

**AccuDiag™**  
**Lyme Disease IgG**  
**ELISA**

**Cat# 1428-2**



<b>Test</b>	<b>Lyme Disease IgG</b>
<b>Method</b>	<b>Enzyme Linked Immunosorbent Assay</b>
<b>Principle</b>	<b>Indirect ELISA : Antigen Coated Plate</b>
<b>Detection Range</b>	<b>Qualitative: Positive, Negative Control &amp; Cut Off</b>
<b>Sample</b>	<b>10µL serum</b>
<b>Specificity</b>	<b>96.40%</b>
<b>Sensitivity</b>	<b>91.70%</b>
<b>Total Time</b>	<b>~ 70 min</b>
<b>Shelf Life</b>	<b>12-18 Months from the manufacturing date</b>

**INTENDED USE**

The Diagnostic Automation B. burgdorferi IgG ELISA Test System is an enzyme-linked immunosorbent assay (ELISA) for the qualitative presumptive detection of IgG antibodies to Borrelia burgdorferi in human serum. This test should only be used for patients with signs and symptoms that are consistent with Lyme disease. Equivocal or positive results must be supplemented by testing with a standardized Western blot procedure. Positive supplemental results are supportive evidence of exposure to B. burgdorferi and can be used to support a clinical diagnosis of Lyme disease.

**SUMMARY AND EXPLANATION**

Borrelia burgdorferi is a spirochete that causes Lyme disease. Ticks of the genus Ixodes transmit the organism. In endemic areas, these ticks reside on vegetation and animals such as deer, mice, dogs, horses, and birds. B. burgdorferi infection shares features with other spirochetal infections (diseases caused by three genera in humans: Treponema, Borrelia, and Leptospira). Skin is the portal of entry for B. burgdorferi and the tick bite often causes a characteristic rash called erythema migrans (EM). EM develops around the tick bite in 60 - 80% of patients. Spirochetemia occurs early with wide spread dissemination through tissue and body fluids.

Lyme disease occurs in stages, often with intervening latent periods and with different clinical manifestations. In Lyme disease there are generally three stages of disease often with overlapping symptoms. Symptoms vary according to the sites affected by the infection such as joints, skin, central nervous system, heart, eye, bone, spleen, and kidney. Late disease is most often associated with arthritis or CNS syndromes. Asymptomatic subclinical infection is possible and infection may not become clinically evident until the later stages.

Patients with early infection produce IgM antibodies during the first few weeks after onset of EM and produce IgG antibodies more slowly (1). Tests only detect IgM during the first month after onset of illness. The majority of patients develop IgG antibodies within one month. Both IgG and IgM antibodies can remain detectable for years.

Isolation of B. burgdorferi from skin biopsy, blood, and spinal fluid has been reported (2). However, these direct culture detection methods may not be practical in the large scale diagnosis of Lyme borreliosis. Serological testing methods for antibodies to B. burgdorferi include indirect fluorescent antibody (IFA) staining, immunoblotting, and enzyme immunoassay (EIA).

B. burgdorferi is antigenically complex with strains that vary considerably. Early antibody responses often are to flagellin that has cross reactive components. Patients in early stages of infection may not produce detectable levels of antibody. In addition, early antibiotic therapy after EM may diminish or abrogate good antibody response. Some patients may never generate detectable antibody levels. Thus, serological tests for antibodies to B. burgdorferi are known to have low sensitivity and specificity and because of such inaccuracy, these tests cannot be relied upon for establishing a diagnosis of Lyme disease (3, 4).

In 1994, the Second National Conference on Serological diagnosis of Lyme disease recommended a two-step testing system toward standardizing laboratory serologic testing for B. burgdorferi. Because EIA and IFA methods were not sufficiently specific to support clinical diagnosis, it was recommended that positive or equivocal results from a sensitive EIA or IFA (first step) should be further tested, or supplemented, by using a standardized Western Blot method (second step) for detecting antibodies to B. burgdorferi (Western Blot assays for antibodies to B. burgdorferi are supplemental rather than confirmatory because their specificity is less than optimal, particularly for detecting IgM). Two-step positive results provide supportive evidence of exposure to B. burgdorferi, which could support a clinical diagnosis of Lyme disease but should not be used as a sole criterion for diagnosis.

