Automated Assay for HER-2/neu in Serum

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Background: The extracellular domain of the HER-2/neu oncogene product is increased in sera of some patients with epithelial cancers. Our aim was to develop an automated serum assay for the extracellular domain of the HER-2/neu protein.

Methods: We used a monoclonal antibody labeled with fluorescein for capture and a monoclonal Fab’ fragment labeled with alkaline phosphatase for detection. Separation of bound and free detection conjugate was performed with magnetizable particles coated with monoclonal antibody to fluorescein. Alkaline phosphatase activity was measured kinetically at 405 or 450 nm.

Results: The assay was linear from 0.1 to 250 μg/L. No hook effect was evident up to 10 000 μg/L. Within-run imprecision (CV) was 0.8–1.2%, and total imprecision was 1.1–1.7%. Cross-reactivity with human epithelial growth factor receptor, which has extensive homology with HER-2/neu extracellular domain, was <0.6%. Human anti-mouse antibodies, heterophilic antibodies, and rheumatoid factor did not interfere, nor did the therapeutic monoclonal antibody Herceptin®. In 51 healthy females, the mean value was 9.3 μg/L with a range of 6.4–14.0 μg/L. No reagent lot-to-lot variability was detected over four lots of reagents tested.

Conclusion: The Bayer Immuno 1™ assay for HER-2/neu was precise and resistant to interferences, characteristics that are essential for longitudinal monitoring of cancer patients.

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The human HER-2/neu oncogene encodes a transmembrane growth factor receptor with a molecular weight of 185 000 (p185). The complete protein consists of three portions: an internal cytoplasmic structure with tyrosine kinase activity, a short hydrophobic transmembrane section, and an extracellular ligand-binding domain (ECD). The ECD is heavily glycosylated and has a 44% sequence homology with human epidermal growth factor receptor (EGFr) (1). The ECD is shed into the blood stream and has a molecular weight of 97 000 to 115 000 (2). Studies with human mammary tumor cell lines have shown that the HER-2/neu gene may be amplified and the concentration of the p185 gene product highly increased (3).

Since 1989, many reports have appeared that evaluated the importance of measuring circulating HER-2/neu ECD of women with breast cancer (4–9). The shed ECD has been shown to be present in the sera of healthy women and to be increased above the health-related reference range in some women with breast cancer and in particular women with metastatic breast cancer. Many of these studies have shown that patients with increased HER-2/neu have a poor prognosis and shorter overall survival. Moreover, it has been shown that the tumors of these breast cancer patients grow more aggressively than those of patients who do not have increased serum concentrations of HER-2/neu. These studies raise the possibility that measuring HER-2/neu serum concentrations may be useful in monitoring patients for early detection of breast cancer recurrence, in predicting the outcome of hormonal therapy, as an aid to decisionmaking about appropriate treatment, and in monitoring the response of patients to treatment. In addition, there may be applications of the serum test to other cancers. Overexpression of this gene has been shown to have potential prognostic significance in patients with ovarian (10), gastric (11), endometrial (12), salivary gland (13), lung (14), colon (15), pancreatic (16), and prostate (17) cancers.

A new therapeutic approach to treatment of metastatic breast cancer is the use of a humanized monoclonal antibody (Mab) to HER-2/neu marketed as Herceptin® by Genentech. This is a murine Mab (Mab 4D5) that was marketed as Herceptin® by Genentech.

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Nonstandard abbreviations: ECD, extracellular domain; EGFr, epidermal growth factor receptor; Mab, monoclonal antibody; OSDI, Oncogene Science Diagnostics, Inc.; MDP, Medical Decision Pool; HAMA, human anti-mouse antibody; and MGG, mouse γ-globulin.
engineered to give a human IgG1 molecule with the murine sequences necessary for binding HER-2/neu ECD retained (18). Clinical studies have demonstrated that Herceptin has value in the treatment of patients with HER-2/neu-overexpressing metastatic breast cancer (19, 20). Measurement of serum HER-2/neu may therefore have potential value in monitoring patients on Herceptin therapy.

