Avidity determination of IgG antibodies against West Nile Virus
Test instruction of the ELISA

ORDER NO.	ANTIBODIES AGAINST	IG-CLASS	SUBSTRATE	FORMAT
EI 2662-9601-1 G	West Nile Virus	IgG	Ag-coated microplate wells	96 x 01 (96)

Intended Use: This test kit is intended for the avidity determination of IgG class antibodies against West Nile virus in human serum or plasma.

Background

The differentiation between fresh and long-standing infections is one of the greatest challenges in serology. Until now this was based mainly on determination of specific antibodies of the immunoglobulin class IgM, which generally only appear initially. However, the detection of these antibodies is often unreliable and problematic due to interfering factors such as persistence of the IgM response, too weak or delayed IgM production, and unspecific IgM production through polyclonal B-cell stimulation.

In recent years additional determination of the antibody avidity has become an established method for identifcation of primary infections. The immune system reacts to an infection by first forming low-avidity antibodies. With continued disease duration, IgG that are more precisely adapted to the antigens are produced – the avidity increases. If high-avidity IgG are detectable in the serum, it can be assumed that the infection is at a late stage.

Contents of the test system: EI 2662-9601-1 G:

Component	Colour	Format	Symbol
1. Test kit Anti-West Nile Virus ELISA (IgG, order number EI 2662-9601 G)	---		
2. Positive control HA High-avidity anti-West Nile Virus (IgG, human), ready for use	red	1 x 1.3 ml	POS CONTROL HA
3. Positive control LA Low-avidity anti-West Nile Virus (IgG, human), ready for use	blue	1 x 1.3 ml	POS CONTROL LA
4. Urea solution for Anti-West Nile Virus ELISA, ready for use	yellow	1 x 12 ml	UREA
5. Phosphate buffer ready for use	light blue	1 x 12 ml	PBS BUFFER
6. Test instruction	---	1 booklet	
7. Protective foil	---	2 pieces	

LOT Lot
IVD In vitro determination
CE
Storage temperature
Unopened usable until

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

The thermostat adjusted ELISA incubator must be set at 37°C +/- 1°C.

- **Controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Urea solution:** Ready for use. The urea solution included in this test system may only be used for the avidity determination of antibodies against West Nile.

- **Phosphate buffer:** Ready for use.

Warning: The calibrators and controls used have been tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 using enzyme immunoassays and indirect immunofluorescence methods. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the toxic agent sodium azide. Avoid contact with the skin.

Preparation and stability of the patient samples

Sample material: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:101** sample buffer. For example: dilute 10 µl serum to 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: The controls are prediluted and ready for use, do not dilute them.

Incubation

Sample incubation: (1. step)
Transfer 100 µl of the controls or diluted patient samples into the individual microplate wells according to the pipetting protocol.
For manual processing of microplate wells, cover the finished test plate with the protective foil. When using an automated microplate processor for incubation, follow the instrument manufacturer´s recommendations with regard to microwell plate sealing
Incubate for **60 minutes at 37°C ± 1°C**.

Wash:
Manual: Remove the protective foil, empty the wells and subsequently wash **1 time** using 300 µl of working strength wash buffer.
Automatic: Wash reagent wells **1 time** with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer “Overflow Modus”).

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer. Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Urea incubation: (2. step)
Pipette 200 µl of urea solution into each of the microplate wells of the first microtiter strip and 200 µl of phosphate buffer into each of the microplate wells of the second microtiter strip.
When using an automated microplate processor for incubation, follow the instrument manufacturer´s recommendations with regard to microwell plate sealing. Incubate for **10 minutes at room temperature (+18°C to 25°C).**

Wash:
Empty the wells. Wash as described above, but wash **3 times** using working strength wash buffer for each wash.

Attention: Residual liquid (> 10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short reaction times) can lead to false high extinction values.

