EUROIMMUN
a PerkinElmer company

Medizinische
Labordiagnostika
AG

ASLM Webinar

EUROIMMUN - SARS-CoV-2 diagnostics
Reliable serological tools

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Laboratory diagnostics

Direct detection ➤ **Serology**

Methods

Direct detection

Diagnosing acute infections

- CE-marked PCR test for specific detection of SARS-CoV-2
- Quick and simple pathogen detection by means of reverse transcription and real-time PCR in one step
- High sensitivity due to simultaneous detection of two SARS-CoV-2 target sequences
- Only one reaction per sample

EUROIMMUN Medizinische Labordiagnostika AG

EURORealTime SARS-CoV-2
Use for research purposes only

ORDER NO. MP 2606-0425   SIZE 4 x 25 (100)

CONTENTS
1. PCR Mix A SARS-CoV-2 (green cap), ready for use   4 x 150   µl
2. PCR Mix B SARS-CoV-2 (yellow cap), ready for use   4 x 150   µl
3. Positive control SARS-CoV-2 (purple cap), ready for use   4 x 400   µl
4. RNA internal control (white cap), ready for use   1 x 1.2   ml
5. Test   1   booklet

PCR MIX A
PCR MIX B
POS CONTROL
INT CONTROL

Exposure
(incubation time 1-14 days/median 5 days)

Contagious

Days: -4   -2   0   2   4   6   8   10   ...14

Laboratory diagnostics

Direct detection .... **Serology**

Methods

Serology

- Tracing infection chains in order to identify previously unrecognized cases

- Epidemiological studies in the population in order to determine the prevalence

- Evaluation of immune response to candidate vaccines

- (Identification of potential convalescent plasma donors)

NOT recommended for:

- Diagnosis of acute/recent COVID-19

- Determination of whether or not a patient has developed protective immunity

Exposure
(incubation time 1-14 days/median 5 days)

Contagious

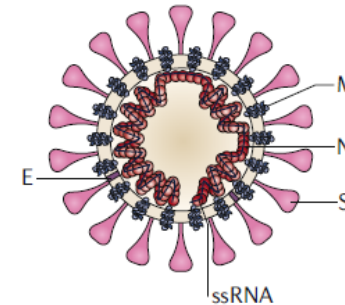Days: -4   -2   0   2   4   6   8   10   ...14

SARS-CoV-2 – Antigen: S1 vs. NCP

Structural proteins incl. envelope glycoproteins
1. spike (S)
2. envelope (E)
3. membrane (M) and nucleocapsid (N)

Viral particle

M
N
E
S
ssRNA

Spike protein S1 domain — S1

Modified nucleocapsid protein — N

- The receptor binding domain (RBD) within S1 domain is the key target for virus neutralisation[1]

- Highly specific antigen: Low S1 domain homology within the coronavirus family

- Excellent correlation between SARS-CoV-2 S1 ELISA and neutralization test[2, 3, 4, 5]

- Most immunogenic antigen of the coronavirus family

- Highly specific due to the use of designer antigen obtained by removal of unspecific epitopes-diagnostically relevant epitopes used

1. Ni et al. 2020; 2. Streeck et al. 2020; 3. Varneite et al. 2020; 4. Kohmer et al. 2020; 5. Harvala et al. 2020

SARS-CoV-2 – EUROIMMUN products I

Anti-SARS-CoV-2 ELISA (IgA and IgG)

Based on recombinant S1 subunit of the spike protein

- Specific detection of IgG against SARS-CoV-2 using the S1 domain of the spike protein including the immunologically relevant receptor binding domain (RBD)[1]

- Excellent performance and good correlation with neutralization tests confirmed in external studies [2, 3, 4]

- Programs for vaccine development worldwide are based on the spike protein as the antigen

- Fully automated processing possible

1. Ni et al. 2020; 2. Streeck et al. 2020; 3. Varneite et al. 2020; 4. Kohmer et al. 2020

Anti-SARS-CoV-2 ELISA (IgA and IgG)

Sensitivity

To determine the diagnostic sensitivity, samples from patients with confirmed SARS-CoV-2 infection were analysed. The following sensitivity therefore corresponds to the prevalence of antibodies against SARS-CoV-2 in COVID-19 infected persons.