Evaluation of the HER-2/neu status of breast cancer patients has been limited to testing tumor tissue, which is done by immunohistochemical staining techniques for overexpression of the p185 protein or by fluorescent in situ hybridization assays for amplification of the HER-2/neu oncogene. These methods limit testing for HER-2/neu status to the time of diagnosis. Once the tumor has been surgically removed, these methods are no longer applicable and cannot be used for testing for recurrence or monitoring metastatic breast cancer patients. This situation may be remedied by development of a test that can accurately determine the circulating concentrations of HER-2/neu ECD.

We have developed a fully automated random access immunoassay for the quantitative measurement of HER-2/neu ECD in serum on the Bayer Immuno 1 immunoanalyzer. Here we describe the development and analytical performance of this assay. As a part of this evaluation, we sought to determine whether Herceptin would interfere with this new method with the purpose of evaluating the future potential use for this test in monitoring patients on Herceptin therapy.

**Materials and Methods**

**Automated Immunoassay for HER-2/neu**

The Bayer Immuno 1 HER-2/neu assay is a magnetic particle separation immunoassay designed for the random access automated Bayer Immuno 1 immunochemistry analyzer. The assay uses two Mabs developed by Oncogene Science Diagnostics, Inc. (OSDI, Cambridge, MA). These Mabs, designated NB-3 and TA-1, bind to independent binding sites on the HER-2/neu ECD. Reagent 1 contains the Mab NB-3, which is conjugated to fluorescein. Reagent 2 contains the Fab’ fragment of Mab TA-1, which is conjugated to alkaline phosphatase. Reagent 1 (65 µL), reagent 2 (65 µL), and patient sample, calibrator, or control (20 µL) are incubated at 37 °C on the system for 20 min. Magnetic particles covalently coated with anti-fluorescein Mab (20 µL) are then added to capture sandwich immunocomplexes. After 28 min, the magnetic particles are washed, a colorimetric substrate reagent containing p-nitrophenyl phosphate is added, and the rate of increase in absorbance at 405 or 450 nm is measured. A cubic-through-zero curve-fitting algorithm is used to construct a calibration curve.

**Calibrators and Controls**

The Bayer Immuno 1 assay uses six calibrators containing 0, 10, 25, 60, 125, or 250 µg/L HER-2/neu ECD. The calibrator matrix is delipidated bovine serum supplemented with detergent and sodium azide. The HER-2/neu ECD used to make the calibrators is a recombinant protein secreted by transfected mouse NIH 3T3 cells and was developed by OSDI. The original cloned DNA containing the sequence for the complete HER-2/neu protein was isolated from human breast carcinoma cells, SK-BR-3, by OSDI. A further modification was used to generate a truncated version coding for the ECD fragment of the HER-2/neu protein, which was inserted in the mouse cell designated as 3-30 (p105). The cells were grown in roller bottles using 100 mL/L bovine calf serum in DMEM. The supernatant was concentrated to give an ECD concentration of ~8000 µg/L and was diluted into the calibrator matrix to give the required final ECD concentrations.

The formulation of the Bayer controls was the same as those of the calibrators at concentrations of 15, 50, and 100 µg/L.

The Medical Decision Pool (MDP) is a serum-based control material manufactured at Bayer Corporation and used internally for product development and routine quality control. This is a pool of human serum to which recombinant HER-2/neu ECD is added to obtain a concentration of ~15 µg/L.

**Blocker Optimization**

Human anti-mouse antibody (HAMA) type 1 and type 2 controls were obtained from Boehringer Mannheim Biochemicals. HAMA type 1 is a pool of sera from healthy donors who have never been immunized with mouse proteins and represents a rare interference that has an occurrence of <0.5%. This control can show severe interference in sandwich assays that use intact IgG/IgG or IgG/Fab’ mouse Mab pairs.

