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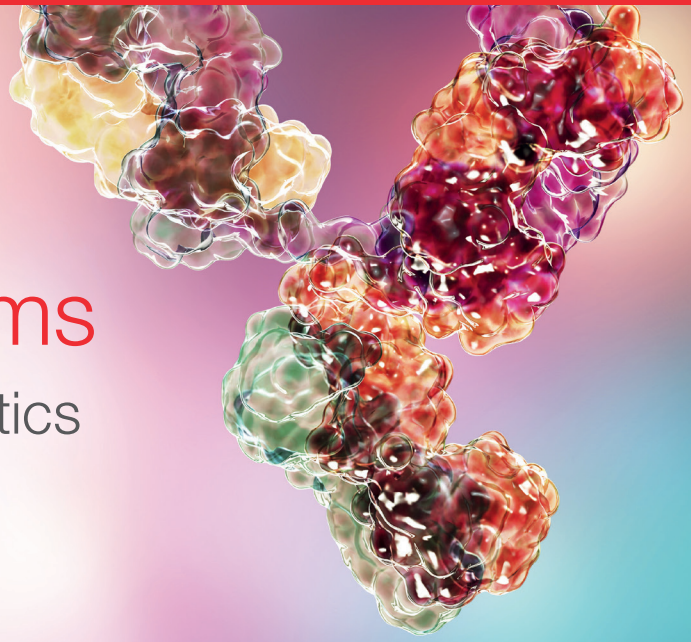
EliA™



EliA test algorithms

for autoimmunity diagnostics

ThermoFisher SCIENTIFIC

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Contact details and additional information

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EliA test algorithms for autoimmunity diagnostics

You are reading the first edition of the EliA™ test algorithms. This booklet has been produced following our customers' requests for a simple and comprehensive overview of test algorithms to support autoimmunity diagnostics.

It was written with those in mind who regularly work in autoimmune diagnostics and aims to help with selecting the appropriate test for laboratory requirements, organizing and dispensing EliA tests in routine laboratory work, as well as with interpreting test results to support diagnoses and plan further suitable follow-up evaluation.

The reference values were selected based on the best available information and their clinical relevance. However, we do not accept liability for their accuracy. The use of this booklet should not replace independent studies, guidelines, medical recommendations or updates subsequent to the publication of this booklet.

Autoimmune diseases are generally caused by a disorder of the immune system. Clinical disease characteristics frequently overlap, which makes a differential diagnosis based on symptoms alone very challenging.

All autoimmune diseases have a fundamental feature in common: the formation of autoantibodies against naturally occurring proteins and target structures. Detecting and accurately determining these autoantibodies can make a vital contribution to clinical diagnosis, and thus to a targeted therapy.

On the following pages, you will find test algorithms based on international guidelines and recommendations in combination with the latest scientific findings. These will help you in diagnosing systemic and organ-specific autoimmune diseases. Please note that the test algorithms shown here are only some of the diagnostic approaches which may be indicated and/or possible, and that additional clinical and diagnostic tests are needed for a final diagnosis.

The EliA test algorithms focus on the serological determination of autoantibodies using, for instance, EliA tests, and in some cases contain further important methods and parameters for laboratory diagnostics. Furthermore, you will find useful information on the different autoimmune diseases and test-specific features.

The chapter entitled “Good to know” contains some general information and unique features of the EliA test principle as well as a complete list of products and antigens.

I wish you the best of success with your autoimmunity diagnostics!

Dr. Christian Fischer
Senior Director Scientific & Medical Affairs

ANA/ENA diagnostics

Indication

Investigation of clinical suspicion of connective tissue diseases (CTD) or antinuclear antibody (ANA) requirements. A positive screening result will be followed up by differentiating antibodies against extractable nuclear antigens (ENA) which could give a clear indication of a specific CTD.

Explanations

CTDs are connective tissue diseases caused by an autoimmune reaction and can, in principle, affect any organ in the body. CTDs include systemic lupus erythematosus (SLE), Sjögren's syndrome (SjS), systemic sclerosis (SSc), autoimmune myopathies (PM/DM) and mixed connective tissue disease (MCTD).[1] These diseases are often associated with non-specific symptoms, which makes them difficult to diagnose.[2] Autoantibodies are found in most CTDs as markers of autoimmune disease.

The determination of these antinuclear antibodies forms the basis of CTD diagnosis, for example using ANA-IIF, an indirect immunofluorescence test (IIF) on HEp-2 cells. In the last few years, automated enzyme immunoassays (EIA) have become more widely used because they offer a comparable degree of sensitivity and a significantly higher specificity for CTD compared to ANA-IIF.[3–9] EIAs can be used in combination with, or as an alternative to, ANA-IIF, considerably reducing the number of false positives thanks to their high specificity.[8,10] Furthermore, EIAs are more sensitive to some specific antibodies, such as Ro52 and Ro60.[4,7,11]

32% of samples taken from the general population will produce false positive ANA-IIF results, and can cause a great deal of uncertainty and anxiety among patients.[12] Furthermore, costly and time-consuming follow-up tests are required before a systemic rheumatic disease can actually be excluded.[13] The worst-case scenario would be misdiagnosis and improper treatment in the long term.[10]

ANAs occur in
Connective tissue diseases
Autoimmune liver diseases
Rheumatoid arthritis
Type 1 diabetes mellitus
Gastrointestinal diseases
Lung- and skin diseases
Infectious diseases
Cancer

ANA positivity in healthy subjects[12]	
32 %	at a titer of 1:40
13 %	at a titer of 1:80
5 %	at a titer of 1:160

ANA requirements

ANA-IIF + EliA CTD Screen

The combination of ANA-IIF and EliA CTD Screen test can significantly reduce the number of false positive and false negative test results compared to individual testing.[10] In addition to the most frequent ENAs, the EliA CTD Screen test also comprises less common, but very specific ENAs such as fibrillarin and Mi-2.

+ test for frequent and rare ENAs
+ few false positive and false negative results

ANA-IIF + EliA Symphony^S + EliA dsDNA

The EliA Symphony^S test comprises the ENAs which are most frequently associated with CTD. The combination of ANA-IIF, EliA Symphony^S and EliA dsDNA tests provides important results for focused follow-up tests to enable diagnosis.

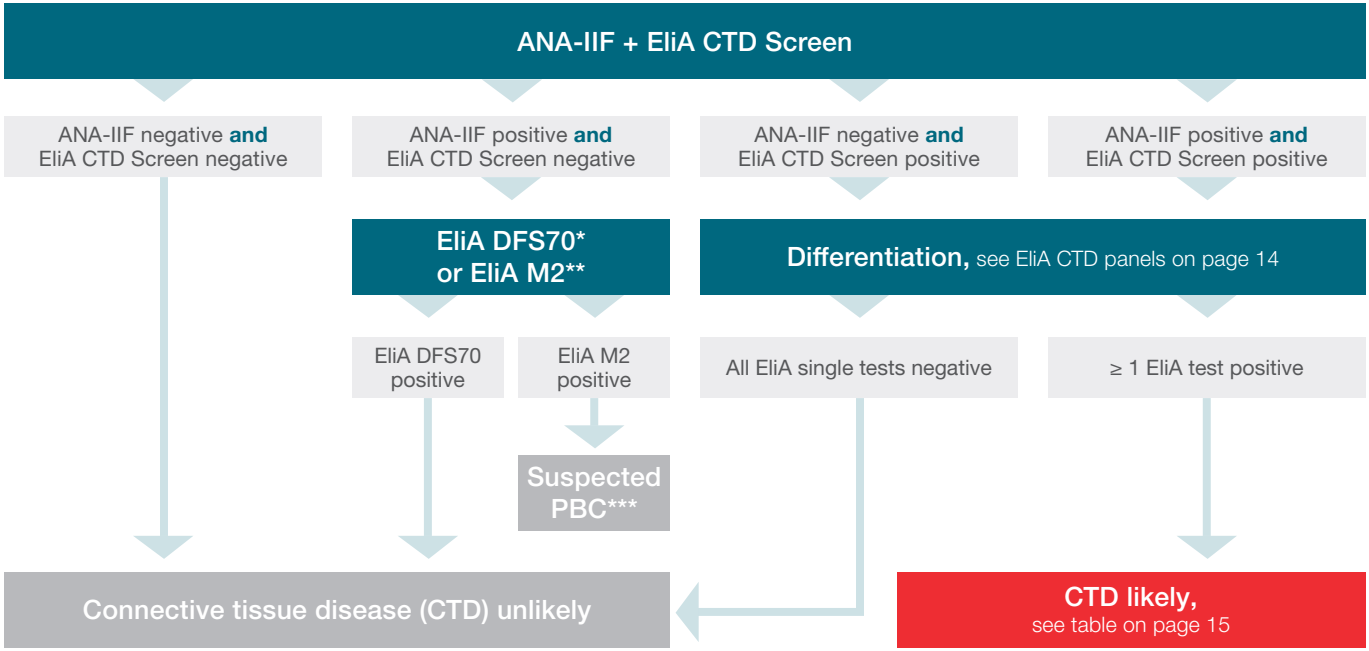
+ testing for the most frequent ENAs
+ starting result for anti-dsDNA antibodies

EliA CTD Screen

In addition to the most frequent ENAs, the EliA CTD Screen test also comprises less frequent, but very specific ENAs such as fibrillarin and Mi-2. Thanks to its high specificity, which has been confirmed by various studies, the number of false positive test results in particular can be significantly reduced compared to an initial screening with ANA-IIF.[10]

+ an automated initial test
+ few false positive results

For the complete antigen lists for the EliA CTD Screen and the EliA Symphony^S tests, please see page 36 et seq.

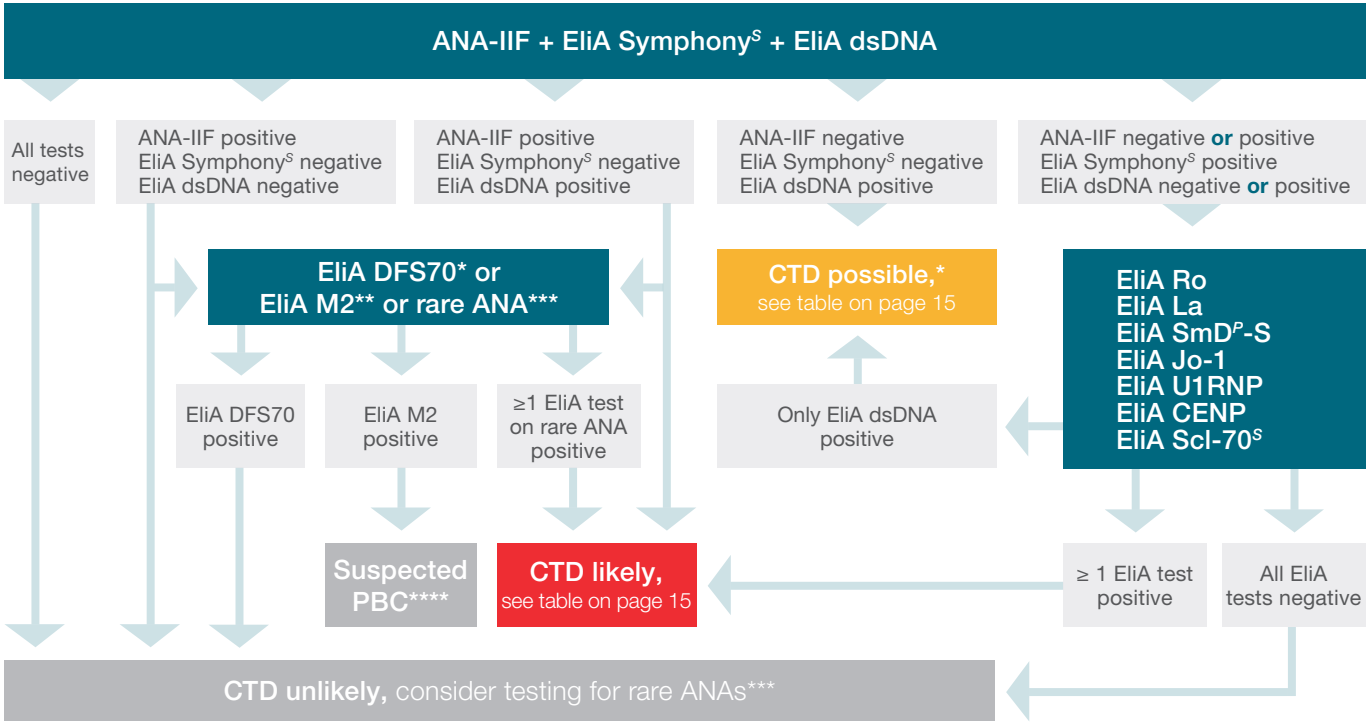


* IIF pattern "dense fine speckled" (AC-2) ** IIF pattern "cytoplasmic/reticular AMA" (AC-21) *** Primary biliary cholangitis (see page 28)

