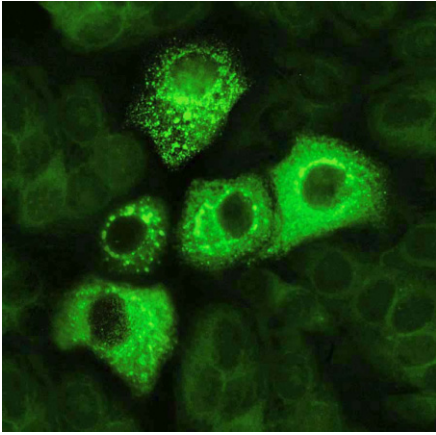


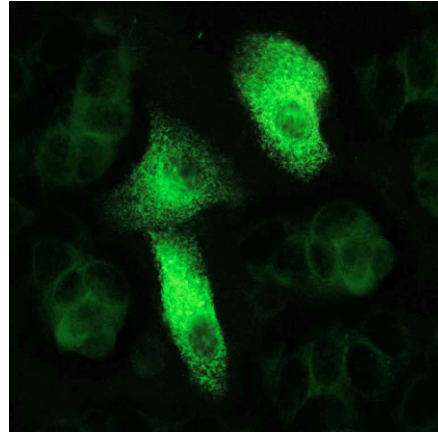


In collaboration with Matthias Niedrig, Robert Koch Institute, Berlin, Germany

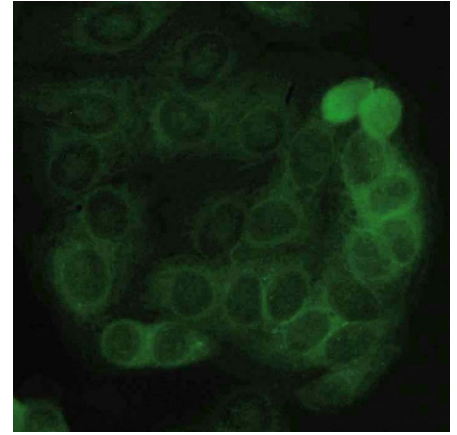
Serological diagnosis of SARS-coronavirus infections by IIFT



Positive: IgG Ab against SARS-CoV



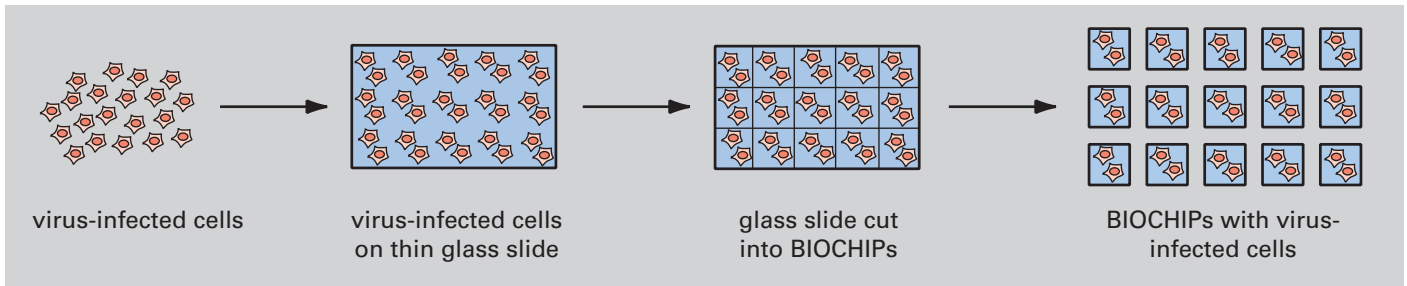
Positive: IgM Ab against SARS-CoV



Negative: no Ab against SARS-CoV

The **indirect immunofluorescence test (IIFT)** allows the detection of antibodies against **severe acute respiratory syndrome coronavirus (SARS-CoV)** in patient serum samples. The test utilizes BIOCHIP slides containing the two substrates SARS-CoV-infected cells and non-infected cells positioned side by side in each reaction field. The virus isolate was kindly provided by the Institute of Medical Virology, Johann Wolfgang Goethe University (Frankfurt, Germany). The BIOCHIPS coated with virus-infected cells have been treated with a disinfecting fixing agent and gamma-irradiated to inactivate infectious virus particles.

