<6% of the differences in immunoactivity among rcTnI-0, rcTnI-6, and rcTnI-14 can be attributed to the use of the Bradford assay. The observed immunoactivity differences among rcTnI-0, rcTnI-6, and rcTnI-14 were much greater than 6%. In the Access assay, immunoactivity was markedly decreased if a leader sequence was present in the TnI molecule. It also seemed that the extent to which immunoactivity was decreased was related to the length of the leader sequence. This tendency was not observed in the Stratus assay, indicating that the presence of leader sequence may affect only certain epitopes and, therefore, certain assays.

It is common practice to add a leader or tag sequence to recombinant proteins to increase expression (7) or to facilitate purification of the expressed product (16). It was not clear whether the leader, the tag, or both would affect the folding status and, thus, the immunoactivity of the resulting recombinant protein (7). Our results suggest that the immunoactivity of a fusion protein can be altered, although the detectability of this alteration may depend on the specific antibodies used.

In conclusion, we have demonstrated for the first time that fused leader sequences affect the immunoactivity of a recombinant protein, presumably by changing its folding status. Therefore, in making controls and calibrators for an immunoassay, the unfused original sequence is highly preferred.

We thank Henry T. Burke and Michael Mingfu Ling for their critical review and valuable discussions.

References

Automated Immunoturbidimetric Method for Measuring Serum Transferrin Receptor, Pauli Suominen, Mika Suominen, Kari Punnonen, Allan Rajamäki, Raimo Majuri, Veli Hänninen, and Kerttu Irjala 1 (Departments of 1 Clinical Chemistry and 2 Hematology, Turku University Central Hospital, FIN-20521 Turku, Finland; 3 Department of Clinical Chemistry, Kuopio University Hospital, 70210 Kuopio, Finland, 4 Orion Diagnostica, 02101 Espoo, Finland; * author for correspondence: Turku University Hospital, Central Laboratory, P.O. Box 52, FIN-20521 Turku, Finland)

Cellular iron uptake is mediated by transferrin receptors (TfRs), which are present on virtually all mammalian cells. A soluble form of TfR (sTfR) can be detected in serum, the concentration of which is closely related to erythroid TfR turnover. The prime determinants of sTfR concentration are cellular iron demands and the erythroid proliferation rate (I). The measurement of sTfR has been introduced as a powerful tool for the diagnosis of iron deficiency (ID) in a variety of clinical situations (1–8). Up-regulation of the expression of cellular TfR occurs as a result of an inadequate tissue supply of iron or increased cellular demand for iron, for example, in the context of chronic loss of blood and compensatorily activated erythropoiesis. In a clinical setting, sTfR measurements offer an attractive amendment to the repertoire of indices of iron status because it has been shown to sensitively detect iron-deficient erythropoiesis early in developing ID and to retain its specificity to changes in iron status irrespective of the concurrent inflammatory status (2, 3, 6, 8, 9). It has been suggested, however, that in cases of noncomplicated ID, the use of sTfR measurements does not provide any relevant additional information compared with ferritin measurements (IO). The sTfR concentration has also been shown to be a more sensitive and less variable index of iron status than the more conventional serum iron, transferrin, and total iron-binding capacity (8, 11, 12).

Several commercially available methods have been introduced to measure sTfR (7, 9). To date, the available methods have been manually performed enzyme immunoassays, which are fairly laborious and time-consuming and require special equipment. Another obstacle for wider implementation of sTfR measurements is the lack of uniform calibrators (7, 9). This study was undertaken to evaluate the analytical and clinical performance characteristics of a new automated immunoturbidimetric assay for sTfR (IDEA® sTfR-IT; Orion Diagnostica). This method is a successor of the manual enzyme immunoassay by the same manufacturer, namely the IDEA® sTfR IEMA assay.
We believe that the automated method described here promotes a more widespread adoption of sTfR as a routine laboratory measurement and renders the method available even to small laboratories.

In this study, we tested the IDeA sTfR-IT application on the Hitachi 911 analyzer (Roche-Boehringer Mannheim). Applications are also available for the Hitachi 917 and Cobas Mira analyzers.