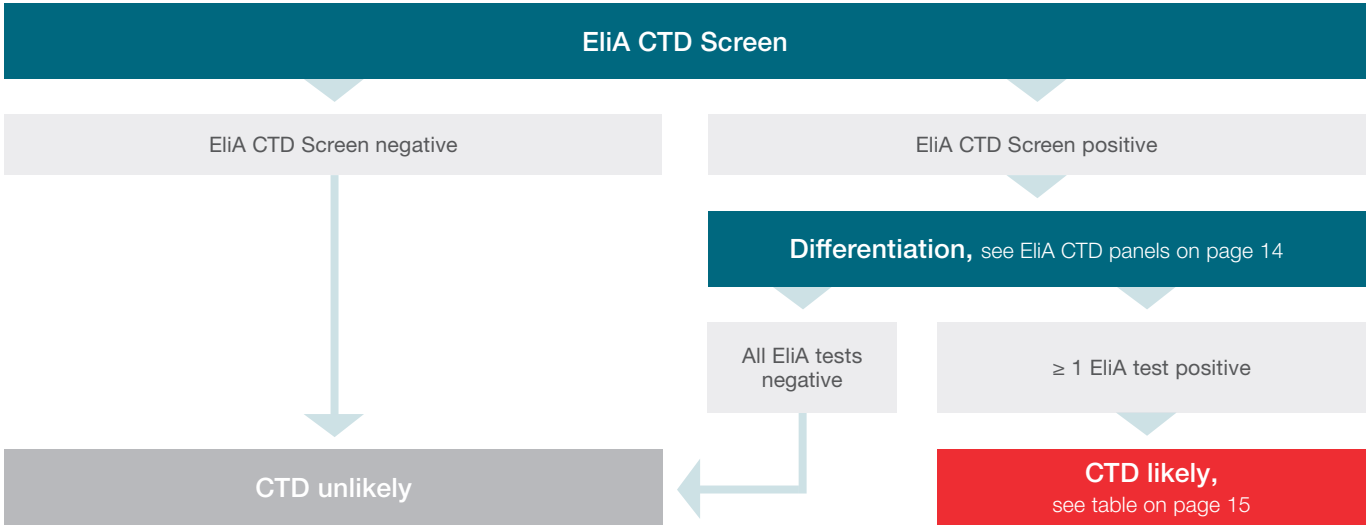
Selected references for this test algorithm:

- "Screening for CTD-associated antibodies by automated immunoassay", Willems et al. 2018 [9]
- "The association of solid-phase assays to immunofluorescence increases the diagnostic accuracy for ANA screening in patients with autoimmune rheumatic diseases", Bizzaro et al. 2018 [4]

ANA diagnostic



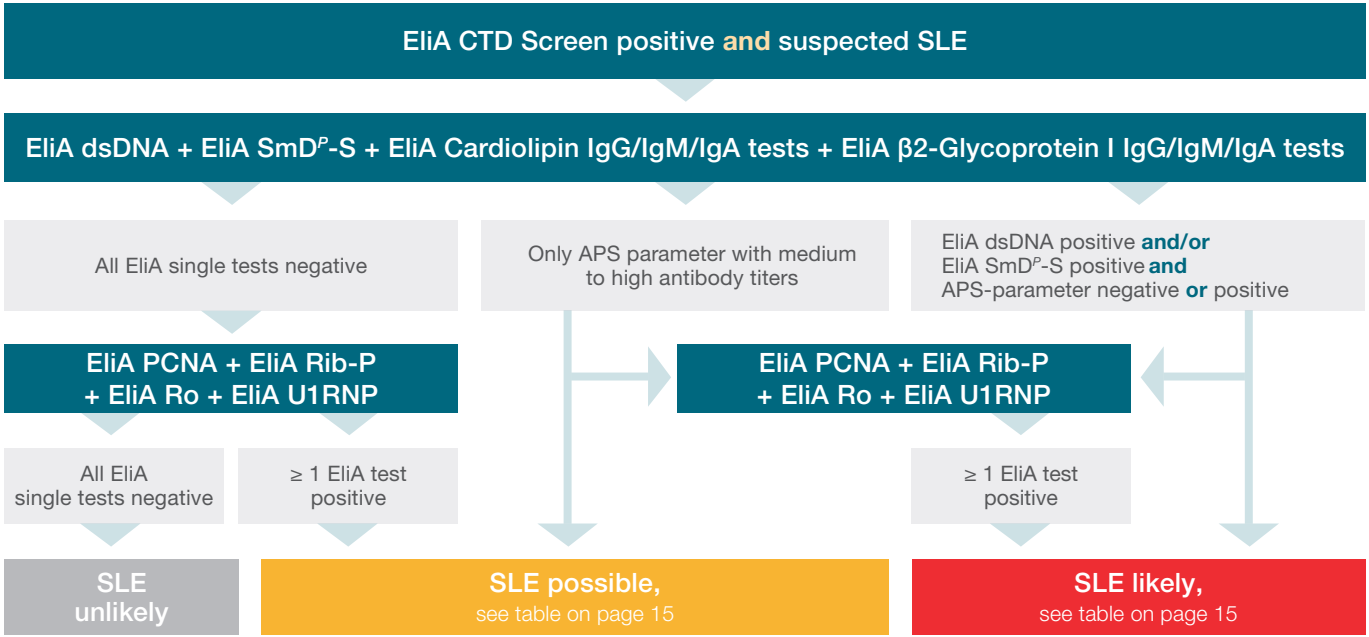
* In case of ANA-IIF negative and EliA dsDNA positive: consider confirmatory test for anti-dsDNA antibodies (e.g. CLIFT, Farr RIA) ** IIF pattern "cytoplasmic/reticular AMA" (AC-21)
*** EliA tests for rare ANAs (see CTD panels page 14): EliA RNA Pol III, EliA Fibrillarin, EliA Rib-P, EliA PM-Scl, EliA PCNA, EliA Mi-2 **** Primary biliary cholangitis (see page 28)



Selected references for this test algorithm:

- "A comparison of a fluorescence enzyme immunoassay versus IIF for initial screening of CTD", Orme et al. 2018 [10]
- "Measurement of antinuclear antibodies and their fine specificities: time for a change in strategy?", Otten et al. 2017 [5]
- "Comparison of the clinical utility of the EliA CTD Screen to IIF on Hep-2 cells", Robier et al. 2016 [7]

Systemic lupus erythematosus



Selected references for this test algorithm:

- "2019 EULAR/ACR classification criteria for systemic lupus erythematosus", Aringer et al. 2019[3]
- "EULAR/ACR SLE classification criteria item performance", Aringer et al. 2021[14]

Systemic lupus erythematosus (SLE) is a classical autoimmune disease characterized by the development of antibodies against components of the cell nucleus in association with various clinical manifestations. Due to the disease complex clinical presentation and unclear etiology, classification criteria for SLE have been defined, with a recent revised edition that was published in 2019. One addition to this most recent version is the recommendation to use a solid-phase immunoassay with comparable performance as an alternative to ANA-IIF for the determination of antinuclear antibodies (ANAs).[3]

Incidence of relevant autoantibodies in SLE patients

In a study published in 2019, of a cohort of 1,137 SLE patients, 92.3% tested ANA-IIF positive, 6.2% tested ANA-IIF negative and 1.5% showed an isolated, positive cytoplasmic or mitotic ANA-IIF pattern (CMP).[15] This table lists the various autoantibodies which can be detected using ANA differentiation according to incidence.

Autoantibodies	ANA-IIF positive N=1049	ANA-IIF negative N=71	CMP positive N=17
Ro60	47.3%	22.5%	29.4%
Ro52	35.9%	21.1%	23.5%
anti-β2-Glycoprotein I	15.0%	15.9%	12.5%
dsDNA	28.4%	11.3%	17.7%
U1RNP	32.4%	11.3%	11.8%
anti-Cardiolipin	12.6%	11.1%	12.5%
Sm	24.7%	5.7%	11.8%
Rib-P	16.1%	5.6%	11.8%
La/SS-B	15.9%	5.6%	11.8%
PCNA	7.3%	1.4%	11,8%

CTD panels and table for interpretation

Standard test panel

- EliA dsDNA*
- EliA Ro*
- EliA SmD^{P}-S
- EliA La
- EliA Jo-1*
- EliA U1RNP
- EliA CENP
- EliA Scl-70^{S}

Rare ANA panel

- EliA RNA Pol III
- EliA Fibrillarin
- EliA Rib-P
- EliA PM-Scl
- EliA PCNA
- EliA Mi-2

Test panel by ANA-IIF pattern**

Nuclear

Homogeneous
- EliA dsDNA* (AC-1)
- EliA Ro*

Nucleolar
- EliA PM-Scl (AC-8)
- EliA Fibrillarin (AC-9)

Pleomorphic
- EliA PCNA (AC-13)

Centromeric
- EliA CENP (AC-3)

Speckled
- EliA DFS70 (AC-2)
- EliA Ro* (AC-4)
- EliA La (AC-4)
- EliA Mi-2 (AC-4)
- EliA U1RNP (AC-5)
- EliA SmD^{P}-S (AC-5)
- EliA RNA Pol III (AC-5)
- EliA RNP70 (AC-5)
- EliA Scl-70^{S} (AC-29)

Cytoplasmic

Speckled
- EliA Rib-P (AC-19)
- EliA Jo-1* (AC-20)

AMA
- EliA M2 (AC-21)

Test panel by indication

SjS
- EliA Ro52*
- EliA Ro60*
- EliA La

PM/DM
- EliA Jo-1*
- EliA Ro52*
- EliA Ro60*
- EliA Mi-2
- EliA PM-Scl

SLE
- EliA dsDNA*
- EliA SmD^{P}-S
- EliA Cardiolipin***
- EliA β2-Glycoprotein I***
- EliA PCNA
- EliA Rib-P
- EliA Ro*
- EliA U1RNP
- EliA RNP70

SSc
- EliA CENP
- EliA Scl-70^{S}
- EliA RNA Pol III
- EliA Ro52*
- EliA Ro60*
- EliA U1RNP
- EliA Fibrillarin
- EliA PM-Scl