Conjugate incubation: (3. step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells.
When using an automated microplate processor for incubation, follow the instrument manufacturer´s recommendations with regard to microwell plate sealing.
Incubate for **30 minutes at room temperature (+18°C to 25°C)**.

Wash:
Empty the wells. Wash as described above, but wash **3 times** using working strength wash buffer for each wash.

Substrate incubation: (4. step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C to 25°C) protect from direct sunlight.

Stopping the reaction:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution.** Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**pos HA**	**pos HA**	**P 7**	**P 7**	**P 15**	**P 15**						
B	**pos LA**	**pos LA**	**P 8**	**P 8**	**P 16**	**P 16**						
C	**P 1**	**P 1**	**P 9**	**P 9**	**P 17**	**P 17**						
D	**P 2**	**P 2**	**P 10**	**P 10**	**P 18**	**P 18**						
E	**P 3**	**P 3**	**P 11**	**P 11**								
F	**P 4**	**P 4**	**P 12**	**P 12**								
G	**P 5**	**P 5**	**P 13**	**P 13**								
H	**P 6**	**P 6**	**P 14**	**P 14**								

The above pipetting protocol is an example of the avidity determination of IgG antibodies in 18 patient samples (P 1 to P 18).

Controls (pos HA and pos LA) as well as the patient samples have been incubated in duplicate in one well each on two different microtiter strips. The reagent wells of the microtiter strips 1, 3, 5 etc. are treated with urea solution after the incubation with patients samples, the reagent wells of the microtiter strips 2, 4, 6 etc. are treated with phosphate buffer.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimizes reagent wastage.
Both positive controls with high-avidity and low-avidity antibodies serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

The presence of low-avidity antibodies in a patient's serum has been proved if the ELISA extinction value is considerably reduced by urea treatment. For an objective interpretation the relative avidity index (RAI) is calculated and expressed in percent using the extinction values with and without urea treatment.

$$\frac{\textbf{Extinction of the sample with urea treatment x 100}}{\textbf{Extinction of the sample without urea treatment}} = \textbf{relative avidity index (RAI) in \%}$$

The upper limit of the range of low-avidity antibodies (**cut-off value**) recommended by EUROIMMUN is 40% RAI. Values below the indicated cut-off are to be considered as an indication of low-avidity antibodies, values between 40% and 60% RAI as equivocal, values above 60% RAI as an indication of high-avidity antibodies. If a result is classified as equivocal, it is recommended to collect a second sample not less than 7 days later and to test it together with the first sample.

RAI < 40%:	indication of low-avidity antibodies
RAI 40% - 60%:	equivocal
RAI > 60%:	indication of high-avidity antibodies

Reliable results in the the measurement of IgG antibody avidity can only be yielded if the patient sample contains a diagnostically significant concentration of specific antibodies. Generally, the determination of the relative avidity index is not helpful in samples which have an O.D. of <0.140 after incubation with urea treatment.

For diagnosis, the clinical symptoms of the patient should always be taken into account alongside the serological results.

Attention:
In some patients with an acute infection, very high titers of IgG antibodies can be found. Even though the specific IgG antibody population is in different maturation stages, both high-avidity and low-avidity, the vacant antigen epitopes are predominantly occupied by high-avidity antibodies in high titer samples. The determination of the avidity of the whole specific IgG antibody population can lead to false high RAI values in results.
False high RAI values were found in some cases of acute infections when the extinction value of the IgG measurement without urea treatment was >1.200.
It is recommended for samples with extinction values of >1.200 to repeat the avidity determination with a higher sample dilution (e. g. 1:401). If low avidity of IgG antibodies is already found at extinction values of >1.200, no further testing is necessary.

Clinical significance

West Nile virus (WNV) is an enveloped single-stranded RNA virus of the Flaviviridae family [1]. This family comprises around 100 virus types that are presently categorized into the three known species Flavivirus, Pestivirus and Hepacivirus [1, 2, 3, 4, 5]. West Nile virus received its name in 1937 when it was first isolated from a blood sample of an elderly woman living in the West Nile district in Uganda, who had fever of unknown cause accompanied by neurological disorders [6]. Further isolates were achieved only in 1951 from the sera of children with weak, unspecific symptoms, namely in Egypt where the virus is endemically distributed. At that time mice and embryonated hen's eggs were used for virus detection [1].