Days after onset of symptoms or positive direct detection	EUROIMMUN Anti-SARS-CoV-2 ELISA (IgA)		
	positive	negative	Sensitivity*
≤ 10	50	33	**60,2 %**
> 10	69	1	**98,6 %**

Days after onset of symptoms or positive direct detection	EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG)		
	positive	negative	Sensitivity*
≤ 10	38	49	**43,7 %**
> 10	68	4	**94,4 %**

*Borderline results (IgA: n = 13; IgG: n = 7) were not considered in the calculation.

Anti-SARS-CoV-2 ELISA (IgA and IgG)

Specificity

Collective	EUROIMMUN Anti-SARS-CoV-2-ELISA				
	n	IgA	n	IgG	
		Specificity*		Specificity*	
Blood donors	849	96.0 %	849	99.5 %	
Pregnant women	199	96.0 %	199	99.5 %	
Children	74	98.6 %	74	100 %	
Elderly patients	97	90.1 %	97	100 %	
Other human coronaviruses	11	100 %	23	100 %	
Influenza (freshly vaccinated, incl. follow ups)	40	97.5 %	40	100 %	
Acute EBV infection & heterophile antibodies	22	81.0 %	22	100 %	
Rheumatoid factor	40	89.2 %	40	100 %	
Total	**1,332**	**92.0 %**	**1,344**	**99.6 %**	

*Borderline results (IgA: n = 59; IgG: n = 7) were not considered in the calculation.

IgA detection not useful for screening purposes - only be applied for follow-up investigations in patients with proven COVID-19 infections.
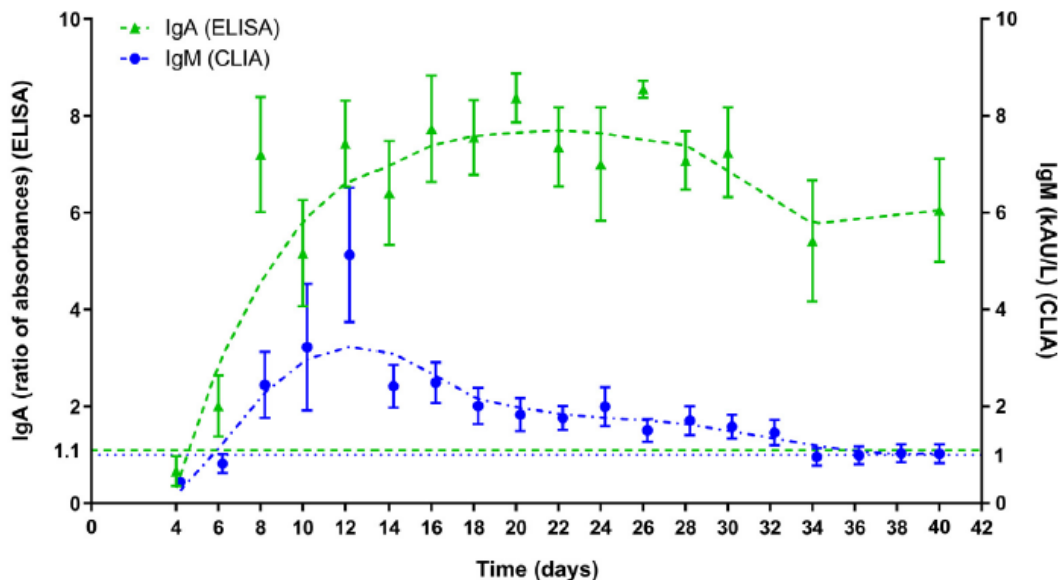
Anti-SARS-CoV-2 ELISA (IgA)

**EUROIMMUN Anti-SARS-CoV-2 ELISA (IgA)
& Snibe MAGLUMI SARS-CoV-2 (IgM)**

IgA-Ab response to spike glycoprotein of SARS-CoV-2 in patients with COVID-19: A longitudinal study

We found that the IgA response appears and grows early, peaks at week 3, and it is stronger and more persistent than the IgM response. Further longitudinal investigations of virus-specific antibodies functions and of their protective efficacy over time are needed.