MCTD
- EliA U1RNP
- EliA RNP70

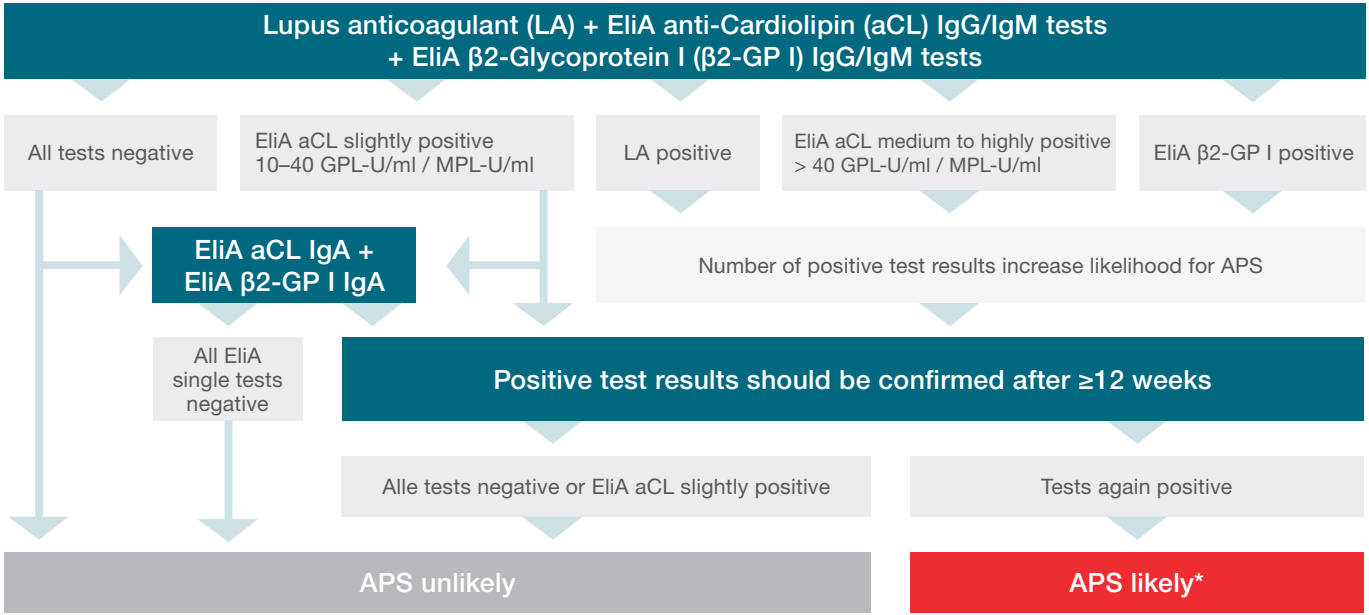
See below for result interpretation

* Antibodies against SS-A/Ro, dsDNA and Jo-1 may be missed by an ANA-IIF test. [4, 7, 16–18] ** According to the nomenclature of the International Consensus on ANA Patterns (ICAP) [19]
*** EliA Cardiolipin IgG, EliA Cardiolipin IgM, EliA Cardiolipin IgA, EliA β2-Glycoprotein I IgG, EliA β2-Glycoprotein I IgM, EliA β2-Glycoprotein I IgA

Autoantibodies/antigens	Test	Sjögren's Syndrome (SjS)	Systemic lupus erythematosus (SLE)	Systemic sclerosis (SSc)	Autoimmune Myopathies (PM/DM)	Mixed connective tissue disease (MCTD)
dsDNA	EliA dsDNA		+			
U1-RNP	EliA U1RNP		+	+		+
RNP70	EliA RNP70		+			+
Sm	EliA SmD^{P}-S		+			
SS-A/Ro	EliA Ro	+	+	+	+	
Ro52	EliA Ro52	+	+	+	+	
Ro60	EliA Ro60	+	+	+	+	
SS-B/La	EliA La	+	+			
Centromere	EliA CENP			+		
Topoisomerase I/Scl-70	EliA Scl-70^{S}			+		
RNA Polymerase III	EliA RNA Pol III			+		
Histidyl-tRNA-synthetase/Jo-1	EliA Jo-1				+	
Fibrillarin/Scl-34/U3-RNP	EliA Fibrillarin			+		
Ribosomal P-protein/Rib-P	EliA Rib-P		+			
PM-Scl-100	EliA PM-Scl			+	+	
Cyclin/PCNA	EliA PCNA		+			
Mi-2	EliA Mi-2				+	
Cardiolipin/aCL	EliA Cardiolipin***		+			
β2-Glycoprotein I	EliA β2-Glycoprotein I***		+			

+ Parameters or autoantibodies which are part of the relevant classification criteria. Overview adapted from Conrad K, Schößler W, Hiepe F and Fritzler MJ (2015)[20]

Antiphospholipid syndrome



* According to the Sydney classification criteria for definite antiphospholipid syndrome (APS), at least one clinical and one laboratory diagnostic criterion must be met.[21]

Selected relevant guidelines and classification criteria for this test algorithm:

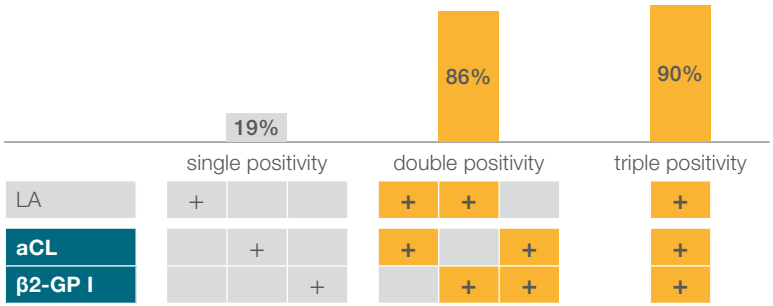
- "Sydney Criteria" as part of the "11th International Congress on antiphospholipid antibodies", Miyakis et al. 2006[21]
- "International Consensus Guidelines on Anticardiolipin and Anti–β2-Glycoprotein I Testing", 2012[22]

Antiphospholipid syndrome (APS) is an autoimmune disease which is characterized by the formation of various antibodies against phospholipids and a blood hypercoagulability. It may occur as an independent disease, or be associated with a range of rheumatic diseases such as SLE, SjS or RA.[23] At least one clinical and one laboratory diagnostic criteria must be present to establish the final diagnosis. The clinical symptoms of APS are usually venous or arterial thrombosis and spontaneous abortion. A laboratory diagnostic classification criterion is regarded as met if two consecutive tests are positive conducted at least 12 weeks apart for lupus anticoagulant (LA) or antiphospholipid antibodies (aPL) against cardiolipin (IgG/IgM) or β2-Glycoprotein I (IgG/IgM).[21] The EULAR 2019 guidelines recommend testing all SLE patients for APS as a frequent comorbidity.[3]

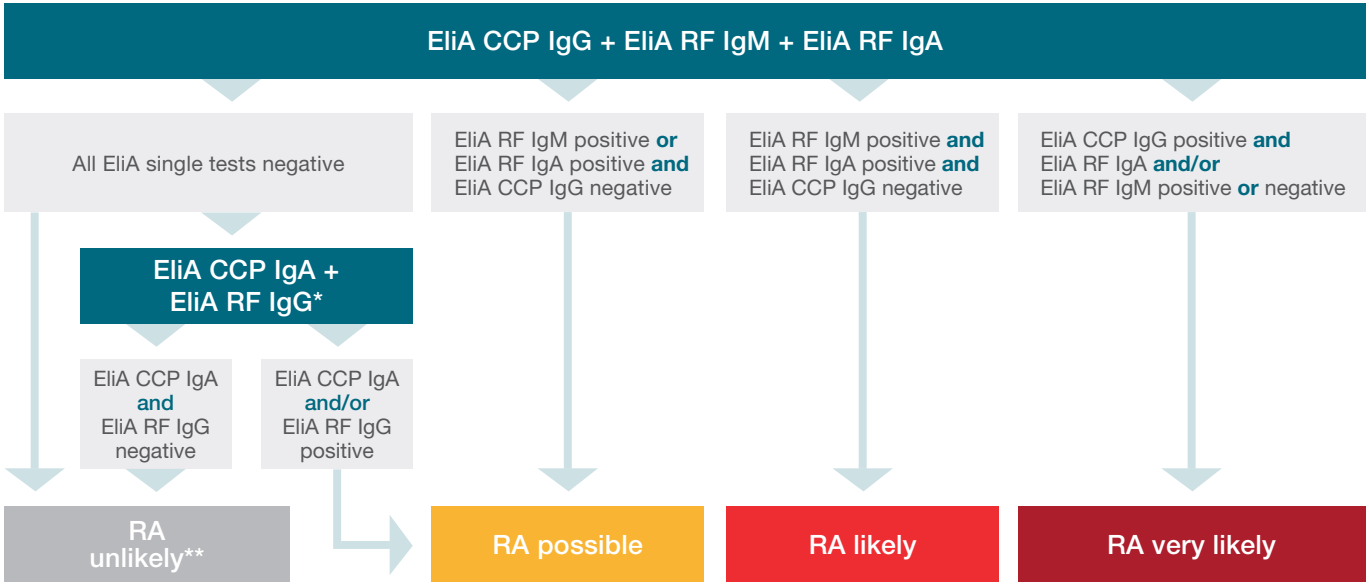
Risk stratification

aPLs play a pathogenic role in APS patients and may be seen as a risk factor for thrombosis or fetal loss. The detection of all three aPLs in high concentrations at the same time is highly relevant for predicting these clinical events.[24] A study with a total of 161 patients was able to show that the occurrence of clinical criteria was strongly associated with double or triple positivity.[25]

Percentage of individuals with aPL positivity who fulfill at least one clinical criterion and were classified as APS patients



Rheumatoid arthritis



* in case of clinical suspicion ** consider possible seronegativity

Selected relevant guidelines and references for this test algorithm:

- "2010 ACR/EULAR classification criteria for rheumatoid arthritis", Aletaha et al. 2010[26]
- "Relationship between rheumatoid factor isotypes and IgG anti-cyclic citrullinated peptide antibodies", Jaskowski et al. 2010[27]
- "Determination of Autoantibody Isotypes Increases the Sensitivity of Serodiagnostics in Rheumatoid Arthritis", Sieghart et al. 2018[28]

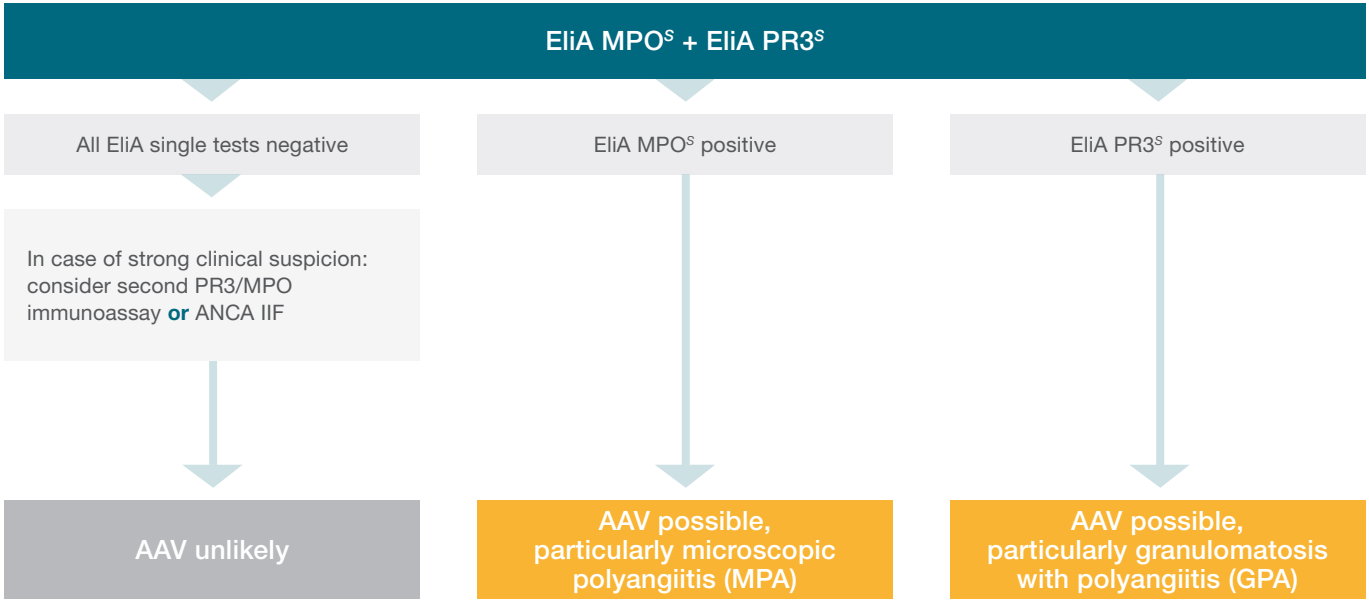
Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by a chronic inflammation of the joints, which often leads to irreversible joint deformities and progressive physical disability in the later stages of the disease.[26] Early diagnosis is vital for better treatment results.[29,30] In addition to clinical history and physical exami-na-tion, imaging and laboratory tests offer additional specificity for diagnosis.[31] The guidelines recommend primary tests for rheumatoid factor (RF) IgM and anti-CCP antibodies (ACPA).[26] CCP antibodies are formed in the early stages of the disease.[32] Rheumatoid factor IgM is the most common RF isotype in RA, and can be detected in 60–80% of all RA patients.[33,34] Positivity for more than one RF isotype is associated with an increased risk of RA.[27,35,36]