The method requires 20 μL of sample, 250 μL of IDeA sTfR-IT buffer, and 20 μL of IDeA sTfR-IT reagent. The reagent consists of polyclonal anti-human TfR F(ab)2 antibodies bound to SVBC-latex particles. In the presence of sTfR, the latex particles are agglutinated in a dose-dependent manner, causing increased turbidity. The increase in turbidity is detected at 660 nm. The amount of sTfR in the sample is determined by means of a calibration curve based on five ready-to-use calibrators, which may be also prepared by automatic dilution of the highest calibrator, which contains 8–9 mg/L sTfR. Two additional controls (IDea sTfR-IT control low and control high) are also provided. Calibrators and controls are derived from human serum, and sTfR is therefore present bound to transferrin, forming complexes comprising two transferrin and two sTfR molecules. The insert states that suitable assay specimens are fresh serum, EDTA plasma, heparin-treated plasma, and citrate plasma. Volume correction for the sTfR result is needed if liquid anticoagulant-containing sample tubes (EDTA and citrate) are used. The results from the IDea sTfR-IT method were compared with results from the Quantikine® IVD® sTfR assay (R&D), which has been approved by the Food and Drug Administration (13).

In addition, the clinical performance of the method was evaluated by using several previously described populations of healthy individuals as well as anemic patients. Sera from 78 patients with iron deficiency anemia (IDA), anemia of chronic disease, and ID in the presence of a complicating inflammatory condition (COMBI) were assayed to evaluate the ability to detect ID in the presence of COMBI. The patient material has been described in detail elsewhere (9). An adult reference interval (95%) was obtained from a population of healthy, nonanemic adults, consisting mainly of laboratory staff and other related personnel. The pediatric reference population consisted of 102 healthy males (n = 64) and females (n = 38), who were undergoing selective short-term surgery in the Turku University Central Hospital. The selection of patients was made on the basis of detailed anamnesis and laboratory tests to exclude the effect of manifest ID, acute or chronic inflammatory conditions, and dietary restrictions. The reference values were calculated by a regression-based method described elsewhere (14). The cutoff value for iron-deficient erythropoiesis was calculated from the population and as described in a previous paper from our group (8).

Table 1. Pediatric reference values.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>2.5% and 97.5% reference limits, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 months–2 years (n = 24)</td>
<td>1.48 (1.36–1.60)–3.51 (3.19–3.88)</td>
</tr>
<tr>
<td>2–4 years (n = 28)</td>
<td>1.40 (1.30–1.50)–3.36 (3.11–3.65)</td>
</tr>
<tr>
<td>4–6 years (n = 23)</td>
<td>1.32 (1.22–1.43)–3.10 (2.88–3.33)</td>
</tr>
<tr>
<td>6–8 years (n = 27)</td>
<td>1.24 (1.12–1.38)–2.92 (2.67–3.20)</td>
</tr>
</tbody>
</table>

\* The 2.5% and 97.5% reference limits with 95% confidence intervals (in parentheses) for these respective limits are displayed separately for each age group as suggested by a regression-based method. The reference values calculated by the same method from the same population on the manual IDeA method are discussed elsewhere (Suominen et al., submitted for publication.)

\* Number of subjects.

The methods correlated well within the respective linear ranges (left). Both methods were calibrated by sTfR derived from human plasma. The Bland-Altman analysis (right) revealed, however, differences between the two methods that are unacceptable for direct comparison of clinical data. The mean difference between the methods was −0.29 mg/L (SD, 0.39 mg/L). The dashed lines in the Bland-Altman plot represent the lines of agreement (mean ± 2 SD). OD, Orion Diagnostica.
Statistical analyses were carried out using the SPSS 7.5 for Windows software (SPSS) and the GraphROC for Windows software (15). The Bland-Altman analysis was performed as described elsewhere (16).

