Anti-Treponema pallidum ELISA (IgM)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG-CLASS	SUBSTRATE	FORMAT
EI 2111-9601 M	Treponema pallidum	IgM	Ag-coated microplate wells	96 x 01 (96)

Indication: Infection with Treponema pallidum, associated diseases: Lues.

Principles of the test: The ELISA test kit provides a semiquantitative in vitro assay for human antibodies of the IgM class against Treponema pallidum in serum or plasma. The test kit contains microtiter strips each with 8 break-off reagent wells coated with purified recombinant antigens of Treponema pallidum. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgM antibodies (also IgA and IgG) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgM (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens: 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator (IgM, human), ready for use	dark red	1 x 2.0 ml	CAL
3. Positive control (IgM, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
4. Negative control (IgM, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
5. Enzyme conjugate peroxidase-labelled anti-human IgM (goat), ready for use	red	1 x 12 ml	CONJUGATE
6. Sample buffer buffer containing IgG/RF-Absorbent (Anti-human IgG antibody preparation obtained from goat), ready for use	green	1 x 100 ml	SAMPLEBUFFER
7. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASHBUFFER 10x
8. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
9. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
10. Test instruction	---	1 booklet	
11. Protocol with reference values	---	1 protocol	
12. Protective foil	---	2 pieces	
LOT Lot	CE	Storage temperature	
IVD In vitro determination		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 should be disposed of according to official 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.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for a minimum of 4 months.

- **Calibrator and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use. The green coloured sample buffer contains IgG/RF absorbent. Serum or plasma samples diluted with this sample buffer are only to be used for the determination of IgM antibodies.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallization occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionized or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The Chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Warning: The controls and calibrators 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 skin contact.

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.

Introduction: Before a patient sample is tested for specific antibodies of the IgM class, antibodies of class IgG must be removed by ultracentrifugation, chromatography or immunoabsorption. This procedure must be carried out in order to prevent any rheumatoid factors from reacting with specifically bound IgG, which would lead to false positive IgM test results, and to prevent specific IgG displacing IgM from the antigen, which would lead to false IgM negative test results.

Functional principle: The sample buffer (green coloured!) contains an anti-human antibody preparation from goat. IgG from a serum sample is bound with high specificity by these antibodies and precipitated. If the sample also contains rheumatoid factors, these will be absorbed by the IgG/anti-human IgG complex.

Separation properties:
- All IgG subclasses are bound and precipitated by the anti-human IgG antibodies.
- Human serum IgG in concentrations of up to 15 mg per ml are removed (average serum IgG concentration in adults: 12 mg per ml).
- Rheumatoid factors are also removed.
- The recovery rate of the IgM fraction is almost 100%.

Performance: The **patient samples** for analysis are diluted **1:101** with sample buffer. For example, add 10 µl serum to 1.0 ml sample buffer and mix well. Incubate the mixture for at least **10 minutes** at room temperature. Subsequently, it can be pipetted into the microplate wells according to the pipetting protocol.

Notes:
- Antibodies of the class IgG should not be analyzed with this mixture.
- It is possible to check the efficacy of the IgG/RF absorbent for an individual patient sample by performing an IgG test in parallel to the IgM test using the mixture. If the IgG test is negative, the IgM result can be considered as reliable.
- The calibrator and controls containing IgM antibodies are pre-diluted and ready for use, do not dilute them.

Incubation

Sample incubation: (1. step)

Transfer 100 µl of the calibrator, positive and negative 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 **60 minutes** at 37°C ± 1°C.

Wash:

Manual: Remove the protective foil and empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Remove the protective foil and empty the wells and subsequently wash 3 times with 450 µl 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.

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.
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.

Conjugate incubation: (2. step)

Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgM) into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18°C to 25°C).

Wash:

Empty the wells. Wash as described above.

Substrate incubation: (3. 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	**C**	**P 6**	**P 14**	**P 22**								
B	**pos.**	**P 7**	**P 15**	**P 23**								
C	**neg.**	**P 8**	**P 16**	**P 24**								
D	**P 1**	**P 9**	**P 17**									
E	**P 2**	**P 10**	**P 18**									
F	**P 3**	**P 11**	**P 19**									
G	**P 4**	**P 12**	**P 20**									
H	**P 5**	**P 13**	**P 21**									

The above pipetting protocol is an example of the semiquantitative analysis of antibodies in 24 patient samples (P 1 to P 24).