HAMA type 2 interference occurs in donors who have been immunized with mouse proteins. It is frequent in special populations. More than 30% of people who have been treated with monoclonal murine IgG develop HAMAs. This control shows strong interference in all sandwich assays based on mouse Mabs in the absence of appropriate blockers.

Poly MAK 33, obtained from Boehringer Mannheim Biochemicals, is a chemically polymerized mixture of whole antibody and Fab fragment of MAK 33 Mab, which is provided as a lyophilized preparation. Mouse γ-globulin (MGG) was obtained as a concentrated solution from Scantibodies Inc.

The Poly MAK 33 and MGG are incorporated into reagent 2. The performance evaluations were all performed with the optimized concentrations of 200 mg/L Poly MAK 33 and 0.5 g/L MGG.

**Patient Samples**

Breast cancer patient serum samples with increased HER-2/neu concentrations were obtained from BioClinical Partners Inc. Serum samples from patients receiving mouse monoclonal therapy were obtained from New
York Medical College. Female Red Cross blood donor serum samples were obtained from South Bend Medical Foundation. The serum samples from healthy females were collected in-house at Bayer Corporation (Elkhart, IN). Rheumatoid factor-positive serum samples were obtained from the Foundation for Blood Research.

INTERFERENCE STUDIES

HAMA interference. The Immunomedics ImmuSTRIP® HAMA IgG ELISA assay for human antibodies to mouse IgG was used to assess the presence of HAMAs in samples from patients receiving mouse monoclonal therapy. HAMA interference in the Bayer Immuno 1 HER-2/neu assay was tested before and after the addition of 5 µL of a stock solution of HER-2/neu ECD (250 µg/L) into 95 µL of HAMA sample. In the calculation of the recovery of the amount of HER-2/neu added to a sample, a correction was made for the dilution of the endogenous HER-2/neu in the sample. The expected recovery was determined from a MDP control to which a similar quantity of HER-2/neu ECD stock solution had been added. The apparent concentrations of the stock HER-2/neu were calculated for each sample and then expressed as a percentage of the value obtained for the MDP.

Herceptin interference. Herceptin, a therapeutic agent approved for use in patients with metastatic breast cancer, was obtained from Genentech. Herceptin is a humanized recombinant DNA-derived antibody that selectively binds with high affinity to the ECD of HER-2/neu. Herceptin is provided as a lyophilized powder that when reconstituted according to the manufacturer’s instructions gives a solution of 21 g/L Herceptin. The stock solution of Herceptin was diluted into samples to give concentrations of 0–420 mg/L. The samples were incubated for 30 min at room temperature to allow antibody-Herceptin complexes to form before the HER-2/neu concentration was determined in the Bayer Immuno 1 assay.

Cross-reactants and endogenous compounds. Lyophilized affinity-purified human EGFr from human carcinoma A431 cells was purchased from Sigma. The material was reconstituted with 100 mL/L glycerol in water to give a stock solution of 370 mg/L. The stock solution was diluted into the MDP or bovine serum to give a stock solution of 0–7500 µg/L for cross-reactivity measurements. The extent of cross-reactivity was calculated from the slope of the linear regression analysis obtained by comparing the concentration of EGFr with the apparent concentration of HER-2/neu obtained in the Bayer Immuno 1 assay.

Potential interfering endogenous compounds were tested at NCCLS-recommended concentrations (21). Human serum from healthy subjects was supplemented with bilirubin (500 mg/L), hemoglobin (7.5 g/L), triglycerides (10 g/L), cholesterol (5 g/L), and human γ-globulin (50 g/L). Each sample was diluted with MDP to give final concentrations representing 100%, 75%, 50%, 25%, 10%, and 0% of initial concentration of the compound. The HER-2/neu results for these samples were compared to that of MDP to which the appropriate diluent had been added, and the percentage of bias between the supplemented and unsupplemented MDPs was calculated to determine interference.