Padoan et al. Clinica Chimica Acta 507 (2020) 164-166

Anti-SARS-CoV-2 ELISA (IgA and IgG)

			Wantai Ig	Wantai IgM	Euroimmun IgG*	Euroimmun IgA*	Liaison
	Specificity	n/N	149/150	148/150	160/161	151/161	68/69
		Value	0.9933	0.9867	0.9938	0.9379	0.9855
		(95% CI)	(0.9632 - 0.9997)	(0.9527 - 0.9976)	(0.9657 - 0.9997)	(0.8895 - 0.9659)	(0.9224 - 0.9993)
Overall	Sensitivity	n/N	75/76	68/76	62/76	74/76	39/53
		Value	0.9868	0.8947	0.8158	0.9737	0.7358
		(95% CI)	(0.9292 - 0.9993)	(0.8058 - 0.9457)	(0.7142 - 0.8870)	(0.9090 - 0.9953)	(0.6042 - 0.8356)
	Positive Predictive Value	Value	0.9868	0.9714	0.9841	0.881	0.975
		(95% CI)	(0.9292 - 0.9993)	(0.9017 - 0.9949)	(0.9154 - 0.9992)	(0.7946 - 0.9340)	(0.8712 - 0.9987)
	Negative Predictive Value	Value	0.9933	0.9487	0.9195	0.9869	0.8293
		(95% CI)	(0.9632 - 0.9997)	(0.9021 - 0.9738)	(0.8695 - 0.9515)	(0.9536 - 0.9977)	(0.7336 - 0.8955)
	Likelihood Ratio		148	67.11	131.3	15.68	50.77
>14 dps	Sensitivity	n/N	26/26	19/26	25/26	26/26	17/18
		Value	1	0.7308	0.9615	1	0.9444
		(95% CI)	(0.8713 - 1.000)	(0.5392 - 0.8630)	(0.8111 - 0.9980)	(0.8713 - 1.000)	(0.7424 - 0.9972)

EUROIMMUN Anti-SARS-CoV-2

	IgA	IgG
Sensitivity	**100 %**	**96.2 %**
Specificity	**93.8 %**	**99.4 %**

GeurtsvanKessel et al. Preprint medRxiv. 2020

Anti-SARS-CoV-2 ELISA (IgA and IgG)

New Results

Comment on this paper

bioRxiv
THE PREPRINT SERVER FOR BIOLOGY

Evaluation of the EUROIMMUN Anti-SARS-CoV-2 ELISA Assay for detection of IgA and IgG antibodies

Scott Matushek, ⓘ Kathleen G. Beavis, Ana Abeleda, Cindy Bethel, Carlissa Hunt, Stephanie Gillen, Angelica Moran, ⓘ Vera Tesic

In this study, the **EUROIMMUN anti-SARS-CoV-2 ELISA test showed excellent sensitivity for the detection of IgA antibodies with 96.7% and IgG antibodies with 100%.** Both ELISA showed **no cross-reactivity with coronavirus positive samples**, including types HKU1, NL63, CV229E and OC43. The **specificity determined for the IgA ELISA is 93.0% for IgG 98.2%**.