WNV is present not only in tropical areas, but also in moderate climate regions [2, 3, 4, 5]. Significant epidemics were observed in 1951/52 and 1957 in Israel and 1974 and 1983/84 in South Africa [1]. In the mid 90's the virus changed its virulence causing an epidemic accumulation of WNV encephalitis in Algeria (1994), Rumania (1996/97), the Czech Republic (1997), the Democratic Republic of Congo (1998), Russia, North America (1999) and Israel (2000) [2, 3, 7, 8, 9, 10, 11, 12]. In the USA 149 infections with 18 cases of death were recorded from 1999 to 2001. In 2002 this number rose to 4156 infections and 284 deaths, in 2003 to 9858 infections and 262 deaths [7, 8, 10]. Currently the virus has been detected in seven Canadian provinces, in 48 USA states and in Mexico, as well as in Puerto Rico, the Dominican Republic, Jamaica, Guadeloupe and El Salvador [3, 9, 11].

Since 1958, when antibodies against the WNV were first detected in the sera of two Albanians, repeated outbreaks of West Nile fever have occurred in Southern and Eastern Europe and meanwhile also in Central and West Europe [8, 9, 10, 12, 14, 15]. Its emergence and rapid spread is credited to world climate change, long-distance travel and globalization of economic trade [9, 12, 13, 15, 16, 17]. Consequent monitoring of West Nile activity by controlling sera of exposed persons is essential [15, 17, 18, 19]. Seroprevalence studies in endemic regions have shown an infection spread of up to 40% [14]. West Nile virus is therefore the Flavivirus with the largest distribution area [2, 3, 4, 5, 9].

WNV is transmitted by a number of mosquitoes. In the Mediterranean region and in Africa mosquitoes of the Culex univittatus complex species are the main arthropod hosts, while in North America WNV could be detected in 37 mosquito species, with Culex pipiens being the main vector [1, 2, 3, 4, 7, 9]. In India Culex vishnui and in France Culex modestus were identified as the main vectors [1]. In total WNV could be found in more than 40 mosquito and in several tick species [3, 4, 5].

Birds represent the vertebrate reservoir [1, 20, 21]. Alone in the USA WNV has been found in more than 162 species of birds. Many of them showed clinical symptoms and thousands of birds died after contracting a natural infection [7, 8, 10, 20, 21]. Birds that survive develop lifelong immunity. Acting as coincidental hosts mammals can also become infected when bitten by an infected mosquito [10, 13, 14]. Transmission has additionally been documented via breast milk, bone marrow transplantations, liver and heart transplants, blood transfusions, lab accidents such as open wounds during handling of infected brain tissue as well as transplacental transmission [2, 3, 4, 5, 8, 22, 23, 24, 25, 26, 27, 28]. Other than humans, mostly only horses became ill after an infection [2, 13, 21]. As well as WNV infection via a mosquito bite, a second natural infection source is possible in animals, namely via feeding on infected prey [3, 5]. An experimental infection of cats was successfully achieved by feeding them infected mice [20, 21].