BIOCHIP Technology



For the production of BIOCHIP slides EUROIMMUN uses a method similar to that used by the electronics industry in the manufacture of microchips. In contrast to conventional production methods, the substrates are no longer applied directly to microscope slides, but initially to thin glass slides. These are mechanically cut into millimetre-sized fragments (BIOCHIPs). The BIOCHIPs are then glued onto microscope slides using automated assembly equipment. With this method it is possible to produce large batches of each substrate, thus reducing fluctuations in quality.

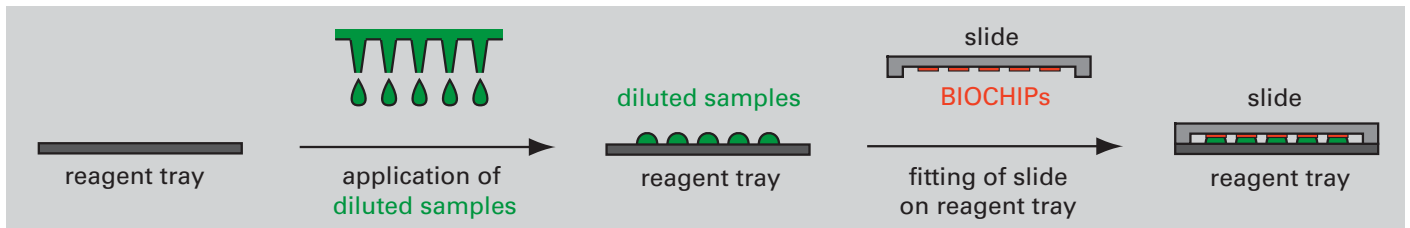
The miniature size of the BIOCHIPs means that the reaction fields of the slides can be supplemented with further BIOCHIP substrates if required (BIOCHIP Mosaic™), for example for determination of antibodies against influenza virus, parainfluenza virus, respiratory syncytial virus, adenovirus, Chlamydia pneumoniae, Legionella pneumophila or Mycoplasma pneumoniae. In this way a patient antibody profile can be obtained with a single incubation.



Anti-SARS-CoV IIFT slide



Titerplane™ Technique



The BIOCHIP slides are incubated using EUROIMMUN's proprietary TITERPLANE™ Technique, which is performed as follows: Diluted samples or labelled antisera are applied to the reaction fields of a reagent tray. The slide is then placed into the recesses of the reagent tray, where all BIOCHIPs come into contact with the drops and the reactions commence simultaneously. As the fluids are confined in a closed space, there is no need to use a conventional humidity chamber. The position and height of the drops is exactly defined by the geometry of the system. In this way, many patient samples can be incubated next to each other simultaneously under identical conditions.

Test procedure

In the first incubation step (30 minutes) the substrates are incubated with diluted patient serum samples. If the sample is positive specific antibodies bind to viral antigens in the infected cells. In the second incubation step (30 minutes) the attached antibodies are stained with fluorescein-labelled anti-human antibodies (IgG or IgM). Results are evaluated visually by fluorescence microscopy.

Initial evaluation data

Sera from 9 patients with probable SARS from Hong Kong, Germany and the United Kingdom were investigated for anti-SARS-CoV antibodies using the EUROIMMUN IIFT. The patients were clinically characterised according to WHO criteria, and their sera were precharacterised using in-house IIFT from the corresponding institutes. Sera from 33 individuals who had been in contact with SARS-CoV-infected persons and 200 healthy blood donors were also tested. All of the patients with probable SARS were positive for anti-SARS-CoV antibodies (IgG). None of the contact persons or healthy blood donors showed antibodies against SARS-CoV.

Panel	n	Anti-SARS-CoV positive (IgG)
SARS patients	9	9 (100%)
Contact persons	33	0
Blood donors	200	0

To demonstrate seroconversion, serum samples taken from two clinically characterized patients with probable SARS (Drosten et al., N Engl J Med 348: 1967-76, 2003) on different days were investigated for anti-SARS-CoV antibodies. Patient 1 had shown symptoms of illness for several days before admission on 15 March 2003. Patient 2 developed symptoms on 16 March after direct contact with patient 1. Seroconversion could be demonstrated in both patients. In the case of patient 2 this had occurred by 9 days after onset of symptoms.

Patient 1 sample	Anti-SARS-CoV	
	IgG titer	IgM titer
18 Mar 2003	1:100	1:10
28 Mar 2003	>1:1000	>1:100

Patient 2 sample	Anti-SARS-CoV	
	IgG titer	IgM titer
25 Mar 2003	1:32	0
26 Mar 2003	1:100	1:10
27 Mar 2003	1:320	1:100
28 Mar 2003	1:1000	>1:100