Method-dependent differentiation and measurement of individual RF isotypes

RF is often detected using nephelometry or turbidimetry, but no distinction is made between the different RF isotypes. However, differentiation – particularly between RF IgA and RF IgM – may provide important additional information.[28,37–39] A high RF IgM titer, for example, correlates with disease activity and extra-articular manifestations.[31,40–42] A high RF IgA titer is a prognostic marker for a severe progression of the disease and an insufficient response to TNF-α inhibitors.[35,37,42]

Method	Signal	Differentiation of RF isotypes
EliA RF IgM/IgA/IgG tests	fluorescence	+
Nephelometry	stray light	–
Turbidimetry	transmission	–

ANCA-associated vasculitides



Selected relevant guideline for this test algorithm:

- "Revised 2017 international consensus on testing of ANCAs in GPA and MPA", 2017[43]

The group of antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitides (AAVs) comprises microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA) and eosinophilic granulomatosis with polyangiitis (EGPA). All of these diseases show features of vasculitis of the small vessels, but otherwise affect various different target organs with varying ANCA positivity. During the active stage of the disease, ANCAs against myeloperoxidase (MPO) and/or proteinase 3 (PR3) can be detected in almost all MPA and GPA patients. Among patients with EGPA, less than 40% have ANCAs; the presence of ANCAs is associated with the typical manifestations of small vessel vasculitis, such as glomerulonephritis. Taking the concentrations of antibodies into account improves clinical interpretation.[43,44]

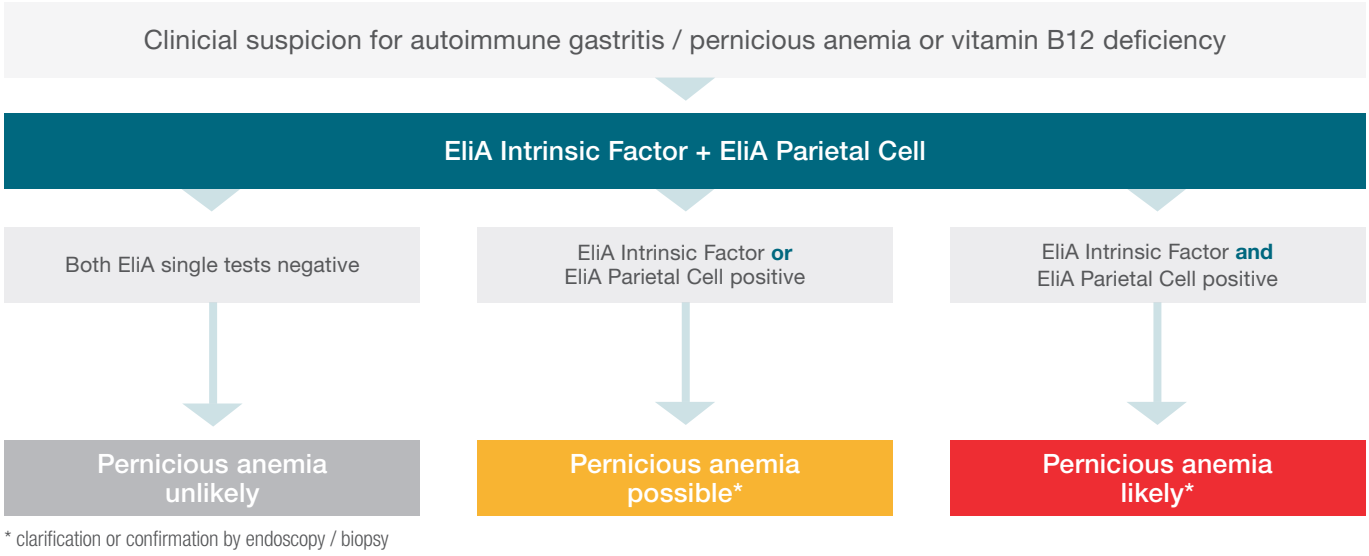
Gating strategy

The 2017 international consensus on ANCA-diagnostics for AAV recommends that in the case of suspected GPA or MPA, anti-MPO and anti-PR3 antibodies should be determined using highly specific immunoassays.[43] This process replaces the previous primary screening using IIF.

Adherence to a strict gating strategy based on the clinical manifestations presented is critical here.[45]

Clinical manifestations for a gating strategy
Chronic-destructive disease of the upper respiratory system
Pulmonary nodules
Nephritis or lung diseases
Rapidly progressive glomerulonephritis
Cutaneous vasculitis with systemic disease
Mononeuritis multiplex
Subglottic tracheal stenosis
Retro-orbital tumor

Autoimmune gastritis / pernicious anemia



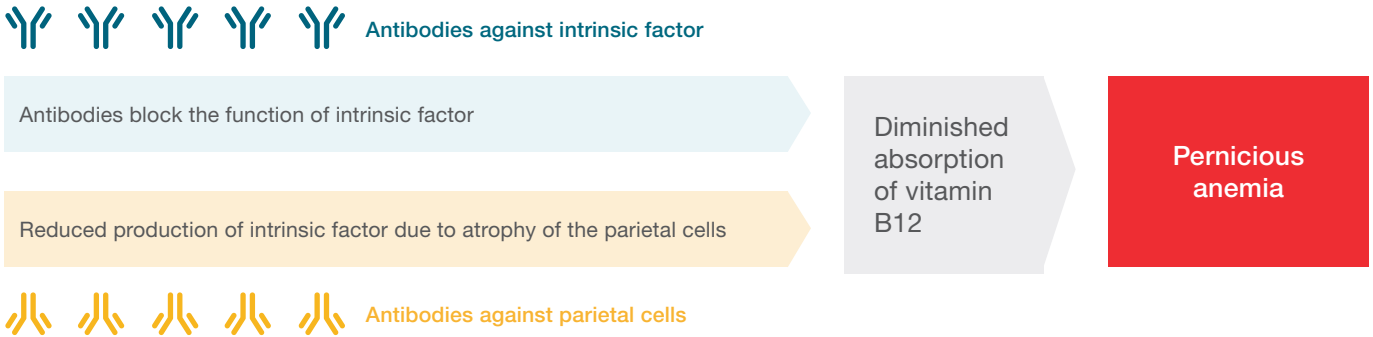
* clarification or confirmation by endoscopy / biopsy

Selected references for this test algorithm:

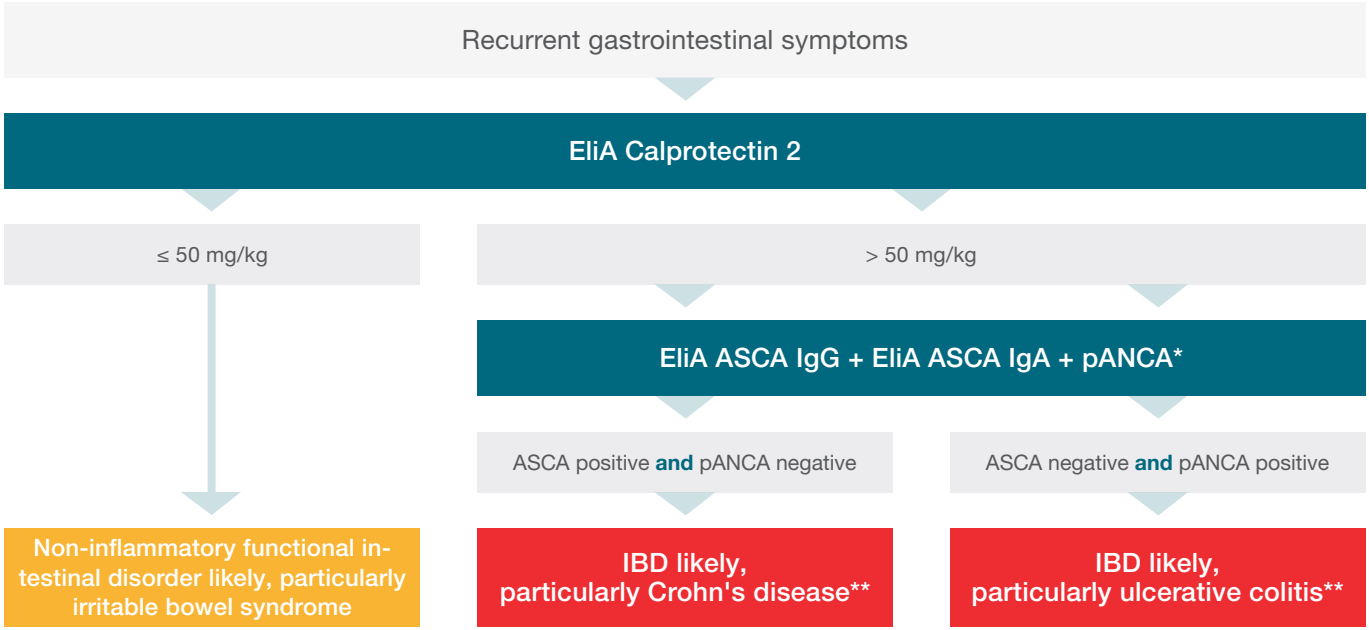
- "Autoimmune Gastritis and Pernicious Anemia", Toh, 2019[46]
- "Guidelines for the diagnosis and treatment of cobalamin and folate disorders", Devalia et al. 2014[47]

Pernicious anemia (PA) is the late stage of autoimmune metaplastic atrophic gastritis (AMAG) and the cause of vitamin B12 deficiency in 20–50% of all adult cases.[48] The prevalence of PA in the overall population is 0.1%, rising to 1.9% above the age of 60.[49] Diagnosing PA properly is critical because these patients require life-long vitamin B12 substitution.[47,50] Anti-intrinsic factor autoantibodies are highly specific for PA while parietal antibodies are highly sensitive.[47,49,51] This is why the combination of these two tests can help achieve an early diagnosis.