70% to 80% of the humans infected with WNV showed no symptoms [8]. In the remaining 20%-30%, signs of sudden flu-like symptoms appear after an incubation period of 2-6 days with fever ranging from 38.5 to 40°C lasting for 3-5 days, nausea, shivering, head and back aches, joint and muscle pain and other unspecific symptoms such as loss of appetite, dizziness, vomiting, diarrhoea, coughing and a sore throat [1, 2, 3, 4, 5].
Typical for epidemical occurring fever are exanthema on the breast, back and upper extremities and general lymph node swelling [1, 3, 26]. Severe clinical cases of WNV infections are characterized by myocarditis, pancreatitis and hepatitis and since 1996, also neurological disorders, as WNV is now capable of crossing the blood-brain barrier [3]. The neurological symptoms begin after a short febrile prodome phase approximately 1-7 days after infection and become manifest in the form of encephalitis and meningoencephalitis accompanied by stiffness, spasms and shivering as the result of damage done to the basal ganglia [3, 8, 29, 30]. Another widespread symptom is general muscle weakness similar to the Guillain-Barré syndrome and also polio-like paralysis [29, 30]. Approximately 4%-14% of the hospitalized patient cases are fatal [29]. High risk factors are old age and a weak immune system [1, 3, 29].

An infection with WNV during pregnancy can cause miscarriages, congenital meningitis, birth defects in approx. 10% of the cases and in an additional 10% of newborns growth disturbances [3, 8, 27, 28].

The diagnosis of WNV can be performed by virus detection or by detection of specific antibodies [1, 4, 17, 18, 19]. As virus isolation from serum or cerebrospinal fluid or virus detection using Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) is usually unsuccessful due to short viraemia and low virus titers, the detection of specific WNV antibodies using ELISA and IFA has gained importance [11, 17, 18, 19, 31, 32, 33, 34, 35, 36, 37, 38].

Specific IgM antibodies in serum can be determined using ELISA or IIFT [19]. Antibodies of class IgM are detectable in serum from the second day after initial symptoms of the illness occur. A four-fold increase in titer of the respective class of antibody is considered proof of a WNV infection.

If the IgM test is negative, even though the symptoms indicate a WNV infection, a second serum sample should be taken and tested for IgM antibodies a few days later. A combination of ELISA and IIFT provides close to 100% reliability [17, 18, 31, 32, 33, 34]. Anti-WNV IgM antibodies persist for 2 to 3 months, often for more than a year [4, 17, 18, 19, 22].

Antibodies of class IgG are detectable approx. 2 days after the appearance of IgM antibodies [11, 19, 32, 33, 34, 40]. Two to four weeks after a positive IgM result the infection can be confirmed and its severity and prognosis evaluated using a qualitative and quantitative test for the detection of specific WNV IgG antibodies in the patient serum [34].

For the reliable differentiation between acute and past infections the detection of low-avidity IgG antibodies gives evidence for a primary or an acute WNV infection, while high-avidity antibodies indicate a past or reactivated WNV infection [39, 40, 41]. EUROIMMUN offers additional test systems for determination of IgG avidity in both ELISA and IIFT formats. The detection of low-avidity antibodies using ELISA and IIFT in parallel is possible for WNV as it is for Toxoplasma gondii, rubella virus, EBV-EA, EBV-CA and Corona virus [39, 40, 41].

As the degree of similarity within the Flavivirus family is high antibody cross reactions can occur [19, 31, 42]. Therefore samples that are positive for specific IgM and/or IgG antibodies against WNV should be titrated and investigated on all relevant Flavivirus IIFT substrates for cross reactions. By comparing the titer strengths the initial result can be confirmed or disproved by the second detection and an infection with another Flavivirus identified as the source of illness [17, 32, 34].

To supplement and extend the current Anti-West Nile Virus ELISA and Anti-West Nile Virus IIFT (each IgG or IgM or avidity) BIOCHIP Mosaics and Profiles for the detection (IIFT) of infections with Flaviviruses and the BIOCHIP Mosaic Fever Profile 1: South-East Asia have been developed. With these tests specific antibodies (IgG and IgM) against several infectious agents can be investigated simultaneously [36, 37, 38, 43, 44, 45]. These supplementary tests allow similar or ambiguous disease symptoms and potential cross reactions to be clarified and differential diagnostic issues to be addressed [1].