**TEST PRINCIPLE**

The Diagnostic Automation B. burgdorferi ELISA test system is designed to detect IgG class antibodies to B. burgdorferi in human sera. Wells of plastic microwell strips are sensitized by passive adsorption with B. burgdorferi antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgG (Fc chain specific) is added to the wells and the plate is incubated. The Conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

**SPECIMEN COLLECTION AND PREPARATION**

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease.
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay. No anticoagulants or



preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.

4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2° and 8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

2. Determine the number of microwells needed. Allow six Control/Calibrator determinations (one Blank, one Negative Control, three Calibrators and one Positive Control) per run. A Reagent Blank should be run on each assay. Check software and reader requirements for the correct Controls/ Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2° and 8°C.

## MATERIALS AND COMPONENTS

### Materials provided with the test kits

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label. **Note: All reactive reagents contain sodium azide as a preservative at a concentration of 0.1% (w/v): Controls, and Calibrators.**

1. **Plate.** 96 wells configured in twelve 1x8-well strips coated with inactivated B. burgdorferi B31 strain antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
2. **Conjugate.** Conjugated (horseradish peroxidase) goat anti-human IgG . Ready to use. One, 15 mL vial with a white cap.
3. **Positive Control** (Human Serum). One, 0.35 mL vial with a red cap.
4. **Calibrator** (Human Serum). One, 0.5 mL a blue cap.
5. **Negative Control** (Human Serum). One, 0.35 mL vial with a green cap.
6. **Sample Diluent.** One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate- buffered-saline.Green solution, ready to use.
7. **TMB:** One 15 mL amber bottle (amber cap) containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use.
8. **Stop solution:** One 15 mL bottle (red cap) containing 1M H<sub>2</sub>SO<sub>4</sub>, 0.7M HCl. Ready to use.
9. **Wash buffer concentrate (10X):** dilute 1 part concentrate + 9 parts deionized or distilled water. One 100 mL bottle (clear cap) containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). **NOTE: 1X solution will have a pH of 7.2 ± 0.2.**

The following components are not kit lot number dependent and may be used interchangeably with the ELISA assays: TMB, Stop Solution, and Wash Buffer.

### Note: Kit also contains:

1. Component list containing lot specific information is inside the kit box.
2. Package insert providing instructions for use.

### Materials required but not provided

1. ELISA microwell reader capable of reading at a wavelength of 450nm.
2. Pipettes capable of accurately delivering 10 to 200µL.
3. Multichannel pipette capable of accurately delivering (50-200µL).
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
10. Paper towels.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant. (example: 10% household bleach, 0.5% sodium hypochlorite.)

## ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20-25°C).

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 3
B	Neg. Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Pos. Control	
G	Patient 1	
H	Patient 2	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum. The Sample Diluent will undergo a color change confirming that the specimen has been combined with the diluent.
4. To individual wells, add 100µL of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.
6. Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5X.

### A. Manual Wash Procedure:

- a. Vigorously shake out the liquid from the wells.
- b. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
- c. Repeat steps a. and b. for a total of 5 washes.
- d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with disinfectant at the end of the day's run.

### B. Automated Wash Procedure:

If using an automated microwell wash system, set the dispensing volume to 300-350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.

8. Add 100µL of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
9. Incubate the plate at room temperature (20-25°C) for 25 + 5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
12. Incubate the plate at room temperature (20-25°C) for 10 to 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after the addition of the Stop Solution.

## RESULTS

### A. Calculations:

#### 1. Correction Factor

A cutoff OD value for positive samples has been determined by the manufacturer and correlated to the Calibrator. The correction factor (CF) will allow you to determine the cutoff value for positive samples and to correct for slight day-to-day variations in test results. The correction factor is determined for each lot of kit components and is printed on the Component List located in the kit box.

#### 2. Cutoff OD Value

To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator determined above.

$$(CF \times \text{mean OD of Calibrator} = \text{cutoff OD value})$$

#### 3. Index Values or OD Ratios

Calculate the Index Value or OD Ratio for each specimen by dividing its OD value by the cutoff OD from step 2.

