Anti-Crimean-Congo fever virus (CCHFV) ELISA (IgG)

- **Specific detection of IgG antibodies against Crimean-Congo fever virus (CCHFV)**
- **For the support of the diagnosis of Crimean-Congo fever virus infection in addition to direct pathogen detection**
- **Fully automatable**

Technical data

Antigen	Recombinant nucleocapsid protein of Crimean-Congo fever virus
Calibration	Quantitative, in relative units per millilitre (RU/ml) Calibration serum 1: 200 RU/ml Calibration serum 2: 20 RU/ml Calibration serum 3: 2 RU/ml Recommended upper threshold of the reference range for non-infected individuals (cut-off): 20 RU/ml Semiquantitative evaluation also possible
Sample dilution	Serum or plasma, 1 : 101 in sample buffer
Reagents	Ready for use, with the exception of the wash buffer (10x); colour-coded solutions, in most cases exchangeable with those in other EUROIMMUN ELISA kits
Test procedure	60 min (37 °C) / 30 min (RT) / 15 min (RT) (sample / conjugate / substrate incubations), fully automated
Measurement	450 nm, reference wavelength between 620 nm and 650 nm
Test kit format	96 break-off wells; kit includes all necessary reagents
Order number	**EI 279a-9601 G**

Clinical significance

Crimean-Congo haemorrhagic fever virus (CCHFV; genus *Orthonairovirus*, family *Nairoviridae*) can be found in parts of Africa and Asia, in Eastern Europe and the Middle East. Humans can get infected by tick bites (primarily *Hyalomma* spec.) and by contact with blood or other body fluids of viraemic animals and humans. CCHFV circulates in an enzootic cycle: tick-vertebrate-tick, but the infection does not manifest in animals. The incubation time of CCHF is typically 1 to 9 days but up to a maximum of 12 to 13 days. Around 90 % of CCHFV infections take a mild or asymptomatic course. In approximately 10 % of cases, a severe haemorrhagic disease with a fatality of 3 % to 30 % occurs.

Initial symptoms of CCHF (approx. 4 to 5 days) are similar to an unspecific febrile disease, typically with sudden fever, myalgia, diarrhoea, nausea and vomiting. This is followed by the haemorrhagic phase (around 2 weeks) with bleeding from various organs. The convalescent phase (around 10 days) is characterised by excessive fatigue, tachycardia with unstable blood pressure, temporary hair loss and memory disorders. Somnolence, significant amounts of blood in the urine, vomiting of blood, black stool, gastrointestinal bleeding and diarrhoea indicate a poor prognosis: intravascular coagulation, shock, multiple-organ failure. The disease takes a milder course in children.

In the acute (viraemic) phase of the infection, diagnosis is made by determination of viral RNA or virus antigen in, for instance, serum, plasma or blood. Viraemia does not subside in patients with a poor prognosis. IgM against the viral nucleocapsid (N) is produced from day 2 or 3 after onset of the disease. Anti-CCHFV IgM remains detectable for up to 4 months but the concentration is highest after 2 to 3 weeks. Anti-CCHFV IgG appears 1 to 2 days after the IgM response and remains detectable for at least 5 years. In severe cases, specific IgG is not produced at all, only in small amounts or with delay. The presence of IgM or a fourfold increase in the IgG titer in two consecutive samples confirms an acute CCHFV infection.

Depending on the geographic region, differential diagnosis should include rickettsiosis, Q fever (*Coxiella burnetii*), leptospirosis and malaria, as well as bacterial sepsis, viral hepatitis and infectious endocarditis.

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Cross-reactivity (analytical specificity)

Cross-reactivity was determined by investigating sera from patients with infections with different pathogens using the Anti-Crimean-Congo fever virus (CCHFV) ELISA (IgG). It should be noted that cross-reactions within the *Bunyavirales* order cannot be excluded. Double infections are possible, especially in endemic regions. In this case, positive results are not caused by a cross-reactivity of the corresponding antibodies.

Antibodies against	n	Positive in the Anti-Crimean-Congo fever virus (CCHFV) ELISA (IgG) from EUROIMMUN
Hantavirus	28	0 %
Sandfly fever virus	12	0 %
Toscana virus	17	0 %

Diagnostic sensitivity (Prevalence)

The diagnostic sensitivity was determined by investigating samples from patients with confirmed CCHFV infections. The sensitivity given below therefore corresponds to the prevalence of antibodies against CCHFV in infected persons. A total of 45 samples from 16 patients from South Eastern Europe with RT-PCR-confirmed CCHFV infections were analysed using the Anti-Crimean-Congo fever virus (CCHFV) ELISA (IgG). The serological investigation was performed on samples collected in the acute (n = 8, 6 to 14 days after symptom onset), the convalescent (n = 8, 15 to 27 days after symptom onset) and the late phase (n = 15, 28 to 600 days after symptom onset) as well as in the long-term phase of infection (n = 14, > 600 days after symptom onset).

Days after symptom onset	EUROIMMUN Anti-Crimean-Congo fever virus (CCHFV) ELISA (IgG)			
	positive	borderline	negative	Prevalence (sensitivity)
6 to 14 days (acute phase)	3	2	3	**62.5 %**
15 to 27 days (convalescence phase)	6	1	1	**87.5 %**
28 to 600 days (late phase)	14	0	1	**93.3 %**
> 600 days (long-term phase)	14	0	0	**100 %**

For samples from donors collected within a time frame of 6 to 14 days (time point after onset of symptoms), a sensitivity of 62.5 % was determined for the Anti-Crimean-Congo fever virus (CCHFV) ELISA (IgG). For samples taken at 15 to 27 days and 28 to 600 days after symptom onset, the sensitivity was 87.5 % and 93.3 %, respectively. For samples collected > 600 days after symptom onset, the sensitivity amounted to 100 %. Borderline results (n = 3) were considered as positive in the calculation.

The specificity of the Anti-Crimean-Congo fever virus (CCHFV) ELISA (IgG) was determined based on 116 patient samples which were positive for other pathogens, autoantibodies or rheumatoid factors. Additionally, samples from 800 healthy German blood donors, pregnant women, children, as well as from 60 healthy blood donors from South Eastern Europe (CCHFV endemic region) were investigated. Borderline results (n = 10) were considered as positive in the calculation of the specificity. The specificity of the Anti-Crimean-Congo fever virus (CCHFV) ELISA (IgG) was 97.7 %.

Panel	EUROIMMUN Anti-Crimean-Congo fever virus (CCHFV) ELISA (IgG)	
	n	Specificity
Blood donors (South Eastern Europe)	60	98.3 %
Blood donors (Germany)	500	97.2 %
Pregnant women	100	99.0 %
Children	200	99.5 %
Autoantibodies	37	94.6 %
Acute EBV infection & heterophile antibodies	22	100 %
Rheumatoid factors	38	97.4 %
Malaria	19	89.5 %
Total	**976**	**97.7 %**

Literature

1. Emmerich P, et al. **Comparison of diagnostic performances of ten different immunoassays detecting anti-CCHFV IgM and IgG antibodies from acute to subsided phases of Crimean-Congo hemorrhagic fever.** PLoS Negl Trop Dis 15(3):e0009280 (2021).
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