A specific antiviral therapy for WNV encephalitis is not available at present [1, 2, 3, 4, 5, 14, 35]. Intensive medical care is the only possibility to positively influence the illness. Eradication of WNV is impossible due to the natural bird-mosquito cycle [1]. A vaccine with formalin inactivated WNV is only available for horses [2, 10]. Therefore public education, individual precautionary measures and protection against insect bites are essential contributions to preventing WNV infections [2, 3, 4, 5, 14, 16].

Literature references

1. Falke D. **Flavi-Viren.** In: Hahn H, Falke D, Kaufmann SHE, Ullmann U. **Medizinische Mikrobiologie und Infektiologie.** Springer-Verlag, 5. Auflage (2004) 523-529.
2. Campbell GL, Marfin AA, Lanciotti RS, Gubler DJ. **West Nile Virus.** Lancet Infect Dis 2 (2002) 519-529.
3. Nosal B, Pellizzari R. **West Nile virus.** CMAJ 168 (2003)1443-1444.
4. Granwehr BP, Lillibridge KM, Higgs S, Mason PW, Aronson JF, Campbell GA, Barrett ADT. West Nile virus: where are we now? The Lancet Infect Dis 4 (2004) 547-551.
5. Stock I. **West nile virus. An unusual flavivirus with increased importance.** [Article in German] Med Monatsschr Pharm 27 (2004) 112-120.
6. Smithburn KC, Hughes TP, Burke AW et al. **A neurotropic virus isolated from the blood of a native of Uganda.** Am J Trop Med Hyg 20 (1940) 471-472.
7. Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P. **West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior.** PLoS Bio 4 (2006) 82.
8. Zeller HG, Schuffenecker I. **West Nile virus: an overview of its spreads in europe and the mediterranean basin in contrast to its spread in the americas.** Eur J Clin Microbiol Infects Dis 23 (2004) 147-156.
9. Rappole JH, Compton BW, Leimgruber P, Robertson J, King DI, Renner SC. **Modeling movement of West Nile virus in the Western hemisphere.** Vector Borne Zoonotic Dis 6 (2006) 128-139.
10. Hubalek Z. **European experience with the West Nile virus ecology and epidemiology: could it be relevant for the New World?** Viral Immunol 13 (2000) 415-426.
11. Tilley PA, Walle R, Chow A, Jayaraman GC, Fonseca K, Drebot MA, Preiksaitis J, Fox J. **Clinical utility of commercial enzyme immunoassays during the inaugural season of West Nile virus activity, Alberta, Canada.** J Clin Microbiol 43 (2005) 4691-4695.
12. Hubalek Z, Halouzka J. **West Nile Fever - a re-emerging mosquito-borne viral disease in europe.** Emerg Infect Dis 5 (1999) 643-650.
13. Pauli G. **West Nile virus. Prevalence and significance as a zoonotic pathogen.** [Article in German] Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz 47 (2004) 653-660.
14. Robert-Koch-Institut. **West-Nil-Fieber.** Epidemiol Bull 13 (2003) 96-99.
15. Epstein PR. **West Nile virus and the climate.** J Urban Health 78 (2001) 367-371.
16. Gubler DJ. **The global emergence/resurgence of arboviral diseases as public health problems.** Arch Med Res 33 (2002) 330-342.
17. Koraka P, Zeller H, Niedrig M, Osterhaus AD, Groen J. **Reactivity of serum from patients with flavivirus infection measured by immunofluorescence assay and ELISA.** Microbes Infect 12 (2002) 1209-1215.
18. Shi PY, Wong SJ. **Serologic diagnosis of West-Nile virus infection.** Expert Rev Mol Diagn 6 (2003) 733-741.

19. Tardei G, Ruta S, Chitu V, Rossi C, Tsai TF, Cernescu C. **Evaluation of immunglobulin M (IgM) and IgG enzyme immunoassays in serologic diagnosis of West Nile virus Infection.** J. Clin. Microbiol 38 (2000) 2232-2239.