Calibrator (C), positive (pos.) and negative (neg.) control as well as the patient samples have been incubated in one well each. The reliability of the ELISA test can be improved by duplicate determinations of each sample.
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 and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

The extinction value of the calibrator defines the upper limit of the reference range of non-infected persons (**cut-off**) recommended by EUROIMMUN. Values above the indicated cut-off are to be considered as positive, those below as negative.

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator. Use the following formula to calculate the ratio:

$$\frac{\textbf{Extinction of the control or patient sample}}{\textbf{Extinction of calibrator}} = \textbf{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0,8:	**negative**
Ratio ≥0,8 to <1,1	**borderline**
Ratio ≥1,1:	**positive**

In cases of borderline test results, an additional patient sample should be taken 7 days later and re-tested in parallel with the first patient sample. The results of both samples allow proper evaluation of titer changes.

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another the sample should be retested.

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

Test characteristics

Calibration: As no quantificated international reference serum exists for antibodies against Treponema pallidum, results are provided in the form of ratios which are a relative measure for the concentration of antibodies in the serum or plasma. The calibration is based on internal reference sera which were used in the evaluation of the test system.The international standard for Human Syphilis Serum; 1. international standard preparation SSI code number HS reacts with ratio 4.0.

For every group of tests performed, the extinction values of the calibrator and the ratio of the positive and negative controls must lie within the limits stated for the relevant test kit lot. A protocol containing these reference values is included. If the values specified for the control sera are not achieved, the test results may be inaccurate and the test should be repeated.

The activity of the enzyme used is temperature-dependent and the extinction values may vary if a thermostat is not used. The higher the room temperature during substrate incubation, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibration sera are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigens: The microplate wells were coated with a mixture of four antigens of Treponema pallidum: p15, p17, p47 and TmpA. The corresponding cDNAs were expressed in E. coli as recombinant antigens.

Detection limit: The detection limit is defined as a value of three times the standard deviation of an analyte-free sample and is the smallest detectable antibody titer. The lower detection limit of the Anti-Treponema pallidum ELISA (IgM) is a ration value of 0.06.

Cross reactivity: The quality of the antigen used ensures a high specificity and sensitiviy of the ELISA. Sera from patients with infections caused by various agents were investigated with the Anti-Treponema pallidum ELISA (IgM). This ELISA showed no cross reactivity.

Parameter	CMV	Measles	Mumps	Toxoplasma gondii	VZV
n	9	8	7	5	8
Anti-Treponema pallidum ELISA (IgM)	0	0	0	0	0

Interference: Haemolytic, lipaemic and icteric samples showed no influence at the result up to a concentration of 10 mg/ml for hemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation, n = 20		
Serum	**Mean value (Ratio)**	**CV (%)**
1	1.1	4.4
2	3.3	4.2
3	6.1	3.9

Inter-assay variation, n = 4 x 6		
Serum	**Mean value (Ratio)**	**CV (%)**
1	1.2	5.8
2	3.5	5.4
3	6.2	4.6

Specificity and sensitivity: 30 clinically characterized patient samples (Interlaboratory test samples of the INSTAND, Germany) were examined with the EUROIMMUN Anti-Treponema pallidum ELISA (IgM). The test showed a specificity of 100 % and a sensitivity of 100 %. Values for 1 of the samples were borderline and were not included in the calculation.

n = 30		INSTAND / LABQUALITY		
		positive	borderline	negative
EUROIMMUN ELISA	positive	8	0	0
	borderline	0	0	0
	negative	0	1	21

Reference range: The levels of the anti-Treponema pallidum antibodies (IgM) were analysed with this EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off ratio of 1.0, 0.4 % of the blood donors were anti-Treponema pallidum positive (IgM) which reflects the known percentage of infections in adults.

Clinical significance

Treponema pallidum is a helically wound bacteria of the Spirochaeta family [1]. This family includes five genera: borrelia, spirochaeta, cristispira, treponema and leptospira. Treponema pallidum is the causative agent of syphilis or lues, a chronic infectious disease. The subspecies T. pallidum endemicum causes veneric syphilis; T. pallidum pertenue leads to a non-veneric infection occuring in tropical regions called framboesia; T. pallidum carateum is the causative agent of Pinta [2, 3, 4].

In 1905 Fritz Schaudinn (German zoologist, 1871-1906) and Erich Hoffmann (German dermatologist, 1868-1959) at the Charité in Berlin were the first to detect the causative agent of syphilis under the microscope. Spirochaeta were first found In 1913 by the Japanese microbiologist and physician Noguchi Hideyo (1876-1928) in the brain tissue of a patient with progressive paralysis [1].