Development of pernicious anemia



Inflammatory bowel disease



* pANCA: antibodies against granulocytes with unknown target antigen ** clarification or confirmation by colonoscopy

Selected relevant guidelines for this test algorithm:

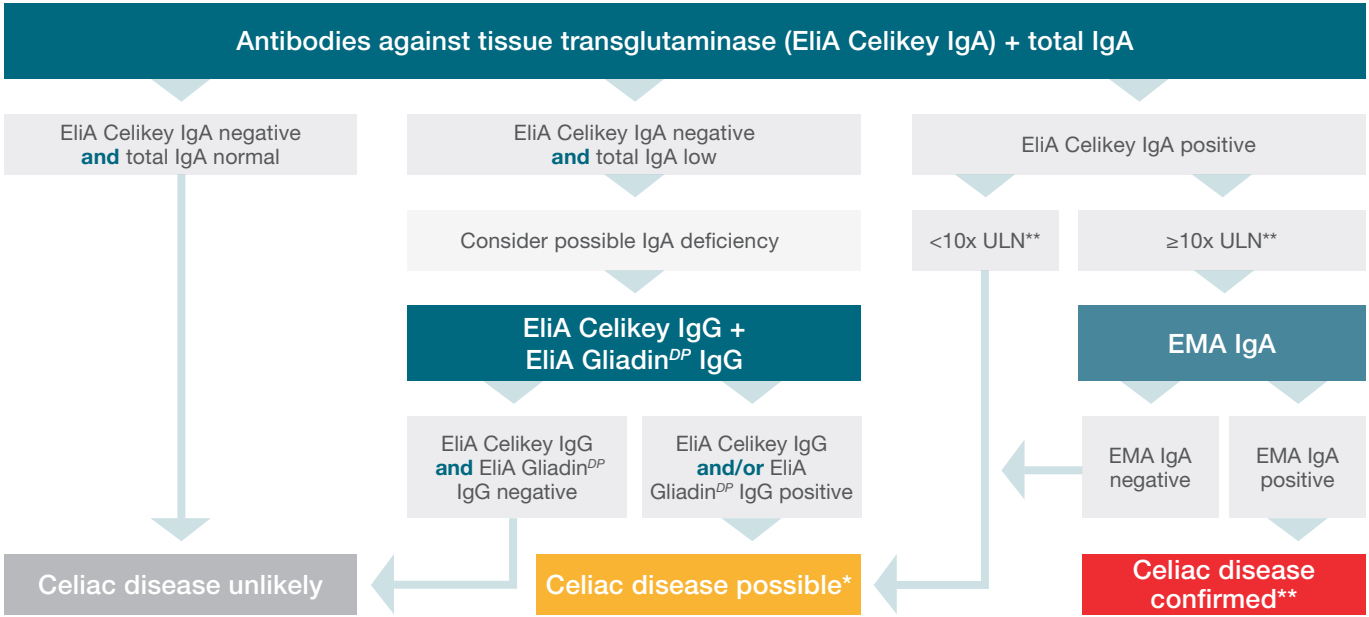
- "ECCO-ESGAR Guideline for Diagnostic Assessment in IBD Part 1: Initial diagnosis, monitoring of known IBD, detection of complications", Maaser et al. 2019[52]
- "World Gastroenterology Organisation Global Guidelines Inflammatory Bowel Disease: Update August 2015", Bernstein et al. 2016[53]

Inflammatory bowel diseases (IBD) constitute a group of chronic inflammatory diseases of the gastrointestinal tract, including, mainly Crohn's disease and ulcerative colitis.[54] Most cases of non-inflammatory functional bowel disease are irritable bowel syndrome (IBS) – a chronic, recurrent and frequently life-long disease with unclear causes.[55] Fecal calprotectin is particularly important as an exclusion marker in a differential diagnosis. As it is a very sensitive marker for inflammatory bowel diseases, a negative test result will exclude an IBD with very high probability. Testing for calprotectin can thus contribute to avoiding or reducing the number of unnecessary invasive follow-up examinations such as colonoscopies, which are frequently associated with complications for the patient.[56,57]

Meaningful follow-up measurements for IBD patients based on fecal calprotectin concentration[58–62]

Concentration of fecal calprotectin…	Ulcerative colitis	Crohn's disease
…and correlation with clinical activity	high	low
…and correlation with endoscopic and histological results (e.g. mucosal healing)	high	
…to predict relapses	possible	

Celiac disease



Selected relevant guidelines and references for this test algorithm:

- "Guidelines for Diagnosing Coeliac Disease" from the European Society Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), Husby et al. 2020[63]
- "European Society for the Study of Coeliac Disease (ESsCD) guideline for coeliac disease and other gluten-related disorders", Al-Toma et al. 2019[64]
- "Accuracy of a no-biopsy approach for the diagnosis of coeliac disease across different adult cohorts", Penny et al. 2020[65]

Celiac disease mainly affects the gastrointestinal tract and is characterized by chronic inflammation of the mucous membrane. This can lead to villous atrophy and malabsorption. With a prevalence of approximately 1%, celiac disease is an under-diagnosed and under-treated disease which is no longer considered the typical presentation of malnutrition in childhood. It can occur at any age as one of several unclear symptoms or as a comorbidity.[64,66,67]

Important

A diet containing gluten is mandatory to receive a meaningful test result for autoantibodies against tissue transglutaminase (tTG). In case a gluten-free diet is pursued at the time of sampling, a gluten intake for several weeks before sampling needs to be followed.

Risk groups

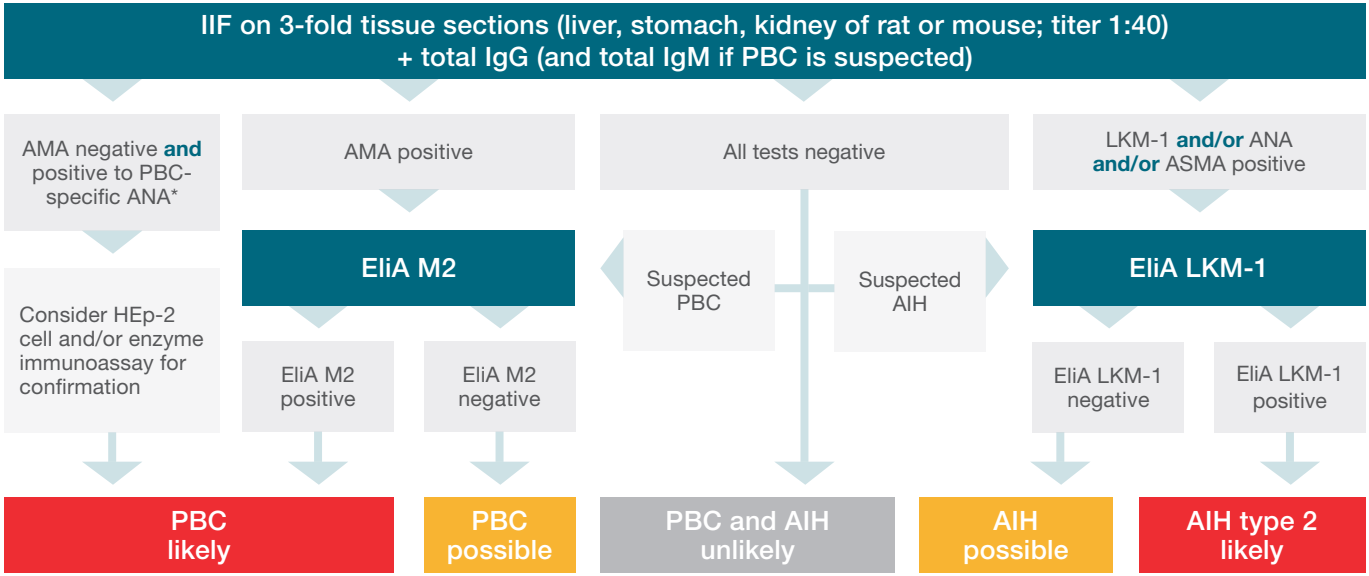
- 1st degree relatives of celiac patients
- Type 1 diabetes mellitus
- Autoimmune thyroid diseases
- Connective tissue diseases
- Trisomy 21 (Down syndrome)
- Irritable bowel syndrome

Consider testing in case of

- Anemia
- Chronic fatigue syndrome
- Dyspepsia
- Flatulence
- Weight loss
- Migraine
- Stomatitis
- Transaminase increase
- Change of bowel habits

* investigation and conformation by specialist: ≥ 4 biopsies from distal duodenum and ≥ 1 biopsy from bulb ** According to the ESPGHAN guideline from 2020, biopsy can be avoided in children and adolescents if the tTG IgA level is more than 10 x ULN (upper limit of normal) and a test for endomysium IgA antibodies using a different blood sample from the same patient is positive.[63] In a study from Werkstetter et al. (2017) EliA Celikey IgA test achieved a positive predictive value (PPV) of 99% with as little as 2 x ULN (20 EliA U/ml).[68]

Primary biliary cholangitis and autoimmune hepatitis



* PBC-specific ANAs can be directed against the nuclear membrane (AC-12, target antigen: gp210) or against nuclear proteins (AC-6, target antigen: sp100). The specific ANA-IIF patterns can be masked by high-titre AMA-IIF patterns.

Selected relevant guideline and references for this test algorithm:

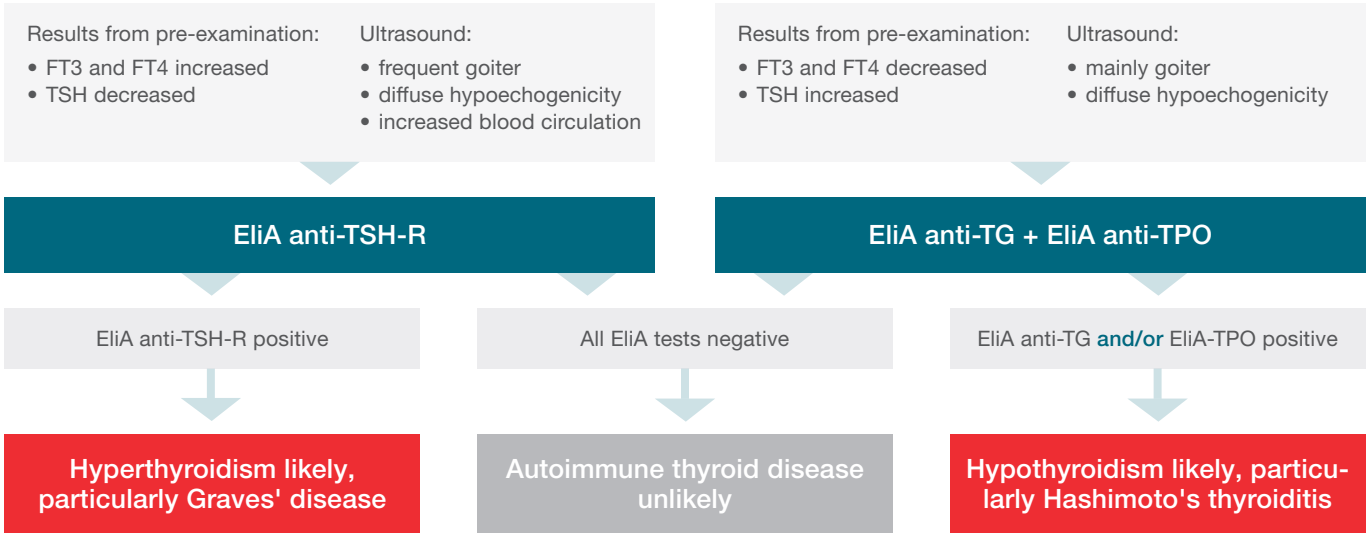
- "EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis", European Association for the Study of the Liver, 2017 [69]
- "EASL Clinical Practice Guidelines: Autoimmune hepatitis", European Association for the Study of the Liver, 2015 [70]
- "Diagnosis and Management of Autoimmune Hepatitis: Current Status and Future Directions", Czaja, 2016 [71]

Autoimmune liver diseases include autoimmune hepatitis (AIH), primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC). The etiology of these diseases is still unknown. Early diagnosis and personalized, often life-long therapy can prevent irreversible damage and possible secondary diseases.[69,72] The criteria for the diagnosis of AIH and PBC include the determination of specific and particularly important autoantibodies. Positive detection of AMA or PBC-specific ANA is one of two required diagnostic criteria for PBC.[69] AIH-specific antibodies can contribute 2 points towards a diagnosis (AIH is probable at 10 points or higher) in the AIH diagnostic score proposed by Hennes et al.[73]