EUROIMMUN Anti-SARS-CoV-2

	IgA	IgG
Sensitivity	96.7 %	100 %
Specificity	93.0 %	98.2 %

Matushek et al. Preprint bioRxiv. 2020

Anti-SARS-CoV-2 ELISA
Correlation with neutralization test

Ni et al. (2020) & Jiang et al. (2020)

The Receptor Binding Domain (RBD, part of the S1 proteins) was identified of being an appropriate antigen triggering the synthesis of antibodies with neutralization capacities

Harvala et al. (2020)

- The strongest correlation was observed between neutralising antibody titres and reactivity in the EUROIMMUN IgG ELISA

Streeck et al. (2020)

- EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) showed a very good correlation with a neutralisation assay (91 %)

Varnaite et al. (2020)

- SARS-CoV-2-specific IgA, IgG and IgM antibody levels (incl. EUROIMMUN ELISAs) positively correlated with SARS-CoV-2-neutralizing antibody titers
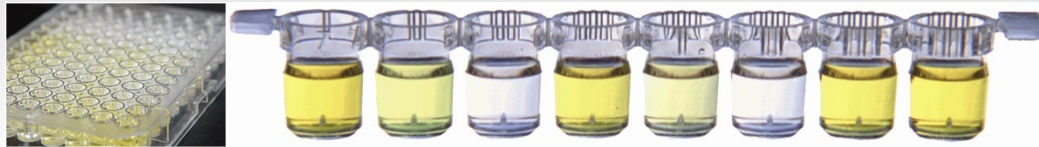
Kohmer et al. (2020)

- The EUROIMMUN ELISA IgG also showed a good correlation to the PRNT, which indicates a possible protective immunity.

More comprehensive studies are required

SARS-CoV-2 – EUROIMMUN products II

Anti-SARS-CoV-2-NCP ELISA (IgG)

Based on recombinant modified nucleocapsid protein

- Sensitive and specific detection of anti-SARS-CoV-2 antibodies
- Antigen with the strongest immunodominance in the coronavirus family
- Highly specific due to the use of designer antigen obtained by removing non-specific epitopes - diagnostically relevant epitopes are used
- Collection of epidemiological data
- Fully automatic processing possible

Anti-SARS-CoV-2-NCP ELISA

Sensitivity & Specificity

Days after onset of symptoms or positive direct detection	EUROIMMUN Anti-SARS-CoV-2-NCP ELISA (IgG)		
	positive	negative	Sensitivity*
≤ 10	12	3	80 %
> 10	53	3	94.6 %

*Borderline results (n=3) were not considered in the calculation.

Collective	n	EUROIMMUN ELISA Anti-SARS-CoV-2-NCP (IgG)
		Specificity*
Blood donors	849	99.8 %
Pregnant women	99	100 %
Children	74	100 %
Elderly patients	97	99 %
Other human coronaviruses	27	100 %
Influenza (freshly vaccinated, incl. follow ups)	40	100 %
Acute EBV infection & heterophile antibodies	22	100 %
Rheumatoid factor	40	100 %
Total	1,248	99.8 %

*Borderline results (n=4) were not considered in the calculation.