Syphilis is transmitted from human to human during sexual acts via the mucosa [1, 5]. Indirect transmission by blood transfusions and wounds is also possible. During pregnancy and at birth the baby can become infected by the mother (syphilis connata) [1, 6, 7, 8, 9]. Syphilis is a known risk factor for abortions and stillbirths [7, 10].

Primary stage: The ulcus durum (hard-edged ulcer) is characteristic of the primary lesion of the syphilis (stage I) and normally occurs 3 weeks after infection, developing at the place of entry of the virus (e.g. penis) [1]. It is a painless ulcer, which contains large quantities of the pathogen and is therefore highly contagious. Typically, the defined, limited fibrous or crusted erosion has a raised hard edge. The possible swelling of the regional lymph nodes is painless and the lymph nodes remain displaceable. From that time on, the disease can be diagnosed e.g. using the TPHA test (Treponema pallidum haemagglutination assay). After 2 to 6 weeks the ulcer heals leaving a scar. The infection generally persists and develops into stage II [1].

Secondary stage: Approximately 8 weeks after the infection, the disease manifests itself with flu-like symptoms such as fever, fatigue or head and joint pains. In addition to a generalised swelling of the lymph nodes, 90% of patients show local or generalised skin disorders, which are accompanied by weak or no itching. At first, light pink patches form, which further evolve into hard, coppery nodules (papules). In the foreground are condylomata lata, broad papules, which mainly affect skin folds [1]. The liquid excreted by open and weeping papules is highly contagious. Additionally, various organ disorders may develop, for example, ketaritis, irititis, hepatitis, vasculitis, and myocardial disorders [1].

All skin disorders (syphilids) heal after approximately 4 months. Secondary syphilis is followed by a clinically silent stage (syphilis latens), which can last for years [1].

Tertiary stage: Typical manifestations of a Treponema pallidum infection in stage III are large papules and ulcers on the skin and mucous membranes, as well as organ or visceral syphilis, including gummatous and interstitial inflammation, perivasculitis, cardiovascular syphilis, neurosyphilis (asymtomatic and symptomatic form), osteitis, and periosteitis.

Quaternary stage: Ten to thirty years after an untreated infection, 8% to 10% of patients experience severe neurological disorders such as neurosyphilis with progressive paralysis and Tabes dorsalis with severe mental and vegetational disorders [1].

The **diagnosis** of syphilis is based on clinical findings according to the disease stage, microscopic detection of the infectious agent (dark field), and the serological detection of antibodies against Treponema pallidum [1].

Treponema pallidum pallidum has a length of 5-15µm and a width of 0.2µm with 10-20 turns and can rotate around its longitudinal axis [1]. Due to its fine structure, it is difficult to be made visible under the microscope by staining. However, living bacteria can be investigated using dark field microscopy. Detection in cultures has not yet been achieved [1].

The TPHA (Treponema pallidum haemagglutination assay) is an assay for the indirect determination of antibodies against Treponema pallidum. Erythrocytes marked with proteins and polysaccharides of Treponema pallidum on their surface are mixed with patient serum. The presence of antibodies against Treponema pallidum in the patient serum causes agglutination of the erythrocytes (haemagglutination), which is visible to the naked eye [1, 11, 12].

If the screening test is positive, additional testing is recommended e.g. using an FTA-ABS assay. For further serological diagnosis, modern serological test methods such as anti-Treponema pallidum ELISA, Westernblot, e.g. EUROLINE WB, and IIFT have become widely accepted and have proven successful [1, 7, 8, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34]. Antibodies can be found in the serum and CSF, particularly in children having congenital syphilis [6, 8, 9, 20, 35]. Antibodies against cardiolipin serve as an activity marker of the infection (VDRL or RPR test, EUROLINE WB) [36, 37, 38, 39, 40, 41, 42].