Parameter / test	AIH type 1	AIH type 2	PBC	PSC	Other
EliA M2			+		
EliA LKM-1		+			HCV
EliA CENP			+		Scleroderma (CREST syndrome), Raynaud's syndrome
ASMA / anti-actin	+				
SLA/LP	+				
LC-1		+			HCV
Sp100, gp210 (ANA specific for PBC)			+		
ANA	+		+	+	HBV, HCV, NAFLD, drug induced toxic hepatitis

HBV: chronic hepatitis B; HCV: chronic hepatitis C; NAFLD: non alcoholic fatty liver disease

Hashimoto's thyroiditis and Graves' disease



Selected relevant guidelines for this test algorithm:

- "European Thyroid Association Guideline for the Management of Graves' Hyperthyroidism", Kahaly et al. 2018[74]
- "European Thyroid Association Guidelines on the Diagnosis and Management of Central Hypothyroidism", Persani et al. 2018[75]

Autoimmune thyroid diseases include various diseases leading to disturbance of the thyroid function due to pathological immune responses. The most clinically frequent autoimmune thyroid diseases are Hashimoto's thyroiditis and Graves' disease.[76] Autoimmune thyroid diseases are among the most frequent autoimmune diseases, with a prevalence of up to 4.6% among women and 2.8% among men.[76,77] Despite the potentially severe consequences of undiagnosed autoimmune thyroid diseases, such as cardiovascular diseases, osteoporosis or infertility, many patients are unaware of their disease.[78]

Differential diagnosis

Patients suffering from an autoimmune thyroid disease might only have one positive antibody test result. Anti-TSH-R has the highest prevalence in untreated Graves' disease whereas anti-TPO is frequently found in Hashimoto's thyroiditis.[79,80] 6% of patients with Hashimoto's thyroiditis have isolated anti-TG positivity.[81]

Anti-TG antibodies can have a negative effect on measurement of TG protein levels for thyroid carcinoma diagnosis.[82]

Auto-antibodies	Graves' disease (untreated)	Hashimoto's thyroiditis	Normal population
Anti-TSH-R	+++	+	±
Anti-TPO	++	+++	+
Anti-TG	+	++	±

Prevalence:[79,80]
± negative to very low; + low; ++ moderate; +++ high

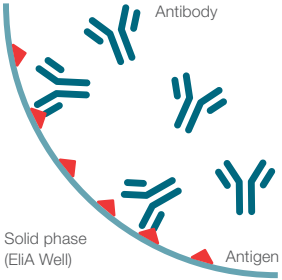
EliA testing principles

The EliA test is a fluorescence enzyme immunoassay (FEIA) based on an indirect enzyme-linked immunosorbent assay (ELISA).

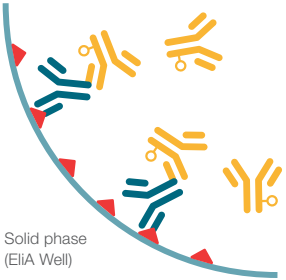
EliA wells are coated with one or several target antigens, which specific autoantibodies recognize and bind to. Such autoantibodies are usually specific markers for certain autoimmune diseases. Different coupling and coating processes are used for each of the specific tests in order to ensure accurate presentation of the relevant epitopes.

If the patient's sample contains the relevant autoantibodies, these will bind to the corresponding target antigen in the EliA well. Following the first washing step, in which non-bound antibodies are removed, enzyme-conjugated secondary antibodies specifically bind to the Fc region of an IgA, IgG or IgM antibody. After a second washing step, in which excess secondary antibodies are removed, a reagent is added to the antigen-antibody complex. This reagent is converted to a fluorescent substrate through an enzymatic reaction. After a set incubation time, the enzymatic reaction is aborted using a stop solution, and the fluorescence is measured with a fluorescence detector in the EliA well.

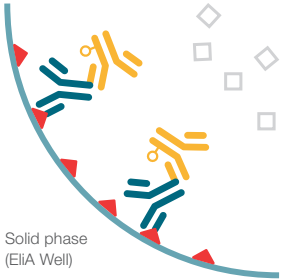
The concentration of antibodies in the patient sample is determined using the previously prepared, standardized calibration curve. This produces a quantitative result and a classification as negative, equivocal or positive.



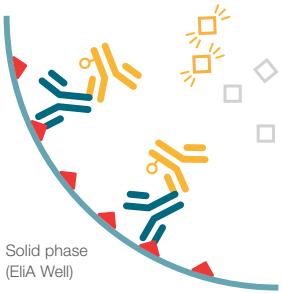
❶ Disease-specific antibodies from the patient sample bind to the target antigens in the EliA well's coating. The specific coupling and coating process ensures that the relevant target antigen epitopes are presented.



❷ Once non-bound and non-specific antibodies have been washed away, enzyme-conjugated antibodies specifically bind to IgA, IgG or IgM antibodies, respectively.

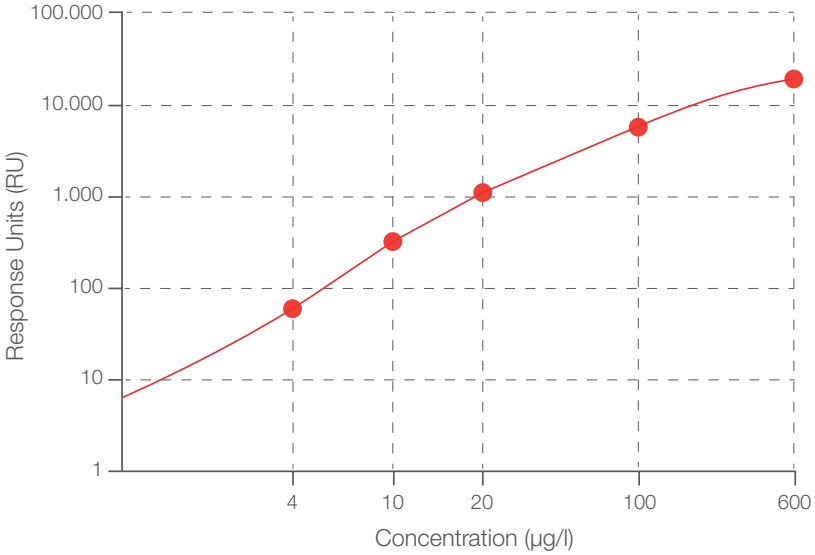


❸ After a second washing step, the bound, enzyme-conjugated antibodies convert the added reagent into a fluorescent substance which is easy to detect.



❹ Adding a stop solution inhibits the enzymatic reaction so that the fluorescence can be determined in the EliA well. The fluorescence measured correlates with the concentration of specific antibodies in the patient sample within a defined measuring range.

EliA calibration method and Phadia Laboratory Systems



Benefits at a glance:

- An immunoglobulin-specific calibration curve has been created and saved in the system
- The immunoglobulin curve is based on the WHO standard
- Only 5 different calibrations are necessary for more than 50 EliA tests
- Quantitative results without test-specific calibration curve
- Isotype-specific measurement
- Fully automated laboratory systems with minimum hands-on time
- Seamless traceability of all reagent lots

The fully automated **Phadia™ Laboratory Systems** have been specially developed for diagnostic tests which can help diagnose allergy and autoimmune diseases. They can be connected to state-of-the-art laboratory information systems (LIS) and laboratory automation systems (LAS) and enable the highly efficient use of laboratory resources with minimal manual steps. They offer a high degree of flexibility through personalized reflex testing, random access functions and the ability to efficiently process short series. The **Phadia™ LabCommunity** offers a direct link and communication with service engineers and application specialists for a quick and efficient service.

Phadia™ 200 instrument

A footprint of less than 0.5 m^2 makes it particularly suitable for laboratories with space constraints.

Phadia™ 250 instrument

More than 2,000 systems are in use for routine processes around the world and are valued for their great reliability and reproducible results.

Phadia™ 2500 series

Laboratories with a very high number of patient samples benefit from the high sample throughput thanks to two independent process lines.

List of products and antigens

Product	Art.-No.	Antigen	Cut-Off			Short-name
			Negative	Equivocal	Positive	
Connective tissue diseases						
EliA CTD Screen Well	14-5596-01	human recombinant U1RNP (RNP70, A, C), SS-A/Ro (60 kDa, 52 kDa), SS-B/La, Centromere B, Scl-70, Jo-1, fibrillarin, RNA Pol III, Rib-P, PM-Scl, PCNA, Mi-2 proteins, Sm proteins and native purified DNA	< 0.7 Ratio	0.7-1.0 Ratio	> 1.0 Ratio	ctd
EliA Symphony Well**	14-5508-01	human recombinant U1RNP (RNP70, A, C), SS-A/Ro (60 kDa, 52 kDa), SS-B/La, Centromere B, Scl-70 and Jo-1 proteins, native purified Sm proteins	< 0.7 Ratio	0.7-1.0 Ratio	> 1.0 Ratio	sy
EliA Symphony^{S} Well	14-5671-01	human recombinant U1RNP (RNP70, A, C), SS-A/Ro (60 kDa, 52 kDa), SS-B/La, Centromere B, Scl-70 and Jo-1 proteins, synthetic SmD_3 peptide	< 0.7 Ratio	0.7-1.0 Ratio	> 1.0 Ratio	sys
EliA dsDNA Well	14-5500-01	double-stranded plasmid DNA	< 10 IU/ml	10-15 IU/ml	> 15 IU/ml	dn
EliA ssDNA Well	14-5629-01	synthetic ssDNA	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	sdn
EliA U1RNP Well	14-5501-01	human recombinant U1RNP (RNP70, A, C) proteins	< 5 EliA U/ml	5-10 EliA U/ml	> 10 EliA U/ml	rn
EliA RNP70 Well	14-5511-01	human recombinant RNP70 protein	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	70
EliA SmD^{P} Well	14-5624-01	synthetic SmD_3 peptide	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	smd
EliA SmD^{P}-S Well	14-5672-01	synthetic SmD_3 peptide	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	sms
EliA Ro Well	14-5503-01	human recombinant SS-A/Ro (60 kDa, 52 kDa) proteins	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	ro
EliA Ro52 Well	14-5598-01	human recombinant SS-A/Ro (52 kDa) protein	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	ro52
EliA Ro60 Well	14-5525-01	human recombinant SS-A/Ro (60 kDa) protein	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	ro60
EliA La Well	14-5504-01	human recombinant SS-B/La protein	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	la
EliA CENP Well	14-5505-01	human recombinant centromere protein B	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	ce
EliA Scl-70^{S} Well	14-5637-01	human recombinant Scl-70 protein	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	scs
EliA RNA Pol III Well*	14-5599-01	human recombinant RNA polymerase III protein	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	pol3
EliA Jo-1 Well	14-5507-01	human recombinant Jo-1 protein	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	jo