The detection limit of the method was 0.05 mg/L, as determined by the concentration corresponding to mean 4 SD above absorbance value of the zero calibrator (9 g/L NaCl). The intraassay CV was 0.3–1.8% for four serum samples (0.6–7.7 mg/L) with 10 replicates. The interassay CVs calculated for four serum samples in 10 subsequent assays over 2 weeks were 2.4–3.5%. The linearity of the method was tested by serially diluting serum samples. The linear range spanned the entire reportable range of the assay (0.3–8.5 mg/L). The measured results were 90.5–107% of expected values within the linear range. Possible interference by potential interfering factors was investigated by adding up to 5 g/L hemoglobin (as hemolysate), up to 30 mg/L l-ascorbic acid, up to 23 mmol/L triglycerides (Intralipid), and up to 5.0 × 10^6 IU/L rheumatoid factor to serum samples. No interference was observed with these concentrations. Samples with up to 1 × 10^6 IU/L rheumatoid factor diluted linearly in IDEIA sTfR-IT assay.

The 95% reference interval for healthy nonanemic adults was 0.85–2.30 mg/L, whereas the corresponding limits for the manual IDEIA method by the same manufacturer were 1.3–3.3 mg/L (9). The pediatric age-related reference limits for the automated method are presented in Table 1. The cutoff concentration for iron-deficient erythropoiesis in a population of 65 healthy adults, from which iron deficiency was excluded by peroral iron supplementation (8), was 1.90 mg/L. For the method’s ability to distinguish between iron deficiency (IDA and COMBI) and anemia related to other causes (anemia of chronic disease), the area under the ROC curve (AUC^ROC) was 0.987 (SE, 0.009), similar to the value observed with the manual IDEIA method (0.973; SE, 0.014) (9). The AUC^ROC values for separating anemia of chronic disease and COMBI patients were 0.950 (SE, 0.010) and 0.918 (SE, 0.047), respectively. The optimal cutoff value for ID as calculated by the GraphROC for Windows software in this material was 2.40 mg/L. The results from 50 patient samples by the sTfR IDEIA-IT assay correlated well with the results obtained with the Quantikine sTfR assay, which thus far is the only sTfR assay that has been approved by the Food and Drug Administration (Fig. 1). However, the Bland-Altman analysis revealed differences between the two methods that are unacceptable for direct comparison of clinical data (Fig. 1). The area between the lines of agreement is wide, and separate reference values are therefore warranted.

The IDEIA sTfR-IT assay is an analytically adequate method, superior to its manual ELISA predecessors in several aspects. Assay time is reduced from ~3 h to 10 min, and the intra- and interassay CVs are two- to threefold lower than the CVs for commercial sTfR-ELISAs. The antibody (polyclonal) used in this new method is different from the antibody in the manual method (monoclonal) produced by the same manufacturer, which accounts for the different values of the results. No sign of matrix effects was seen in the IDEIA sTfR-IT assay when patient samples presenting compromised linearity on the manual IDEIA IEMA assay were tested with the IDEIA sTfR-IT assay (data not shown).

The measurement of sTfR has become a widely used tool in assessing iron status, but its use has mainly been restricted to academic and scientific research. The need for special equipment has been a major setback to the usefulness of the manually operated sTfR assays based on enzyme immunnoassay format. The method evaluated here can be operated on several commonplace clinical chemistry automated analyzers and is, therefore, an attractive upgrade to the repertoire of markers of ID in virtually any laboratory. Although calibration in this new automated IDEIA sTfR-IT method is based on human serum sTfR, which is an important step toward making sTfR even more widely accepted and documented, the pressure toward establishing international guidelines for the calibration of sTfR assays is obvious. This issue is underscored by the results presented here. The good correlation between the IDEIA sTfR-IT and Quantikine assays suggests that they probably measure the same complex (sTfR-transferrin heterodimer in calibrator); however, the difference in results is clinically unacceptable, as indicated by the wide area between the lines of agreement (Fig. 1). We believe that the difference observed between these two assays may be attributable not only to the different antibodies used but to differences in the preparation of the calibrator specimen and could, perhaps, be overcome by establishing a common calibrator.

References
Routine Workflow for Use of Urine Strips and Urine Flow Cytometer UF-100 in the Hospital Laboratory,1

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None of the methods currently available for evaluating hematuria and leukocyturia is perfect. Urinalysis by test strip combined with automated particle counting is an attractive approach that may identify false-negative results of both techniques. We wished to evaluate the possibility that microscopic evaluation of urine samples could be substantially reduced by this approach.