Limitations

Example
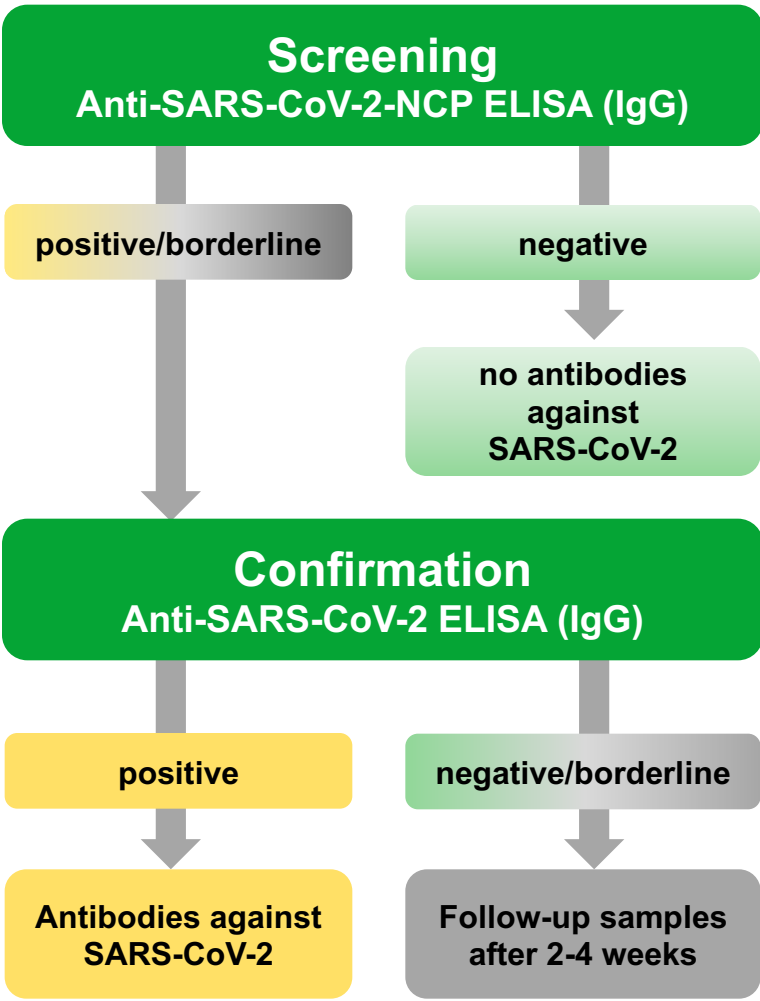> Prevalence of 1%: **0.1 true positive result** on 1000 examinations
>
> Specificity of 99.6-99.8%: **2-4 false positive results** on 1000 examinations

IMPORTANT

- As the prevalence increases, does the positive predictive value (PPV)

- The results are more accurate without changing the test

Prevalence	Specificity	False positive	Treu positive	False positive
1 %	99.0 %	1	1	50 %
5 %	99.0 %	1	5	14 %
10 %	99.0 %	1	10	9 %
50 %	99.0 %	1	50	2 %

Two-Step diagnostic strategy EUROIMMUN IgG ELISA

Screening
Anti-SARS-CoV-2-NCP ELISA (IgG)

positive/borderline | negative

no antibodies against SARS-CoV-2

Confirmation
Anti-SARS-CoV-2 ELISA (IgG)

positive | negative/borderline

Antibodies against SARS-CoV-2 | Follow-up samples after 2-4 weeks

Collective	EUROIMMUN ELISA Anti-SARS-CoV-2-NCP (IgG) & Anti-SARS-CoV-2 (IgG)	
	n	Specificity
Blood donors	849	100 %
Pregnant women	99	100 %
Children	90	100 %
Elderly patients	97	100 %
Other human coronaviruses	23	100 %
Influenza (freshly vaccinated, incl. follow ups)	40	100 %
Acute EBV infection & heterophile antibodies	22	100 %
Rheumatoid factor	40	100 %
Total	1,260	100 %

Increased specificity from 99.8 % and 99.6 % to **100 %** by combined application – relevant especially in populations with low prevalences

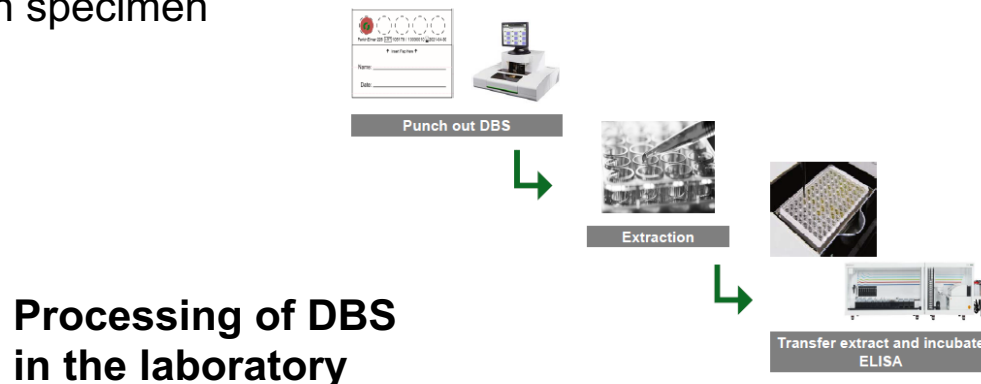# Dried blood spots (DBS) – EUROIMMUN IgG ELISAs