EliA Fibrillarin Well	14-5605-01	human recombinant fibrillarin protein	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	fib
EliA Rib-P Well	14-5521-01	human recombinant Rib-P proteins (P0, P1, P2)	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	rp
EliA PM-Scl Well	14-5602-01	human recombinant PM-Scl protein	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	pmsc
EliA PCNA Well	14-5603-01	human recombinant PCNA protein	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	pcna
EliA Mi-2 Well	14-5604-01	human recombinant Mi-2 protein	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	mi
EliA DFS70 Well*	14-5673-01	human recombinant DFS70 protein	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	dfs
Rheumatoid arthritis						
EliA CCP Well	14-5515-01	citrullinated synthetic peptides, second generation antigen	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	cp
EliA CCP IgA Well	14-5615-01	citrullinated synthetic peptides, second generation antigen	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	Acp
EliA RF IgM Well	14-5600-01	aggregated rabbit IgG	< 3.5 IU/ml	3.5-5.0 IU/ml	> 5.0 IU/ml	Mrf
EliA RF IgA Well	14-5601-01	aggregated rabbit IgG	< 14 IU/ml	14-20 IU/ml	> 20 IU/ml	Arf
EliA RF IgG Well	14-5617-01	rabbit IgG	< 28 IU/ml	28-40 IU/ml	> 40 IU/ml	Grf
Vasculitis and Goodpasture syndrome						
EliA PR3^{S} Well	14-5536-01	human purified proteinase 3	< 2.0 IU/ml	2.0-3.0 IU/ml	> 3.0 IU/ml	prs
EliA MPO^{S} Well	14-5537-01	human purified myeloperoxidase	< 3.5 IU/ml	3.5-5.0 IU/ml	> 5.0 IU/ml	mps
EliA GBM Well	14-5514-01	human recombinant α3 chain of collagen IV	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	gb
Antiphospholipid syndrome						
EliA Cardiolipin IgG Well	14-5529-01	bovine cardiolipin and bovine β2-glycoprotein I as co-factor	< 10 GPL-U/ml	10-40 GPL-U/ml***	>40 GPL-U/ml	Gcl
EliA Cardiolipin IgM Well	14-5530-01	bovine cardiolipin and bovine β2-glycoprotein I as co-factor	< 10 MPL-U/ml	10-40 MPL-U/ml***	>40 MPL-U/ml	Mcl
EliA Cardiolipin IgA Well	14-5528-01	bovine cardiolipin and bovine β2-glycoprotein I as co-factor	< 14 APL-U/ml	14-20 APL-U/ml	>20 APL-U/ml	Acl
EliA β2-Glycoprotein I IgG Well	14-5532-01	human purified β2-glycoprotein I antigen	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	Gb2
EliA β2-Glycoprotein I IgM Well	14-5533-01	human purified β2-glycoprotein I antigen	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	Mb2
EliA β2-Glycoprotein I IgA Well	14-5531-01	human purified β2-glycoprotein I antigen	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	Ab2

* not available on Phadia 100 instrument ** not available on Phadia 200 instrument *** Cut-off weak positive instead of equivocal

List of products and antigens

Product	Art.-No.	Antigen	Cut-Off			Shortname
			Negative	Equivocal	Positive	
Celiac disease						
EliA Celikey IgA Well	14-5517-01	human recombinant tissue transglutaminase	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	Acy
EliA Celikey IgG Well	14-5518-01	human recombinant tissue transglutaminase	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	Gcy
EliA Gliadin^{DP} IgA Well	14-5538-01	synthetic deamidated gliadin peptides	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	Agp
EliA Gliadin^{DP} IgG Well	14-5539-01	synthetic deamidated gliadin peptides	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	Ggp
Inflammatory bowel disease						
EliA Calprotectin Well**	14-5610-01	monoclonal antibodies to calprotectin	≤ 50 mg/kg	-	> 50 mg/kg	cn
EliA Calprotectin 2 Well*	14-6748-01	monoclonal antibodies to calprotectin	≤ 50 mg/kg	-	> 50 mg/kg	cn2
EliA ASCA IgG Well	14-5635-01	mannan of *S. cerevisiae*	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	Gsc
EliA ASCA IgA Well	14-5633-01	mannan of *S. cerevisiae*	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	Asc
Pernicious anemia						
EliA Intrinsic Factor Well*	14-5668-01	human gastric intrinsic factor	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	inf
EliA Parietal Cell Well*	14-5669-01	H+/K+ ATPase of gastric cells	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	par
Autoimmune thyroid disease						
EliA anti-TG Well*	14-5642-01	human thyroglobulin antigen	< 40 IU/ml	40-60 IU/ml	> 60 IU/ml	thg
EliA anti-TPO Well*	14-5641-01	human recombinant thyroid peroxidase	< 25 IU/ml	25-35 IU/ml	> 35 IU/ml	tpo
EliA anti-TSH-R Well*	14-5639-01	human recombinant TSH receptor antigen	< 2.9 IU/l	2.9-3.3 IU/l	> 3.3 IU/l	tsr

Autoimmune liver diseases						
EliA LKM-1 Well*	14-6648-01	human recombinant LKM-1 protein	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	lkm
EliA M2 Well	14-5649-01	native pyruvate dehydrogenase complex from mitochondria and human recombinant M2 protein	< 4 IU/ml	4-6 IU/ml	> 6 IU/ml	m2G
Immunodeficiency						
EliA Anti-IgA Well	14-5535-01	purified human IgA	< 7 EliA U/ml	7-10 EliA U/ml	> 10 EliA U/ml	aiga

* not available on Phadia 100 instrument ** not available on Phadia 200 instrument

The antigens and EliA tests are developed at the Phadia GmbH Biotechnikum in Freiburg, Germany, and produced under GMP conditions. Careful documentation of all process steps and various quality controls ensure that our EliA tests meet the rigorous criteria connected with CE and FDA approvals.

High lot consistency, which is particularly important in routine laboratories, is ensured by the highly automated production process. Phadia GmbH is certified according to ISO 13485 for the “Design and development and manufacturing of in vitro diagnostics for autoimmunity”.

	Production of recombinant antigens using insect cells (Sf9) for ultimate purity and for complex epitopes, which may require post-translational modifications.
	Production of recombinant antigens using bacteria (*E. coli*) for high reproducibility and large output volumes.
	Production of synthetic antigens using chemical peptide production for ultimate purity and reproducibility.
	Production of native antigens using human or animal materials such as blood plasma for complex epitopes.

References

1. Gaubitz M. Epidemiology of connective tissue disorders. Rheumatology (Oxford). 2006;45 Suppl 3(3):iii3-4.
2. Damoiseaux J, Olschowka N, Shoenfeld Y. EASI - European Autoimmunity Standardisation Initiative: facing the challenges of diagnostics in autoimmunity. Clin Chem Lab Med. 2018;56(10):1620-3.
3. Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, et al. 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. Ann Rheum Dis. 2019;78(9):1151-9.
4. Bizzaro N, Brusca I, Previtali G, Alessio MG, Daves M, Platzgummer S, et al. The association of solid-phase assays to immunofluorescence increases the diagnostic accuracy for ANA screening in patients with autoimmune rheumatic diseases. Autoimmun Rev. 2018;17(6):541-7.
5. Otten HG, Brummelhuis WJ, Fritsch-Stork R, Leavis HL, Wisse BW, van Laar JM, et al. Measurement of antinuclear antibodies and their fine specificities: time for a change in strategy? Clinical and Experimental Rheumatology. 2017;35(3):462-70.
6. Robier C, Amouzadeh-Ghadikolai O. The clinical significance of borderline results of the Elia CTD Screen assay. Clin Chem Lab Med. 2018;56(12):2088-92.
7. Robier C, Amouzadeh-Ghadikolai O, Stettin M, Reicht G. Comparison of the clinical utility of the Elia CTD Screen to indirect immunofluorescence on Hep-2 cells. Clin Chem Lab Med. 2016;54(8):1365-70.
8. van der Pol P, Bakker-Jonges LE, Kuijpers J, Schreurs MWJ. Analytical and clinical comparison of two fully automated immunoassay systems for the detection of autoantibodies to extractable nuclear antigens. Clin Chim Acta. 2018;476:154-9.
9. Willems P, De Langhe E, Claessens J, Westhovens R, Van Hoeyveld E, Poesen K, et al. Screening for connective tissue disease-associated antibodies by automated immunoassay. Clin Chem Lab Med. 2018;56(6):909-18.
10. Orme ME, Andalucia C, Sjolander S, Bossuyt X. A comparison of a fluorescence enzyme immunoassay versus indirect immunofluorescence for initial screening of connective tissue diseases: Systematic literature review and meta-analysis of diagnostic test accuracy studies. Best Pract Res Clin Rheumatol. 2018;32(4):521-34.
11. Op De Beeck K, Vermeersch P, Verschueren P, Westhovens R, Marien G, Blockmans D, et al. Detection of antinuclear antibodies by indirect immunofluorescence and by solid phase assay. Autoimmun Rev. 2011;10(12):801-8.
12. Tan EM, Feltkamp TE, Smolen JS, Butcher B, Dawkins R, Fritzler MJ, et al. Range of antinuclear antibodies in "healthy" individuals. Arthritis Rheum. 1997;40(9):1601-11.
13. Fritzler MJ. The antinuclear antibody test: last or lasting gasp? Arthritis Rheum. 2011;63(1):19-22.
14. Aringer M, Brinks R, Dorner T, Daikh D, Mosca M, Ramsey-Goldman R, et al. European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) SLE classification criteria item performance. Ann Rheum Dis. 2021.
15. Choi MY, Clarke AE, St Pierre Y, Hanly JG, Urowitz MB, Romero-Diaz J, et al. Antinuclear Antibody-Negative Systemic Lupus Erythematosus in an International Inception Cohort. Arthritis Care Res (Hoboken). 2019;71(7):893-902.
16. Bossuyt X, Luyckx A. Antibodies to extractable nuclear antigens in antinuclear antibody-negative samples. Clin Chem. 2005;51(12):2426-7.
17. Pourmand N, Blomberg S, Ronnblom L, Karlsson-Parra A, Pettersson I, Wahren-Herlenius M. Ro 52kD autoantibodies are detected in a subset of ANA-negative sera. Scand J Rheumatol. 2000;29(2):116-23.
18. Shiboski CH, Shiboski SC, Seror R, Criswell LA, Labetoulle M, Lietman TM, et al. 2016 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Primary Sjogren's Syndrome: A Consensus and Data-Driven Methodology Involving Three International Patient Cohorts. Arthritis Rheumatol. 2017;69(1):35-45.
19. Chan EK, Damoiseaux J, Carballo OG, Conrad K, de Melo Cruvinel W, Francescantonio PL, et al. Report of the First International Consensus on Standardized Nomenclature of Antinuclear Antibody HEp-2 Cell Patterns 2014-2015. Front Immunol. 2015;6:412.
20. Conrad K, Schößler W, Hiepe F, Fritzler MJ. Autoantibodies in Systemic Autoimmune Diseases – A Diagnostic Reference. 3rd ed: Pabst Science Publishers; 2015.
21. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost. 2006;4(2):295-306.