PRECISION

Imprecision of the HER-2/neu assay was evaluated by analysis of the MDP, three Bayer controls, and Immuno 1 calibrators. Samples were run in duplicate in 20 independent analyses over 10 days with two lots of reagents and two Bayer Immuno 1 systems at the same site. Data were analyzed for variance components to give statistical estimates of total and within-run imprecision.

LINEARITY

To validate linearity over the dynamic range of the assay (0.1–250.0 µg/L), two human serum samples (245.6 and 193.5 µg/L) were diluted with the level 1 calibrator to generate a series of dilutions over the entire calibration range. Final concentrations representing 100%, 75%, 50%, 25%, 10%, and 0% of each sample were assayed. Linear regression analysis of the diluted sample results compared to the expected values was used to assess linearity.

LOWER LIMIT OF DETECTION

The lower limit of detection was evaluated by determination of the minimum detectable concentration of HER-2/neu that could be statistically distinguished from the concentration of the level 1 calibrator. Results were collected with two reagent lots, two calibrator lots, and two magnetic particle reagent lots on two systems to provide a total of 16 combinations. Measurements of the level 1 calibrator response rates (mA/min) were made on 4 days. The rates were pooled for each combination. The overall minimum detectable concentration is defined as the mean HER-2/neu concentration calculated from the mean of twice the pooled SDs for each of the 16 combinations.

METHOD COMPARISON

The quantitative HER-2/neu microtiter plate ELISA (product no. OSDI-10) was obtained from OSDI and used according to the manufacturer’s instructions. The development and performance of this assay has been described previously (22). For the method comparison study, 43 serum samples from breast cancer patients were tested in duplicate using both the Bayer Immuno 1 and OSDI methods. Assay values generated from the methods were compared by least-squares regression statistics.

LOT-TO-LOT VARIABILITY

To evaluate reproducibility in the reagent manufacturing process, four lots of reagents made in the Bayer Diagnostics manufacturing facility in Bridgend, Wales were used to assay 17 samples from breast cancer patients.
SAMPLE STABILITY

Fresh serum samples were collected from 10 healthy women. The HER-2/neu concentrations in these samples ranged from 3.8 to 11.5 \( \mu \text{g/L} \). Aliquots of the samples were stored frozen at \(-20^\circ\text{C}\) and refrigerated at 2–8 \(^\circ\text{C}\). The HER-2/neu values were tested within 5 h after the blood was drawn and on days 1, 8, 15, and 30. In addition, the stability at room temperature of fresh serum samples from three women was evaluated by testing immediately after collection and at 3, 6, and 24 h. The effect on sample stability of allowing the blood sample to stand for 3 and 6 h at room temperature before separating the serum was tested for the same three women.

HIGH-DOSE HOOK EFFECT

To evaluate high-dose hook effect, MDP to which recombinant HER-2/neu ECD concentrate had been added to give a final concentration of 10 000 \( \mu \text{g/L} \) was tested in the assay. We concluded that there is no high-dose hook at the test sample concentration when that sample gives a rate greater than that of the level 6 calibrator.

Optimization of assay to interferences

MGG and Poly MAK 33 blocker concentrations were optimized using HAMA type 1 and type 2 control sera to test the system. The results obtained with these blockers are shown in Table 1. Both HAMA type 1 and HAMA type 2 produced severe interference when only base buffer was used. It was assumed that the true endogenous concentrations of these control interference samples were within the reference range and close to the mean concentration of 9.3 \( \mu \text{g/L} \) found for a group of healthy females with the Bayer Immuno 1 assay. Poly MAK 33 was more effective in eliminating interference from HAMA type 1, whereas MGG was more effective with HAMA type 2. Neither Poly MAK 33 nor MGG were completely effective alone at the highest concentrations used. The lowest concentration achieved with HAMA type 1 with MGG alone was 12.9 \( \mu \text{g/L} \) compared with 7.0 \( \mu \text{g/L} \) with Poly MAK 33 alone. The lowest concentration achieved for HAMA type 2 with Poly MAK 33 alone was 29.1 \( \mu \text{g/L} \) compared with 7.7 \( \mu \text{g/L} \) with MGG alone. The combination of Poly MAK 33 and MGG was the most effective formulation, with 6.9 \( \mu \text{g/L} \) and 7.3 \( \mu \text{g/L} \) obtained for HAMA type 1 and type 2, respectively. Because these concentrations were well within the expected reference range, we concluded that this combination had eliminated all significant interference from these HAMA controls.