20. Rappole JH, Hubalek Z. **Migratory birds and West Nile virus.** J Appl Microbiol 94 (2003) 47-58.

21. Kilpatrick AM, Daszak P, Jones MJ, Marra PP, Kramer LD. **Host heterogeneity dominates West Nile virus transmission.** Proc Biol Sci 22 (2006) 2327-2333.

22. Prince HE, Tobler LH, Lape-Nixon M, Foster GA, Stramer SL, Busch MP. **Development and persistence of West Nile virus-specific immunoglobulin M (IgM), IgA, and IgG in viremic blood donors.** J Clin Microbiol 43 (2005) 4316-4320.

23. Iwamoto M, Jernigan DB, Guasch A, Trepka MJ, Blackmore CG, Hellinger WC, Pham SM, Zaki S, Lanciotti RS, Lance-Parker SE, DiazGranados CA, Winquist AG, Perlino CA, Wiersma S, Hillyer KL, Goodman JL, Marfin AA, Chamberland ME, Petersen LR. **West Nile Virus in Transplant Recipients Investigation Team. Transmission of West Nile virus from an organ donor to four transplant recipients.** N Engl J Med 348 (2003) 2196-2203.

24. Montgomery SP, Brown JA, Kuehnert M, Smith TL, Crall N, Lanciotti RS, Macedo de Oliveira A, Boo T, Marfin AA, 2003 West Nile Virus Transfusion-Associated Transmission Investigation Team. **Transfusion-associated transmission of West Nile virus, United States 2003 through 2005.** Transfusion 46 (2006) 2038-2046.

25. Cameron C, Reeves J, Antonishyn N, Tilley P, Alport T, Towns D, Lane D, Saldanha J. **West Nile virus in Canadian blood donors.** Transfusion 45 (2005) 487-491.

26. Anderson RC, Horn KB, Hoang MP, Gottlieb E, Bennin B. **Punctate exanthem of West Nile Virus infection: report of 3 cases.** J Am Acad Dermatol 51 (2004) 820-823.

27. O'Leary DR, Kuhn S, Kniss KL, Hinckley AF, Rasmussen SA, Pape WJ, Kightlinger LK, Beecham BD, Miller TK, Neitzel DF, Michaels SR, Campbell GL, Lanciotti RS, Hayes EB. **Birth outcomes following West Nile Virus infection of pregnant women in the United States: 2003-2004.** Pediatrics 117 (2006) 537-545.

28. Paisley JE, Hinckley AF, O'Leary DR, Kramer WC, Lanciotti RS, Campbell GL, Hayes EB. **West Nile virus infection among pregnant women in a northern Colorado community, 2003 to 2004.** Pediatrics 117 (2006) 814-820.

29. Guarner J, Shieh WJ, Hunter S, Paddock CD, Morken T, Campbell GL, Marfin AA, Zaki SR. **Clinicopathologic study and laboratory diagnosis of 23 cases with West Nile virus encephalomyelitis.** Hum Pathol 35 (2004) 983-990.

30. Bouffard JP, Riudavets MA, Holman R, Rushing EJ. **Neuropathology of the brain and spinal cord in human West Nile virus infection.** Clin Neuropathol 23 (2004) 59-61.

31. Hogrefe WR, Moore R, Lape-Nixon M, Wagner M, Prince HE. **Performance of immunoglobulin G (IgG) and IgM enzyme-linked immunosorbent assays using a West Nile virus recombinant antigen (preM/E) for detection of West Nile virus- and other flavivirus-specific antibodies.** J Clin Microbiol 42 (2004) 4641-4648.

32. Niedrig M, Sonnenberg* K, Steinhagen* K, Paweska JT (*EUROIMMUN AG). **Comparison of ELISA and immunoassays for measurement of IgG and IgM antibody to West Nile virus in human sera against virus neutralisation.** J Virol Meth in press (2006).

33. Malan AK, Martins TB, Hill HR, Litwin CM. **Evaluations of commercial West Nile virus immunoglobulin G (IgG) and IgM enzyme immunoassays show the value of continuous validation.** J Clin Microbiol 42 (2004) 727-733.