Literature references

1. Hahn H, Miksits K. **Treponemen.** In: Hahn, Falke, Kaufmann, Ullmann. Medizinische Mikrobiologie und Infektiologie. Springer 5. Auflage (2005).
2. Centurion-Lara A, Molini BJ, Godornes C, Sun E, Hevner K, Van Voorhis WC, Lukehart SA. **Molecular differentiation of Treponema pallidum subspecies.** J Clin Microbiol 44 (2006) 3377-3380.
3. Leader BT, Hevner K, Molini BJ, Barrett LK, Van Voorhis WC, Lukehart SA. **Antibody responses elicited against the Treponema pallidum repeat proteins differ during infection with different isolates of Treponema pallidum subsp. Pallidum.** Infect Immun 71 (2003) 6054-6057.
4. Sun ES, Molini BJ, Barrett LK, Centurion-Lara A, Lukehart SA, Van Voorhis WC. **Subfamily I Treponema pallidum repeat protein family: sequence variation and immunity.** Microbes Infect 6 (2004) 725-737.
5. Pajaro MC, Barberis IL, Godino S, Pascual L, Aguero M. **Epidemiology of sexually transmitted diseases in Rio Cuarto, Argentina.** Rev Latinoam Microbiol 43 (2001) 157-160.
6. Sanchez PJ, Wendel GD, Norgard MV. **IgM antibody to Treponema pallidum in cerebrospinal fluid of infants with congenital syphilis.** Am J Dis Child 146 (1992) 1171-1175.
7. Pankratov OV, Saluk YV, Klimova LV. **Epidemiology of syphilis in pregnant women and congenital syphilis in Belarus.** Acta Dermatovenerol Alp Panonica Adriat 15 (2006) 35-38.
8. Michelow IC, Wendel GD Jr, Norgard MV, Zeray F, Leos NK, Alsaadi R, Sanchez PJ. **Central nervous system infection in congenital syphilis.** N Engl J Med 346 (2002) 1792-1798.

9. Rawstron SA, Mehta S, Bromberg K. **Evaluation of a Treponema pallidum-specific IgM enzyme immunoassay and Treponema pallidum western blot antibody detection in the diagnosis of maternal and congenital syphilis.** Sex Transm Dis 31 (2004) 123-126.
10. Folgosa E, Osman NB, Gonzalez C, Hagerstrand I, Bergstrom S, Ljungh A. **Syphilis seroprevalence among pregnant women and its role as a risk factor for stillbirth in Maputo, Mozambique.** Genitourin Med 72 (1996) 339-342.
11. Castro R, Prieto ES, Joao Aguas M, Jose Manata M, Botas J, Araujo C, Borges F, Aldir I, Exposto Fda L. **Evaluation of the Treponema pallidum particle agglutination technique (TP.PA) in the diagnosis of neurosyphilis.** J Clin Lab Analm20 (2006) 233-238.
12. Manavi K, Young H, McMillan A. **The sensitivity of syphilis assays in detecting different stages of early syphilis.** Int J STD AIDSm17 (2006) 768-771.
13. Lewis LL, Taber LH, Baughn RE. **Evaluation of immunoglobulin M western blot analysis in the diagnosis of congenital syphilis.** J Clin Microbiol 28 (1990) 296-302.
14. Kaiser R, Rauer S. **Serodiagnosis of neuroborreliosis: comparison of reliability of three confirmatory assays.** Infectionm27 (1999) 177-182.
15. Lewis LL, Taber LH, Baughn RE. **Evaluation of Immunoglobulin M Western Blot Analysis in the Diagnosis of Congenital Syphilis.** J Clin Microbiol 28 (1990) 296-302.
16. Muller F. **Treponema pallidium IgM enzyme-linked immunosorbent assay (TP-IgM-ELISA). Determination of organism-specific Treponema IgM antibodies in the serum of syphilis patients with and without central nervous system involvement of the infection.** [Article in German] Z Hautkr 58 (1983) 1689-1708.
17. Merlin S, Poncet F, Alacoque B, Guinet R, Andre J. **Treponemal antigens detected by immunoblotting with serum and CSF antibodies of neurosyphilitic patients.** Ann Inst Pasteur Microbiol 138 (1987) 709-717.
18. Rawstron SA, Mehta S, Bromberg K. **Evaluation of a Treponema pallidum-specific IgM enzyme immunoassay and Treponema pallidum western blot antibody detection in the diagnosis of maternal and congenital syphilis.** Sex Transm Dis 31 (2004) 123-126.
19. Kaiser R, Rauer S. **Serodiagnosis of neuroborreliosis: comparison of reliability of three confirmatory assays.** Infection 27 (1999) 177-182.
20. Schmitz JL, Gertis KS, Mauney C, Stamm LV, Folds JD. **Laboratory diagnosis of congenital syphilis by immunoglobulin M (IgM) and IgA immunoblotting.** Clin Diagn Lab Immunol 1 (1994) 32-37.
21. Marangoni A, Sambri V, Storni E, D'Antuono A, Negosanti M, Cevenini R. **Treponema pallidum surface immunofluorescence assay for serologic diagnosis of syphilis.** Clin Diagn Lab Immunol 7 (2000) 417-421.
22. Muller I, Brade V, Hagedorn HJ, Straube E, Schorner C, Frosch M, Hlobil H, Stanek G, Hunfeld KP**. Is serological testing a reliable tool in laboratory diagnosis of syphilis? Meta-analysis of eight external quality control surveys performed by the german infection serology proficiency testing program.** J Clin Microbiol 44 (2006) 1335-1341.
23. Meyer W, Scheper T, Lehmann Heike (Erfinder). **Selbstklebende Blotmembran.** EUROIMMUN Medizinische Labordiagnostika AG. Patent. Aktenzeichen 20215268.5 (2002).
24. Meyer W, Scheper T (Erfinder). **Vorrichtung zur Antikörperdiagnose mit kombinierten Membranen.** EUROIMMUN Medizinische Labordiagnostika AG. Patent. Aktenzeichen 20215270.7 (2002).
25. Morrin M, Rateike Martin, Müller M (Erfinder). **Lichtquelle für ein Auflichtfluoreszenzmikroskop.** EUROIMMUN Medizinische Labordiagnostika AG. Patent. Aktenzeichen 202004010121.1 (2004).
26. Müller M, Wessel S, Morrin M (Erfinder**). Konstante Lichtquelle für die Fluoreszenzmikroskopie.** EUROIMMUN Medizinische Labordiagnostika AG. Patent. Aktenzeichen DE 10 2006 027 518.7 (2006).