A comparison was made between the resistance to interference obtained with Mab TA-1 alkaline phosphatase conjugate made with whole IgG or Fab fragment. The conjugates were used in formulations with and without the combination of Poly MAK 33 and MGG present.

Rheumatoid factor-positive serum samples with titers of 51 000–1 330 000 IU/L were used to test the assay as shown in Table 2. A total of 20 of 42 samples gave significantly increased results with whole antibody and no blockers. Interference was frequent with the IgG conjugate in the absence of blockers. In none of these cases was there any evidence of interference when the Fab'
fragment conjugate was used, even in the absence of blockers. The presence of blockers made no difference to the results with Fab' fragment conjugate but produced results with the IgG conjugate that were equivalent to those with the Fab'. There was only one sample in which interference was not eliminated, but the apparent HER-2/neu concentration was reduced from 153 μg/L to 12.5 μg/L compared with the result of 8.7 μg/L with Fab' fragment conjugate plus blockers. The data in Table 2 show that the Fab' fragment alkaline phosphatase conjugate used in the assay provides protection against rheumatoid factor interference.

INTERFERENCE STUDIES

HAMA samples. Table 3 shows that recovery of HER-2/neu from individual HAMA-positive serum samples was 85–111%. There was no correlation between the HAMA concentration and recovery of HER-2/neu. The HER-2/neu concentrations of all the samples were within the reference range.

Herceptin. Table 4 shows that Herceptin, when added to HER-2/neu-positive patient samples at therapeutic concentrations and higher, had no effect on the measured HER-2/neu concentration.

Cross-reactants and endogenous compounds. The cross-reactivity with EGFr was estimated as 0.05% when EGFr was added into MDP and 0.06% when it was added into calibrator 1. Bilirubin (500 mg/L), hemoglobin (7.5 g/L), triglycerides (10 g/L), cholesterol (5 g/L), and human γ-globulin (50 g/L) were added into human serum from healthy subjects up to the concentrations indicated. All HER-2/neu results were within 98–108% of the expected value.

PRECISION

Precision data for the MDP, three levels of Bayer controls, and six calibrators are presented in Table 5. Pooled within-run CVs were 0.8–1.6%, and total pooled CVs were 1.1–1.7%.

LINEARITY

Linearity was examined by measuring HER-2/neu in five serial dilutions of two positive patient serum samples diluted with level 1 calibrator. In one case, the range of concentrations obtained was 0.1–245.6 μg/L; in the other, it was 0.1–193.5 μg/L. The clinical samples diluted linearly as determined by regression analysis, which yielded slopes of 1.0022 and 1.0015 ($r^2 = 0.999$).

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### Table 3. HAMA sample interference.

<table>
<thead>
<tr>
<th>HAMA, μg/L</th>
<th>Untreated</th>
<th>HER-2/neu added*</th>
<th>Estimated concentration of stock solution</th>
<th>Recovery compared with MDP,* %</th>
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<tbody>
<tr>
<td>121</td>
<td>6.9</td>
<td>19.3</td>
<td>255</td>
<td>104</td>
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<tr>
<td>148</td>
<td>4.8</td>
<td>17.5</td>
<td>259</td>
<td>105</td>
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<td>171</td>
<td>11.2</td>
<td>24.3</td>
<td>273</td>
<td>111</td>
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<td>252</td>
<td>7.8</td>
<td>20.4</td>
<td>260</td>
<td>106</td>
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<td>384</td>
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<td>235</td>
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<td>18.0</td>
<td>27.6</td>
<td>210</td>
<td>85</td>
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<td>11.8</td>
<td>24.2</td>
<td>260</td>
<td>106</td>
</tr>
<tr>
<td>1249</td>
<td>6.6</td>
<td>19.5</td>
<td>265</td>
<td>108</td>
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<td>1251</td>
<td>14.3</td>
<td>26.7</td>
<td>262</td>
<td>107</td>
</tr>
<tr>
<td>MDP</td>
<td>15.8</td>
<td>27.3</td>
<td>246</td>
<td>100</td>
</tr>
</tbody>
</table>

