al., 2002).

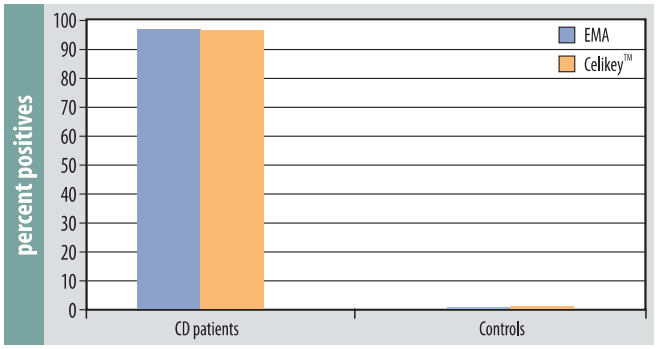

Figure 2:
Comparison of EMA and Celikey™ in a study population of 208 biopsy positive CD patients and 157 biopsy negative disease controls (data taken from Bürgin-Wolff et al., 2002).

These data were also confirmed in a screening study with schoolchildren. 3651 of 3654 results (99.9 %) were concordant for EMA and Celikey™ (Mäki et al., 2003).

Celikey™ in the literature – experiences

»We consider *Celikey™* to be the method of choice for screening for coeliac disease, since it provides good sensitivity and specificity.« *(Collin et al. 2005)*

»*Celikey™* outperforms both the guinea pig based tissue transglutaminase assay and anti-endomysium antibodies when screening for coeliac disease.« *(Wolters et al., 2002)*

»We found that *Celikey™* was as reliable and sensitive as the endomysial antibody test, which is based on indirect immunofluorescence.« *(Mäki et al., 2003)*

»*Celikey™* has equivalent sensitivity and specificity to EMA and is therefore an appropriate first-line test.« *(Hill et al. 2004)*

»*Celikey™* can be used as an accurate observer-independent alternative to EMA in diagnosing or monitoring CD.« *(Bürgin-Wolff et al., 2002)*

»In conclusion, IgG anti-tTG antibodies measured with *Celikey™* IgG are highly reliable serum markers of coeliac disease in IgA deficient subjects.« *(Korponay-Szabó et al. 2003)*

The EliA™ Gliadin Assays complement EliA™ Celikey™

Tests for gliadin antibodies can be useful in unclear cases and provide help in both diagnosis and monitoring of CD, even if they do not reach the specificity of EMA or anti-tTG tests.

Children under 2 years of age who suffer from CD may have not yet developed antibodies to tTG, while those to gliadin are already present and can provide aid in diagnostic decisions (Ascher et al., 1995; Bürgin-Wolff et al., 1991; Grodzinsky et al., 1995).

Gliadin IgA antibodies are of particular interest for monitoring the success of gluten-free diet, as they disappear much faster than tTG antibodies. In contrast, gliadin IgG antibodies seem to respond faster to a new gluten-challenge than those to tTG, and thus are useful for monitoring the patients' compliance (Bürgin-Wolff et al., 1991).

Anti-gliadin tests are also useful to support the anti-tTG results and so can add to safer diagnostic decisions.

We designed our EliA™ Gliadin assays to be as specific as possible in order to prevent unnecessary intestinal biospies. An internal clinical study using sera from 98 CD patients and 101 disease controls suffering from other gastrointestinal disorders revealed that our anti-gliadin assays are very specific and thus show a comparatively high positive predictive value which aids in deciding whether or not to send the patient to gastroscopy (Table 3).

The EliA™ Celikey™ and EliA™ Gliadin assays make the differential diagnosis of celiac disease easier and surer.

	EliA™ Gliadin IgA	EliA™ Gliadin IgG
Specificity	97.0%	80.2 %
Positive predictive value	95.8 %	77.5 %

Table 4: *Clinical specificity and positive predictive value (PPV) of EliA™ Gliadin IgA and IgG.*

The EliA™ Celikey™ and EliA™ Gliadin tests can be performed on two instruments: the ImmunoCAP™ 100€ for low to medium throughput and the ImmunoCAP™ 250 for high throughput. Even though the two instruments are different in their features, the EliA™ Celikey™ and EliA™ Gliadin tests give identical results on both systems.

The EliA™ Celikey™ and EliA™ Gliadin tests give identical results on ImmunoCAP 100€ and ImmunoCAP™ 250

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Technical Data

Products	EliA™ Celikey™ IgA (tissue transglutaminase IgA) EliA™ Celikey™ IgG (tissue transglutaminase IgG) EliA™ Gliadin IgA EliA™ Gliadin IgG				
Antigens	EliA™ Celikey™ tests:	human recombinant tissue transglutaminase produced in the baculovirus/insect cell system			
	EliA™ Gliadin tests:	purified wheat gliadin			
Standardisation	six point IgA or IgG standard curves; results in U/ml				
Cut-off/ measuring range		negative	equivocal	positive	measuring range
	EliA™ Celikey™ IgA	< 7 U/ml	7 - 10 U/ml	> 10 U/ml	0.1 – ≥ 128 U/ml
	EliA™ Celikey™ IgG	< 7 U/ml	7 - 10 U/ml	> 10 U/ml	0.5 – ≥ 600 U/ml
	EliA™ Gliadin IgA	< 7 U/ml	7 - 10 U/ml	> 10 U/ml	0.2 – ≥ 213 U/ml
	EliA™ Gliadin IgG	< 7 U/ml	7 - 10 U/ml	> 10 U/ml	0.2 – ≥ 192 U/ml
Dilution		1:100			
Sample material		Serum, Plasma (EDTA, heparin, citrate)			
Normal distribution	(95% / 99% percentile)		EliA™ Celikey™ IgA		3.2 / 7.4
			EliA™ Celikey™ IgG		2.6 / 4.2
			EliA™ Gliadin IgA		4.1 / 5.8
			EliA™ Gliadin IgG		5.0 / 12.6
Reproducibility	Intra-run variance*	2.2 % - 5.8 %			
	Inter-run variance*	1.6 % - 7.5 %			

** for details see directions for use*

Ordering Information

	Package size	*Article No.*
EliA™ Celikey™ IgA Well	4 x 12	14-5517-01
EliA™ Celikey™ IgG Well	2 x 12	14-5518-01
EliA™ Gliadin IgA Well	4 x 12	14-5519-01
EliA™ Gliadin IgA Well	4 x 12	14-5520-01

For EliA™ specific reagents and general reagents please refer to the Phadia product catalogue.

Phadia

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