34. Malan AK, Stipanovich PJ, Martins TB, Hill HR, Litwin CM. **Detection of IgG and IgM to West Nile virus. Development of an immunofluorescence assay.** Am J Clin Pathol 119 (2003) 508-515.

35. Sanchez MD, Pierson TC, McAllister D, Hanna SL, Puffer BA, Valentine LE, Murtadha MM, Hoxie JA, Doms RW. **Characterization of neutralizing antibodies to West Nile virus.** Virology 25 (2005) 70-82.

36. EUROIMMUN AG. Stöcker W, Wessel S, Morrin M, Müller M. **Konstante Lichtquelle für die Fluoreszenzmikroskopie.** Deutsche Patentanmeldung (Offenlegungsschrift) DE 10 2006 027 518.7 (2006).

37. Stöcker* W, Fauer* H, Krause* C, Barth E, Martinez A (*EUROIMMUN AG). **Verfahren zur Optimierung der automatischen Fluoreszenzerkennung in der Immundiagnostik.** Deutsche Patentanmeldung (Offenlegungsschrift) DE 10 2006 027 516.0 und WO2007140952 (2006).

38. EUROIMMUN AG. Stöcker W. **Process for analyses to be carried out on immobilized biological tissue.** USA-Patent Nr. US4647543 (1983/1987).

39. Levett PN, Sonnenberg* K, Sidaway F, Shead S, Niedrig M, Steinhagen* K, Horsman GB, Drebot MA (*EUROIMMUN AG). **Use of Immunoglubulin G Avidity Assays for Differentiation of Primary from Previous Infections with West Nile Virus.** Journal of Clinical Microbiology Vol 43 (2005) 5873-5875.

40. Fox JL, Hazell SL, Tobler LH, Busch MP. **Immunoglobulin G avidity in differentiation between early and late antibody responses to West Nile virus.** Clin Vaccine Immunol 13 (2006) 33-36.

41. Levett PN, Sonnenberg* K, Shead S, Sidaway F, Niedrig M, Kreher* P, Arp* B, Erdmann* S, Jahn A, Steinhagen* K, Drebot M, Horsman GB (*EUROIMMUN AG). **IgG avidity determinations but not IgM tests are capable to differentiate recent from previous infections with West Nile Virus.** Poster zum 21. Annual clinical virology symposium and annual meeting Pan American society for clinical virology, USA, Florida, Clearwater beach (2005).

42. Stiasny K, Kiermayr S, Heinz FX. **Cryptic properties of a cluster of dominant flavivirus cross-reactive antigenic sites.** J Virol 80 (2006) 9557-9568.

43. Stöcker* W, Horbach E, Slenczka W, Schaper J, Böttger K, Herrmann C (*EUROIMMUN AG). **Automatische Bestückung von Objektträgern mit „BIOCHIPS“: Eine neue Technologie für die indirekte Immunfluoreszenz.** Lab. med. (1989) 171-172.

44. Sonnenberg* K, Hinrichs E, Müller-Kunert* E, Schlumberger* W, Stöcker* W (*EUROIMMUN AG). **Einsatz der BIOCHIP-Technologie in der Serologie von Influenza-, EBV- und Legionella-Infektionen.** Poster zum 3. Deutschen Kongress für Infektions- und Tropenmedizin, 15. bis 18. März 1995 in Berlin-Hohenschönhausen. Abstrakt im Kongressband (1995).

45. EUROIMMUN AG. Stöcker W. **Die BIOCHIP-Technologie: Ein neuer Weg der Produktion Festphase-gebundener Bioreagenzien für das medizinische und biologische Laboratorium.** Dokumentation zum Vorschlag des Rektors der Medizinischen Universität Lübeck für den Schmidt-Römhild-Technologiepreis 1989. Eigenverlag der Firma EUROIMMUN (1990).