Example:	
Mean OD of Calibrator	0.793
Correction Factor (CF)	0.25
Cut off OD	$0.793 \times 0.25 = 0.198$
Unknown Specimen OD	0.432
Specimen Index Value or OD Ratio	$0.432 / 0.198 = 2.18$

### B. Interpretations:

Index Values or OD ratios are interpreted as follows:

	Index Value or OD Ratio
Negative Specimens	$\leq 0.90$
Equivocal Specimens	0.91 to 1.09
Positive Specimens	$\geq 1.10$

- An OD ratio  $< 0.90$  indicates no significant amount of antibodies to B. burgdorferi IgG detected. If exposure to B. burgdorferi is suspected, a second sample should be collected and tested two to four weeks later (15).
- An OD ratio  $> 1.10$  indicates that antibodies specific to B. burgdorferi were detected. This indicates presumptive evidence of probable exposure. Per current recommendations, the result cannot be further interpreted without supplemental Western Blot testing. Western Blot assays for antibodies to B. burgdorferi are supplemental rather than confirmatory because their specificity is less than optimal, particularly for detecting IgM. Do not report results until the supplemental testing is completed.
- Specimens with OD ratio values in the equivocal range (0.91 - 1.09) should be retested in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal specimen by the second step IgG and/or IgM Western blot.

## QUALITY CONTROL

1. Each time the assay is run the Calibrator must be run in triplicate. A reagent blank, Negative Control, and Positive Control must also be included in each assay.
2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
3. The mean OD value for the Calibrator and the OD values for the Positive and Negative Controls should fall within the following ranges:

#### OD Range

Negative Control	$\leq 0.250$
Calibrator	$\geq 0.300$
Positive Control	$\geq 0.500$

- a. The OD of the Negative Control divided by the mean OD of the Calibrator should be  $\leq 0.9$ .
  - b. The OD of the Positive Control divided by the mean OD of the Calibrator should be  $\geq 1.25$ .
  - c. If the above conditions are not met the test should be considered invalid and should be repeated.
4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.
  5. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
  6. Refer to NCCLS document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices.

## PERFORMANCE CHARACTERISTICS

### A. Comparative Study

The Diagnostic Automation, Inc. B. burgdorferi antibody IgG ELISA test system was compared to a commercially available indirect fluorescent antibody (IFA) test

procedure for detection of IgG antibodies to B. burgdorferi. Two hundred seven (207) serum samples were tested by the two procedures. The results of this study are summarized in Table 1 below:

		B. Burgdorferi IFA Procedure	
		Pos.	Neg.
Diagnostic Automation, Inc. B. burgdorferi IgG ELISA	Pos.	54	12
	Neg.	2	133
	Equivocal *	1	5

**Relative Sensitivity** = 96.4 % (54/56)

**Relative Specificity** = 91.7 5 (133/145)

**Concordance** = 93.0 % (187/201)

\*Equivocal samples were excluded from the calculations for sensitivity, specificity, and concordance.

Table 2 shows test results obtained using a serum panel from the CDC. The results are presented as a means to convey further information on the performance of this assay with a masked, characterized serum panel. This does not imply an endorsement of the assay by the CDC.

#### The CDC B. burdorferi Disease Serum Panel Stratified by Time After Onset

Time from onset	Pos	+ / -	Neg.	Total	% agreement with clinical diagnosis
Normals	0	0	5	5	100%
< 1 month	1	1	4	6	20%
1-2 months	2	1	5	8	28%
3-12- months	8	3	9	20	47%
> 1 year	8	0	0	8	100%
Total	19	5	23	47	57%



**B. Reproducibility**

To assess intra-an inter-assay variation of the test procedure seven serum samples ranging from negative to high positive were tested by the Diagnostic Automation, Inc. Lyme IgG ELISA. Eight replicates of each sample were tested on three consecutive days. The mean OD ratio and coefficient of variation (CV) were calculated for each sample. These data are shown below:

Serum #	Intra-Assay (n= 8)						Inter-Assay (n= 3)	
	Day # 1		Day # 2		Day # 3		Mean Ratio	CV
	Mean Ratio	CV	Mean Ratio	CV	Mean Ratio	CV		
Serum # 1	1.75	8.2%	1.76	6.0%	1.50	9.6%	1.67	7.2%
Serum # 2	1.22	3.9%	1.21	6.5%	1.12	3.4%	1.18	3.8%
Serum # 3	4.25	2.8%	4.30	6.7%	3.84	2.1%	4.13	5.0%
Serum # 4	2.73	3.5%	3.14	2.1%	2.53	4.1%	2.80	9.1%
Serum # 5	2.69	2.5%	2.97	7.1%	2.64	4.3%	2.77	15.2%
Serum # 6	0.49	5.5%	0.60	5.1%	0.59	8.1%	0.56	8.9%
Serum # 7	0.35	7.4%	0.43	2.1%	0.39	8.1%	0.39	8.4%

**C. Cross Reactivity**

Serum specimens from patients with North American borreliosis, yaws, pinta, leptospirosis, autoimmune diseases, and syphilis may crossreact.