The fully automated urine flow cytometer UF-100 classifies urinary particles on the basis of their light scattering, fluorescence, and impedance properties. The instrument counts erythrocytes, leukocytes, bacteria, epithelial cells, and casts and flags the presence of pathological casts, small round cells (SRCs), yeast-like cells, crystals, and spermatozoa. The instrument is intended to replace, to an extent, routine urine microscopy. The operating principles of the UF-100 (1); its precision, accuracy, and analytical sensitivity (2); and its potential for differentiation between renal and postrenal hematuria (3) have been published previously (4).

The precision of particle counting in microscope chambers is poorer than counting by flow cytometry (5). The analysis of urine samples by UF-100 and the test strip analyzer Clinitek Atlas showed discordant results in a small but not negligible number of cases. This result has also been described by Ben-Ezra et al. (4). We therefore rechecked the false results in erythrocyte and leukocyte counts by examining discrepant results from both instruments by microscopic evaluation and evaluated a workflow for the routine urine analysis using the combination of UF-100 and Clinitek Atlas.

We analyzed 288 mainly pathological urine specimens by both test strip analyzer (Clinitek Atlas; Bayer Diagnostics) and UF-100 (Sysmex). The samples were examined within 3 h of arriving at the laboratory; no preservatives were used. We additionally reviewed 261 by microscopy.

The ranking system was used to compare the results from UF-100 and Clinitek Atlas (Table 1A). This ranking system was devised because cells were counted very precisely by UF-100 and, therefore, a differentiated classification was required to compare the test strips results.

The workflow strategy was to use both methods together and to detect implausible results by rank value differences between the Clinitek Atlas and UF-100 for erythrocytes and leukocytes separately. Differences in rank values of two or more were regarded as medically relevant. Specimens exhibiting this difference were reviewed by microscopy.

Discordant cases with lower rank values for strip results and higher rank values for flow cytometry were reviewed by microscopy. Microscopy was also performed in cases with higher ranks for strip results if large numbers of cells were seen. If cells were not seen in the UF-100, fresh samples were requested and examined. In some cases, the presence of lysed cells was assumed or excluded in concordance with the disease. Microscopic review was also performed when warning messages indicated that manufacturer specifications were being exceeded. The microscopy counts were performed after centrifugation (500g for 5 min; Madaus System). The cells were stained with Sternheimer supravital solution (Alcian Blue and Pyronin B; Oy Reagena) (6, 7).

Our workflow strategy and rank classifications were then tested prospectively on 635 unselected routine specimens obtained over a 5-day period.

The dysmorphism and isomorphism criteria were examined on the UF-100 in 120 patients with confirmed renal or postrenal hematuria. The diagnostic sensitivity of these criteria for evaluating the localization of the hematuria was determined against phase contrast microscopy and established clinical diagnoses (8–10).

Statistical calculations were carried out using SPSS for Windows (11) and the frequencies of binomial distribution (12).

Red blood cell (RBC) and white blood cell counts were compared using both methods in 288 urine samples with a high frequency of pathologic results. In most cases (263 of 288 samples), there was agreement between the two methods based on our ranking system. The valid results were within the limits of 1.5 rank difference (see Table 1B). The 25 discordant cases are presented in Table 1B. The traditional workflow would have required microscopic review in 67% of the samples. Using the new combined approach, we reduced the microscopic review rate significantly, to 9% (P <0.05).

This approach was further validated using 635 random samples. In this case, the microscopic review rate was 36% (n = 230). The use of both instruments reduced the review rate to 6% (n = 39; P <0.05). Microscopy also clarified the situation in 25 specimens with discordant results (Table 1B).

Of the 263 concordant cases between the test strip and the UF-100, 248 were checked microscopically. Differing results for 6 samples for erythrocytes and 6 samples for leukocytes were obtained from 11 urine samples. In all cases, microscopic review revealed more cells than did the instruments.

The review algorithm of the UF-100 suggested microscopy in 142 samples (133 manufacturer-defined flags and

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1 Dedicated to Prof. Dr. E. Köttgen on the occasion of his 60th birthday.