27. Stöcker W, Rateike M, Morrin M. (Erfinder). **Verfahren zur Herstellung Festphasen-gebundener Bioreagenzien.** EUROIMMUN Medizinische Labordiagnostika AG. Patent. Aktenzeichen PCT/EP2005/000974 - EP 1718 948 (2005).

28. Young H, Moyes A, McMillan A, Patterson J**. Enzyme immunoassay for anti-treponemal IgG: screening or confirmatory test?** J Clin Pathol 45 (1992) 37-41.

29. Enders M, Hagedorn HJ. **Syphilis in pregnancy.** [Article in German] Z Geburtshilfe Neonatol 206 (2002) 131-137.

30. Hagedorn HJ. **MiQ 16/2001 Qualitätsstandards in der mikrobiologisch-infektiologischen Diagnostik: Syphilis.** Urban & Fischer Verlag München Jena. (2001).

31. Hagedorn HJ. **Aktuelle Aspekte der Syphilisdiagnostik.** Immun Infekt 21 (1993) 94-99.

32. Hagedorn HJ. **Serodiagnostik der Syphilis.** Diagnose u. Labor 45 (1995) 94-104.

33. Müller F, Hagedorn HJ. **Syphilis.** In: Thomas L (Hrsg) Labor und Diagnose. TH-Books Frankfurt/Main (1998) 1232-1241.

34. Schmidt BL, Edjlalipour M, Luger A**. Comparative evaluation of nine different enzyme-linked immunosorbent assays for determination of antibodies against Treponema pallidum in patients with primary syphilis.** J Clin Microbiol 38 (2000) 1279-1282.

35. Meyer PM, Eddy T, Baughn RE. **Analysis of Western Blotting (Immunoblotting) Technique in Diagnosis of Congenital Syphilis.** J Clin Microbiol 32 (1994) 629-633.

36. Angue Y, Yauieb A, Mola G, Duke T, Amoa AB. **Syphilis serology testing: a comparative study of Abbot Determine, Rapid Plasma Reagin (RPR) card test and Venereal Disease Research Laboratory (VDRL) methods.** P N G Med J 48 (2005) 168-173.

37. Dang Q, Feng J, Lu X, Zhang X, Xu H, Liu C, Nu X**. Evaluation of specific antibodies for early diagnosis and management of syphilis.** Int J Dermatol 45 (2006) 1169-1171.

38. Stöcker W. TITERPLANE **Vorrichtung zur Durchführung von Mikroanalysen.** Europäisches Patent Nr. EP 0 018 435 (1979). Übersetzt ins Englische, Französische und Schwedische.

39. Stöcker W. TITERPLANE **Apparatus and method for simultaneously mixing specimens for performing microanalyses.** USA-Patent Nr. 4,339,241 (1980/1982); patentiert auch in Japan.

40. Mabey D, Peeling RW, Ballard R, Benzaken AS, Galban E, Changalucha J, Everett D, Balira R, Fitzgerald D, Joseph P, Nerette S, Li J, Zheng H. **Prospective, multi-centre clinic-based evaluation of four rapid diagnostic tests for syphilis.** Sex Transm Infect 82 (2006) 13-16.

41. Serwin AB, Kohl PK, Chodynicka B. **The centenary of Wassermann reaction--the future of serological diagnosis of syphilis, up-to-date studies.** [Article in Polish] Przegl Epidemiol 59 (2005) 633-640.

42. Cervera R, Asherson RA. **Antiphospholipid syndrome associated with infections: clinical and microbiological characteristics.** Immunobiology 210 (2005) 735-741.