22. Lakos G, Favaloro EJ, Harris EN, Meroni PL, Tincani A, Wong RC, et al. International consensus guidelines on anticardiolipin and anti-beta2-glycoprotein I testing: report from the 13th International Congress on Antiphospholipid Antibodies. Arthritis Rheum. 2012;64(1):1-10.
23. Saigal R, Kansal A, Mittal M, Singh Y, Ram H. Antiphospholipid antibody syndrome. J Assoc Physicians India. 2010;58:176-84.
24. Sciascia S, Cosseddu D, Montaruli B, Kuzenko A, Bertero MT. Risk Scale for the diagnosis of antiphospholipid syndrome. Ann Rheum Dis. 2011;70(8):1517-8.
25. Pengo V, Ruffatti A, Del Ross T, Tonello M, Cuffaro S, Hoxha A, et al. Confirmation of initial antiphospholipid antibody positivity depends on the antiphospholipid antibody profile. J Thromb Haemost. 2013;11(8):1527-31.
26. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis. 2010;69(9):1580-8.
27. Jaskowski TD, Hill HR, Russo KL, Lakos G, Szekanecz Z, Teodorescu M. Relationship between rheumatoid factor isotypes and IgG anti-cyclic citrullinated peptide antibodies. J Rheumatol. 2010;37(8):1582-8.
28. Sieghart D, Platzer A, Studenic P, Alasti F, Grundhuber M, Swiniarski S, et al. Determination of Autoantibody Isotypes Increases the Sensitivity of Serodiagnostics in Rheumatoid Arthritis. Front Immunol. 2018;9:876.
29. Smolen JS, Landewe R, Bijlsma J, Burmester G, Chatzidionysiou K, Dougados M, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. Ann Rheum Dis. 2017;76(6):960-77.
30. Suresh E. Diagnosis of early rheumatoid arthritis: what the non-specialist needs to know. Journal of the Royal Society of Medicine. 2004;97(9):421-4.
31. Littlejohn EA, Monrad SU. Early Diagnosis and Treatment of Rheumatoid Arthritis. Prim Care. 2018;45(2):237-55.
32. Bizzaro N. Antibodies to citrullinated peptides: a significant step forward in the early diagnosis of rheumatoid arthritis. Clin Chem Lab Med. 2007;45(2):150-7.
33. Berglin E, Padyukov L, Sundin U, Hallmans G, Stenlund H, Van Venrooij WJ, et al. A combination of autoantibodies to cyclic citrullinated peptide (CCP) and HLA-DRB1 locus antigens is strongly associated with future onset of rheumatoid arthritis. Arthritis Res Ther. 2004;6(4):R303-8.
34. Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum. 2000;43(1):155-63.
35. de Brito Rocha S, Baldo DC, Andrade LEC. Clinical and pathophysiologic relevance of autoantibodies in rheumatoid arthritis. Adv Rheumatol. 2019;59(1):2.
36. Jonsson T, Thorsteinsson J, Valdimarsson H. Elevation of only one rheumatoid factor isotype is not associated with increased prevalence of rheumatoid arthritis--a population based study. Scand J Rheumatol. 2000;29(3):190-1.
37. Bas S, Perneger TV, Kunzle E, Vischer TL. Comparative study of different enzyme immunoassays for measurement of IgM and IgA rheumatoid factors. Ann Rheum Dis. 2002;61(6):505-10.
38. Bobbio-Pallavicini F, Caporali R, Alpini C, Avalle S, Epis OM, Klersy C, et al. High IgA rheumatoid factor levels are associated with poor clinical response to tumour necrosis factor alpha inhibitors in rheumatoid arthritis. Ann Rheum Dis. 2007;66(3):302-7.
39. Shakiba Y, Koopah S, Jamshidi AR, Amirzargar AA, Massoud A, Kiani A, et al. Anti-cyclic citrullinated peptide antibody and rheumatoid factor isotypes in Iranian patients with rheumatoid arthritis: evaluation of clinical value and association with disease activity. Iran J Allergy Asthma Immunol. 2014;13(3):147-56.
40. de Angelis V, Meroni PL. Rheumatoid Factors. In: Shoenfeld Y, Gershwin ME, Meroni PL, editors. Autoantibodies (Second Edition): Elsevier; 2007. p. 755-62.
41. Mikuls TR, O'Dell JR, Stoner JA, Parrish LA, Arend WP, Norris JM, et al. Association of rheumatoid arthritis treatment response and disease duration with declines in serum levels of IgM rheumatoid factor and anti-cyclic citrullinated peptide antibody. Arthritis Rheum. 2004;50(12):3776-82.
42. Song YW, Kang EH. Autoantibodies in rheumatoid arthritis: rheumatoid factors and anticitrullinated protein antibodies. QJM. 2010;103(3):139-46.

References

43. Bossuyt X, Cohen Tervaert JW, Arimura Y, Blockmans D, Flores-Suarez LF, Guillevin L, et al. Position paper: Revised 2017 international consensus on testing of ANCAs in granulomatosis with polyangiitis and microscopic polyangiitis. Nat Rev Rheumatol. 2017;13(11):683-92.
44. Csernok E, Moosig F. Current and emerging techniques for ANCA detection in vasculitis. Nat Rev Rheumatol. 2014;10(8):494-501.
45. Savige J, Gillis D, Benson E, Davies D, Esnault V, Falk RJ, et al. International Consensus Statement on Testing and Reporting of Antineutrophil Cytoplasmic Antibodies (ANCA). Am J Clin Pathol. 1999;111(4):507-13.
46. Toh B-H. Autoimmune Gastritis and Pernicious Anemia. In: Rose NR, Mackay IR, editors. The Autoimmune Diseases (Sixth Edition): Academic Press; 2019. p. 833-47.
47. Devalia V, Hamilton MS, Molloy AM, British Committee for Standards in H. Guidelines for the diagnosis and treatment of cobalamin and folate disorders. Br J Haematol. 2014;166(4):496-513.
48. Toh BH, Alderuccio F. Pernicious anaemia. Autoimmunity. 2004;37(4):357-61.
49. Andres E, Serraj K. Optimal management of pernicious anemia. J Blood Med. 2012;3:97-103.
50. Varbanova M, Frauenschlager K, Malfertheiner P. Chronic gastritis - an update. Best Pract Res Clin Gastroenterol. 2014;28(6):1031-42.
51. Rusak E, Chobot A, Krzywicka A, Wenzlau J. Anti-parietal cell antibodies - diagnostic significance. Adv Med Sci. 2016;61(2):175-9.
52. Maaser C, Sturm A, Vavricka SR, Kucharzik T, Fiorino G, Annese V, et al. ECCO-ESGAR Guideline for Diagnostic Assessment in IBD Part 1: Initial diagnosis, monitoring of known IBD, detection of complications. J Crohns Colitis. 2019;13(2):144-64.
53. Bernstein CN, Eliakim A, Fedail S, Fried M, Gearry R, Goh KL, et al. World Gastroenterology Organisation Global Guidelines Inflammatory Bowel Disease: Update August 2015. J Clin Gastroenterol. 2016;50(10):803-18.
54. Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology. 2012;142(1):46-54.
55. Harkness EF, Harrington V, Hinder S, O'Brien SJ, Thompson DG, Beech P, et al. GP perspectives of irritable bowel syndrome--an accepted illness, but management deviates from guidelines: a qualitative study. BMC Fam Pract. 2013;14:92.
56. Tibble JA, Sigthorsson G, Foster R, Forgacs I, Bjarnason I. Use of surrogate markers of inflammation and Rome criteria to distinguish organic from nonorganic intestinal disease. Gastroenterology. 2002;123(2):450-60.
57. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. BMJ. 2010;341:c3369.
58. Chew TS, Mansfield JC. Can faecal calprotectin predict relapse in inflammatory bowel disease: a mini review. Frontline Gastroenterol. 2018;9(1):23-8.
59. Costa F, Mumolo MG, Ceccarelli L, Bellini M, Romano MR, Sterpi C, et al. Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. Gut. 2005;54(3):364-8.
60. Roseth AG, Aadland E, Grzyb K. Normalization of faecal calprotectin: a predictor of mucosal healing in patients with inflammatory bowel disease. Scand J Gastroenterol. 2004;39(10):1017-20.
61. Schoepfer AM, Beglinger C, Straumann A, Trummler M, Renzulli P, Seibold F. Ulcerative colitis: correlation of the Rachmilewitz endoscopic activity index with fecal calprotectin, clinical activity, C-reactive protein, and blood leukocytes. Inflamm Bowel Dis. 2009;15(12):1851-8.
62. Theede K, Holck S, Ibsen P, Kallemose T, Nordgaard-Lassen I, Nielsen AM. Fecal Calprotectin Predicts Relapse and Histological Mucosal Healing in Ulcerative Colitis. Inflamm Bowel Dis. 2016;22(5):1042-8.
63. Husby S, Koletzko S, Korponay-Szabo I, Kurppa K, Mearin ML, Ribes-Koninckx C, et al. European Society Paediatric Gastroenterology, Hepatology and Nutrition Guidelines for Diagnosing Coeliac Disease 2020. J Pediatr Gastroenterol Nutr. 2020;70(1):141-56.
64. Al-Toma A, Volta U, Auricchio R, Castillejo G, Sanders DS, Cellier C, et al. European Society for the Study of Coeliac Disease (ESsCD) guideline for coeliac disease and other gluten-related disorders. United European Gastroenterol J. 2019;7(5):583-613.

65. Penny HA, Raju SA, Lau MS, Marks LJ, Baggus EM, Bai JC, et al. Accuracy of a no-biopsy approach for the diagnosis of coeliac disease across different adult cohorts. Gut. 2020.
66. Choung RS, Khaleghi S, Cartee AK, Marietta EV, Larson JJ, King KS, et al. Community-Based Study of Celiac Disease Autoimmunity Progression in Adults. Gastroenterology. 2020;158(1):151-9 e3.
67. Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA, American College of G. ACG clinical guidelines: diagnosis and management of celiac disease. Am J Gastroenterol. 2013;108(5):656-76; quiz 77.
68. Werkstetter KJ, Korponay-Szabo IR, Popp A, Villanacci V, Salemme M, Heilig G, et al. Accuracy in Diagnosis of Celiac Disease Without Biopsies in Clinical Practice. Gastroenterology. 2017;153(4):924-35.
69. EASL. EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis. J Hepatol. 2017;67(1):145-72.
70. European Association for the Study of the L. EASL Clinical Practice Guidelines: Autoimmune hepatitis. J Hepatol. 2015;63(4):971-1004.
71. Czaja AJ. Diagnosis and Management of Autoimmune Hepatitis: Current Status and Future Directions. Gut Liver. 2016;10(2):177-203.
72. Sebode M, Hartl J, Vergani D, Lohse AW, International Autoimmune Hepatitis G. Autoimmune hepatitis: From current knowledge and clinical practice to future research agenda. Liver Int. 2018;38(1):15-22.
73. Hennes EM, Zeniya M, Czaja AJ, Pares A, Dalekos GN, Krawitt EL, et al. Simplified criteria for the diagnosis of autoimmune hepatitis. Hepatology. 2008;48(1):169-76.
74. Kahaly GJ, Bartalena L, Hegedus L, Leenhardt L, Poppe K, Pearce SH. 2018 European Thyroid Association Guideline for the Management of Graves' Hyperthyroidism. Eur Thyroid J. 2018;7(4):167-86.
75. Persani L, Brabant G, Dattani M, Bonomi M, Feldt-Rasmussen U, Fliers E, et al. 2018 European Thyroid Association (ETA) Guidelines on the Diagnosis and Management of Central Hypothyroidism. Eur Thyroid J. 2018;7(5):225-37.
76. Bliddal S, Nielsen CH, Feldt-Rasmussen U. Recent advances in understanding autoimmune thyroid disease: the tallest tree in the forest of polyautoimmunity. F1000Res. 2017;6:1776.
77. Garmendia Madariaga A, Santos Palacios S, Guillen-Grima F, Galofre JC. The incidence and prevalence of thyroid dysfunction in Europe: a meta-analysis. J Clin Endocrinol Metab. 2014;99(3):923-31.
78. Tomer Y, Huber A. The etiology of autoimmune thyroid disease: a story of genes and environment. J Autoimmun. 2009;32(3-4):231-9.
79. Saravanan P, Dayan CM. Thyroid autoantibodies. Endocrinol Metab Clin North Am. 2001;30(2):315-37, viii.
80. Zophel K, Roggenbuck D, Schott M. Clinical review about TRAb assay's history. Autoimmun Rev. 2010;9(10):695-700.
81. Tozzoli R, Villalta D, Kodermaz G, Bagnasco M, Tonutti E, Bizzaro N. Autoantibody profiling of patients with autoimmune thyroid disease using a new multiplexed immunoassay method. Clin Chem Lab Med. 2006;44(7):837-42.
82. Ringel MD, Nabhan F. Approach to follow-up of the patient with differentiated thyroid cancer and positive anti-thyroglobulin antibodies. J Clin Endocrinol Metab. 2013;98(8):3104-10.

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