*a HER-2/neu stock solution (5 μL) was added to 95 μL of HAMA sample or MDP.
*b MDP, Medical Decision Pool.

### Table 4. Effect of Herceptin.

<table>
<thead>
<tr>
<th>Herceptin, mg/L</th>
<th>Fresh serum*</th>
<th>Sample 1282</th>
<th>Sample 1245</th>
<th>Sample 1390</th>
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<tr>
<td></td>
<td>μg/L</td>
<td>% control</td>
<td>μg/L</td>
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<tr>
<td>0</td>
<td>8.5</td>
<td>100</td>
<td>37.7</td>
<td>100</td>
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<td>53</td>
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<td>101</td>
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<td>38.1</td>
<td>101</td>
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</table>

*a Serum collected from healthy subjects.
The lower limit of detection, defined as the concentration 2 SD above the value for the level zero calibrator, was 0.1 μg/L.

No high-dose hook effect was seen when recombinant HER-2/neu ECD was added to samples up to a concentration of 10,000 μg/L (data not shown).

The results obtained with samples from 51 healthy women Red Cross blood donors are shown in Fig. 1. The range of results was 6.4–14.0 μg/L with a mean (± SD) of 9.3 ± 1.9 μg/L.

A total of 43 serum samples from breast cancer patients were tested in duplicate in both the Bayer Immuno 1 assay and the OSDI quantitative HER-2/neu microtiter plate ELISA. Fig. 2 shows the regression plot for the means of all the sample results. The concentration range for the Bayer Immuno 1 HER-2/neu assay was 10.3–231.9 μg/L. The regression equation for this range was \( y = 0.88x + 0.9 \), with a \( S_{\text{y|x}} \) of 4.3 μg/L and correlation coefficient of 0.995. For the narrower range of 10.3–72.0 μg/L into which most of the samples fell (n = 39), the regression equation was \( y = 0.96x - 1.8 \), with a \( S_{\text{y|x}} \) of 3.3 μg/L and a correlation coefficient of 0.983.

The regression analysis statistics for a comparison of results with a panel of serum samples from 17 breast cancer patients using four distinct lots of reagents are shown in Table 6. There was no significant bias among the results for these reagent lots.

### Table 5. Imprecision of the Immuno 1 HER-2/neu assay.\(^a\)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean, μg/L</th>
<th>SD, μg/L</th>
<th>CV, %</th>
<th>SD, μg/L</th>
<th>CV, %</th>
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<td>MDP</td>
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<td>0.19</td>
<td>1.2</td>
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<td>Control</td>
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<td>Calibrator</td>
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<td>247.5</td>
<td>3.21</td>
<td>1.3</td>
<td>3.49</td>
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</table>

\(^a\)Results were collected in duplicate for two lots of reagents on two systems over 10 days for a total of 160 determinations for each sample.

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**Fig. 1.** HER-2/neu concentrations for serum samples from 51 female Red Cross blood donors.

**Fig. 2.** Comparison of results from the Immuno 1 and the OSDI HER-2/neu methods.

The equation for the line is: \( y = 0.88x + 0.9; r = 0.995; S_{\text{y|x}} = 4.3 \text{ μg/L}; n = 43.\)
The use of Fab’ fragment abolished the interference even without the addition of specific blockers. Thus, the combination of Fab’ fragment and specific blockers provides a potent resistance to rheumatoid factor interference. The effectiveness of the blockers was also demonstrated by the elimination of interference when they were combined with the whole antibody conjugate. In the analysis of these results, it was assumed that all of the rheumatoid factor samples contained endogenous HER-2/Neu concentrations within the reference interval. In all cases, the use of the Fab’ fragment conjugate produced concentrations in the expected range. Moreover, the fact that the same results were obtained for the Fab’ fragment conjugate with and without blockers is a strong indication that all interference was eliminated.

