success of antimicrobial therapy. Logical monitoring could thus also be employed to control the usual therapy with antacidic and H2 blocking agents. Sero-kill the germs, and these should complement or replace the interest for the therapy. Bismuth salts and antibiotics use to found. The detection of Helicobacter pylori is of remarkable Western Blot, it should be possible to diagnose an acute in-bodies against specific proteins of Helicobacter pylori in the lins in ELISA and by the detection of IgG, IgA and IgM antibodies against specific proteins of Helicobacter pylori can also be detected sero-logically by the performance of an enzyme immunoassay (EIA) or by Western Blot. Patients with confirmed exposi-tion to Helicobacter pylori often show a positive serological result. Since antibodies persist for a longer time after a Helicobacter pylori infection, seropositive individuals are also found among symptom-free patients. The ratio of seropositive values rises with age. By the determination of immunoglobulins in ELISA and by the detection of IgG, IgA and IgM antibo-dies against specific proteins of Helicobacter pylori in the Western Blot, it should be possible to diagnose an acute in-fection with Helicobacter pylori, even if no germs can be found. The detection of Helicobacter pylori is of remarkable interest for the therapy. Bismuth salts and antibiotics use to kill the germs, and these should complement or replace the usual therapy with antacidic and H2 blocking agents. Sero-logical monitoring could thus also be employed to control the success of antimicrobial therapy.
REAGENTS PROVIDED

Store kit components at 2-8°C and do not use after the expiry date on the box outer label. Before use, all components should be allowed to warm up to ambient temperature (18-25°C). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. The opened kit should be used within three months.

**Microtiter Strips:** 12 strips with 8 breakable wells each, coated with a purified natural Helicobacter pylori antigen (contains Cag, VAC, Urease A + B). Ready-to-use.

**Calibrator A (Negative Control):** 2 mL, protein solution diluted with PBS, contains no IgG antibodies against Helicobacter. Addition of 0.01% methylisothiazolone and 0.01% bromonitrodioxane. Ready-to-use.

**Calibrator B (Cut-Off Standard):** 2 mL, human serum diluted with PBS, contains a low concentration of IgG antibodies against Helicobacter. Addition of 0.01% methylisothiazolone and 0.01% bromonitrodioxane. Ready-to-use.

**Calibrator C (Weak Positive Control):** 2 mL, human serum diluted with PBS, contains a moderate concentration of IgG antibodies against Helicobacter. Addition of 0.01% methylisothiazolone and 0.01% bromonitrodioxane. Ready-to-use.

**Calibrator D (Positive Control):** 2 mL, human serum diluted with PBS, contains a high concentration of IgG antibodies against Helicobacter. Addition of 0.01% methylisothiazolone and 0.01% bromonitrodioxane. Ready-to-use.

**Enzyme Conjugate:** 15 mL, anti-human-IgG-HP (rabbit), in protein-containing buffer solution. Addition of 0.01% methylisothiazolone and 0.01% bromonitrodioxane. 5 mg/L Proclin™. Ready-to-use.

**Substrate:** 15 mL, TMB (tetramethylbenzidine). Ready-to-use.

**Stop Solution:** 15 mL, 0.5 M sulfuric acid. Ready-to-use.

**Sample Diluent:** 60 mL, PBS/BSA buffer. Addition of 0.095% sodium azide. Ready-to-use.

**Washing Buffer:** 60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

**Plastic Foils:** 2 pieces to cover the microtiter strips during the incubation.

**Plastic Bag:** Resealable, for the dry storage of non-used strips.

ASSAY PROCEDURE

1. **Reagent And Sample Preparation**
   **Washing Solution:** dilute before use 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.
   1) Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
   2) All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
   3) Calibrators and samples should be assayed in duplicates.
   4) A calibration curve should be established with each assay.
   5) Return the unused microtiter strips to the plastic bag and store them dry at 4-8°C.

2. **Assay Steps**
   1) Prepare a sufficient amount of microtiter wells for the calibrators, controls and samples in duplicate as well as for a substrate blank.
   2) Pipet 100 μL each of the diluted (1:101) samples and the ready-to-use calibrators and controls respectively into the wells. Leave one well empty for the substrate blank.
   3) Cover plate with the enclosed foil and incubate at room temperature for 60 minutes.
   4) Empty the wells of the plate (dump or aspirate) and add 300 μL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
   5) Pipet 100 μL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.
   6) Cover plate with the enclosed foil and incubate at room temperature for 30 minutes.
   7) Empty the wells of the plate (dump or aspirate) and add 300 μL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
   8) Pipet 100 μL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.
   9) Cover plate with the enclosed foil and incubate at room temperature for 20 minutes in the dark (e.g. drawer).
   10) To terminate the substrate reaction, pipet 100 μL each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.
   11) After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.
**EVALUATION**

The mean values for the measured absorptions are calculated after subtraction of the substrate blank value. The difference between the single values should not exceed 10%.

1. **Qualitative Evaluation**
   
The calculated absorptions for the patient sera, as mentioned above, are compared with the value for the cut-off standard. If the value of the sample is higher, there is a positive result. For a value below the cut-off standard, there is a negative result. It seems reasonable to define a range of +/-20% around the value of the cut-off as a grey zone. In such a case the repetition of the test with the same serum or with a new sample of the same patient, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run. The positive control must show at least the double absorption compared with the cut-off standard.

2. **Quantitative Evaluation**
   
The ready-to-use standards and controls of the Helicobacter pylori antibody kit are defined and expressed in arbitrary units (U/ml). This results in an exact and reproducible quantitative evaluation. Consequently for a given patient follow-up controls become possible. The values for controls and standards in units are printed on the labels of the vials. For a quantitative evaluation the absorptions of the standards and controls are graphically drawn against their concentrations. From the resulting reference curve the concentration values for each patient sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs. The dilution factor of the samples (1:101) has already been reconsidered in the concentration given for the standards.

**ASSAY CHARACTERISTICS**

<table>
<thead>
<tr>
<th>Helicobacter pylori ELISA</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-Assay-Precision</td>
<td>8.5 %</td>
<td>8.1 %</td>
<td>8.1 %</td>
</tr>
<tr>
<td>Inter-Assay-Precision</td>
<td>6.3 %</td>
<td>9.4 %</td>
<td>11.3 %</td>
</tr>
<tr>
<td>Inter-Lot-Precision</td>
<td>3.6-10.8%</td>
<td>3.3-10.5%</td>
<td>4.4-12.7%</td>
</tr>
<tr>
<td>Analytical Sensitivity</td>
<td>1.16 U/mL</td>
<td>1.04 U/mL</td>
<td>1.03 U/mL</td>
</tr>
<tr>
<td>Recovery</td>
<td>90-93%</td>
<td>113-126%</td>
<td>88-107%</td>
</tr>
<tr>
<td>Linearity</td>
<td>82-118%</td>
<td>77-125%</td>
<td>84-122%</td>
</tr>
<tr>
<td>Clinical Specificity</td>
<td>96 %</td>
<td>95 %</td>
<td>99 %</td>
</tr>
<tr>
<td>Clinical Sensitivity</td>
<td>96 %</td>
<td>83 %</td>
<td>85 %</td>
</tr>
</tbody>
</table>

Cross-Reactivity: No cross-reactivity to Yersinia enterocolitis Interferences: No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL.

**REFERENCES**