**LIMITATIONS OF PROCEDURE**

1. Sera from patients with other spirochetal diseases (syphilis, yaws, pinta, leptospirosis, and relapsing fever), or infectious mononucleosis and systemic lupus erythematosus may give false positive results (8, 9). In cases where false positive reactions are observed, extensive clinical epidemiologic and laboratory workups should be carried out to determine the specific diagnosis. False positive sera from syphilis patients can be identified by running an RPR and a treponemal antibody assay on such specimens (10). True B. burgdorferi disease positive sera will be negative in these assays.
2. False negative results may be obtained if serum samples are drawn too early after onset of disease before antibody levels have reached significant levels (8). Also, early antibiotic therapy may abort an antibody response to the spirochete (11).
3. Interpret all data in conjunction with clinical symptoms of disease, epidemiologic data, exposure in endemic areas, and results of other laboratory tests.
4. Do not perform screening of the general population. The positive predictive value depends on the pretest likelihood of infection. Only perform testing when clinical symptoms are present or exposure is suspected.
5. The performance characteristics of the DAI ELISA B. burgdorferi IgG Test System are not established with samples from individuals vaccinated with B. Burgdorferi antigens.

**EXPECTED RESULTS**

Only 10 - 40% of patients with EM alone have detectable levels of antibody to B. burgdorferi (1, 12, and 13). The IgM response usually peaks from three to six weeks following infection and is often not detectable during the first two weeks of infection. The IgG antibody response is frequently not detectable for four to six weeks after infection. A more complete serological picture may be obtained by testing acute and convalescent sera. Most patients (94-97%) with neurological complications and essentially all patients with arthritis have elevated IgG titers to the spirochete (12, 14). In later stages, a positive antibody test may help distinguish Lyme disease from viral meningitis or unexplained nerve palsies. A positive antibody test may be particularly useful in differentiating Lyme arthritis from rheumatoid arthritis, juvenile arthritis and

Reiter's Syndrome (11). Patients without signs or clinical features of Lyme disease should test negative with the DAI ELISA B. Burgdorferi IgG Test System.

**PRECAUTIONS**

1. For In Vitro diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, consider the strips potentially biohazardous materials and handle accordingly.
4. The Controls are potentially biohazardous materials. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, handle these products at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": Current Edition; and OSHA's Standard for Bloodborne Pathogens (5).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The Sample Diluent, Controls, Conjugate and Wash Buffer contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions upon hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Do not use reagents from other sources or manufacturers.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with Conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.



25. Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate's enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.

**STORAGE**

1. Store the unopened kit between 2° and 8°C.
2. Coated microwell strips: Store between 2° and 8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage. Strips are stable for 60 days after the envelope has been opened and properly resealed and the indicator strip on the desiccant pouch remains blue.
3. Conjugate: Store between 2° and 8°C. DO NOT FREEZE.
4. Calibrator, Positive Control and Negative Control: Store between 2° and 8°C.
5. TMB: Store between 2° and 8°C.
6. Wash Buffer concentrate (10X): Store between 2° and 25°C. Diluted wash buffer (1X) is stable at room temperature (20° to 25° C) for up to 7 days or for 30 days between 2° and 8°C.
7. Sample Diluent: Store between 2° and 8°C.
8. Stop Solution: Store between 2° and 25°C.

<p><b>ISO 13485</b> <b>ISO 9001</b></p>  <p><b>Diagnostic Automation/ Cortez Diagnostics, Inc.</b>  <b>23961 Craftsman Road, Suite E/F, Calabasas, California 91302 USA</b></p>			
<b>Date Adopted</b>	<b>Cat # 1428-2-FD</b>		
<b>2013-04-29</b>	<b>AccuDiag™- Lyme Disease IgG ELISA -2013</b>		
<table border="1"> <tr> <td style="padding: 2px;">EC</td> <td style="padding: 2px;">REP</td> </tr> </table>	EC	REP	<p><b>CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands.</b>  <a href="http://www.cepartner4u.eu">www.cepartner4u.eu</a></p>
EC	REP		
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