We concluded that EGFr would have an insignificant effect on HER-2/Neu measurements for the following reasons. Partanen et al. (23) measured mean EGFr concentrations of $336 \pm 228$ pmol/L and $636 \pm 299$ pmol/L in the serum of populations of healthy subjects and cancer patients, respectively. The concentrations in cancer patients were increased, with the highest serum concentration of EGFr reported to be 4583 pmol/L. In comparison, the concentration of HER-2/Neu ECD in healthy subjects was $9.3 \pm 1.9$ μg/L, or $88.6 \pm 18.1$ pmol/L. The cross-reactivity of EGFr in the Bayer Immunol 1 HER-2/Neu assay was 0.06%. Thus, a serum EGFr concentration of 4583 pmol/L would produce an apparent HER-2/Neu concentration of 2.7 pmol/L or 0.3 μg/L in addition to the endogenous HER-2/Neu.

The absence of interference by Herceptin is an important result that will allow the value of the assay in monitoring patients being treated with this drug to be assessed. Herceptin binds to the ECD of HER-2/Neu as do the Mabs TA-1 and NB-3 used in the Bayer Immunol 1 assay. Our interpretation of the results is that Herceptin binds at a site independent from those of the two Mabs used in the assay with no overlap or steric interference. The Herceptin concentrations tested were present in 5.4- to 34.5-fold molar excess over the measured HER-2/Neu in the samples used. According to product information provided by Genentech, Herceptin serum concentrations under steady-state conditions give mean trough and peak concentrations of ~79 and 123 mg/L, respectively.

Because no interference was found at 420 mg/L, we concluded that therapeutic concentrations of Herceptin in serum will not interfere in the accurate measurement of HER-2/Neu using the Bayer Immunol 1 assay.

Lot-to-lot variation can be an important source of analytical error in an immunoassay. Van Dalen (24, 25) has stressed the importance of standardization in the manufacture of immunoassay reagents and has recommended that manufacturers report lot-to-lot variation for their tumor marker assays. The results reported here for the Immunol 1 HER-2/Neu assay demonstrate that the reagents exhibit insignificant lot-to-lot variation.

The Bayer Immunol 1 HER-2/Neu assay has the important attributes needed for monitoring cancer patients. The total imprecision is at most 1.7%. Combined with the resistance to interference and low lot-to-lot variability,

### Table 6. Reagent lot-to-lot variability.

<table>
<thead>
<tr>
<th>Reagent lot (x)</th>
<th>A</th>
<th>A</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent lot (y)</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>Slope, μg/L</td>
<td>0.99</td>
<td>1.00</td>
<td>0.99</td>
</tr>
<tr>
<td>Intercept, μg/L</td>
<td>0.1</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9999</td>
<td>0.9999</td>
<td>0.9998</td>
</tr>
<tr>
<td>$S_{xy}$, μg/L</td>
<td>0.5</td>
<td>0.6</td>
<td>0.9</td>
</tr>
</tbody>
</table>

* A panel of 17 serum samples from breast cancer patients with HER/Neu concentrations in the range 17.4–239.0 μg/L was tested with four reagent lots. Linear regression analysis statistics are shown.
this makes the assay very suitable for longitudinal monitoring over extended periods. Accurate results can be expected at widely separated time points. This is the first description of a fully automated assay for HER-2/neu in serum. The Bayer Immuno 1 HER-2/neu assay may be a valuable tool to aid oncologists in the management of cancer patients as more and more studies, particularly for breast cancer patients, show the value of knowledge of HER-2/neu status for prognosis, selection of therapy, and monitoring.

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References