- Only a few drops of blood are required for the analysis
- Blood retrieval from the finger pad or the ear lobe – no venous puncture, no qualified personnel necessary
- High analytical stability and minimal space requirements – uncomplicated storage and transport of samples
- Low infection risk – simpler transport as exempt human specimen

**Available for
Anti-SARS-CoV-2 ELISA (IgG) & Anti-SARS-CoV-2 NCP ELISA (IgG)**

DBS card:
EUROIMMUN blood collection card
ZV 9711-01100 (100 pcs)

Coming soon: Dried blood spot set
ZV 9701-0101 (1 pcs)

Punch out DBS

Extraction

Transfer extract and incubate ELISA

**Processing of DBS
in the laboratory**

Dried blood spots (DBS) – EUROIMMUN IgG ELISAs

		EUROIMMUN Anti-SARS-CoV-2-NCP ELISA (IgG) Venous blood	
		positive	negative
EUROIMMUN Anti-SARS-CoV-2-NCP ELISA (IgG) DBS	positive	63	1
	negative	0	135

Correlation: 99.5 % (PPA: 98.4 % & NPA: 100 %)

		EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) Venous blood	
		positive	negative
EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) DBS	positive	61	0
	negative	0	147

Correlation: 100 % (PPA: 100 % & NPA: 100 %)

*Borderline results were not considered in the calculation.

EUROIMMUN Anti-SARS-COV-2 ELISA

Automation

- **Possible with common open ELISA processors**
- **Validation files available for devices**
  - EUROLabWorkstation ELISA
  - EUROIMMUN Analyzer I and I-2P
  - Dynex DSX

Summary – EUROIMMUN IgG ELISAs

Anti-SARS-CoV-2 ELISA (IgG)

- Specific detection of IgG against SARS-CoV-2 using the S1 domain of the spike protein including the immunologically relevant receptor binding domain (RBD)

- Excellent performance and good correlation with neutralization tests confirmed in external studies – **Protective Immunity? -> More comprehensive studies are required**

- Programs for vaccine development worldwide are based on the spike protein as the antigen

Anti-SARS-CoV-2-NCP ELISA (IgG)

- Based on recombinant modified nucleocapsid protein

- Most immunogenic antigen of the coronavirus family

- Highly specific due to the use of designer antigen obtained by removal of unspecific epitopes- diagnostically relevant epitopes used

Combination

➢ **Increased specificity** from 99.8 % and 99.6 % to **100 %** by combined application – relevant especially in populations with low prevalences

Further features of EUROIMMUN SARS-CoV-2 ELISAs

- Optimized automation solutions

- Reliable diagnostics using dried blood spots (DBS) – serology without venous puncture

SARS-CoV-2 Dx developments

SARS-CoV-2 – EUROIMMUN products III

NEW

Anti-SARS-CoV-2-NCP ELISA (IgM)

Based on recombinant modified nucleocapsid protein

- Sensitive and specific detection of anti-SARS-CoV-2 antibodies

- Antigen with the strongest immunodominance in the coronavirus family

- Highly specific due to the use of designer antigen obtained by removing non-specific epitopes - diagnostically relevant epitopes are used

- Supplement to direct pathogen detection

- Use to collect epidemiological data

Thank you for your attention!