was performed 8 days after coupling (Fig. 2 in the online Data Supplement). However, on interpolation of data points from the calibration curve, the trend was reversed such that the highest signal was observed for experiment 3 and the lowest for experiment 1.

In parallel studies, antigen-conjugated beads stored at 4 °C gave consistent fluorescent emission within a period of 1 month, whereas antibody-conjugated beads showed diminished fluorescent emission (data not shown).

We identified two limitations of this methodology: The inherent instability of antibody-coupled beads and the occurrence of data points from test samples outside the linear portion of the semilogarithmic calibration curve. To resolve the latter issue, serum samples exhibiting fluorescent output within the plateau of the trendline should be reassayed after further dilution. Problems with long-term stability of protein-conjugated bead sets were evident when the beads were stored at 4 °C. On the Luminex platform, antibody-conjugated beads were viable for approximately 3 weeks. Antigen-conjugated beads exhibited slightly greater longevity, although decoding of the fluorescent signatures was problematic after storage at 4 °C beyond 1 month. The constituents of the storage buffer may have a detrimental effect on the fluorescent dyes within the microspheres.

The reversal of the signal output profile suggests that antibody-bound beads were more liable to degradation than antigen-coupled bead sets within the same timescale. The more elaborate structural complexity of antibodies compared with antigens may account for the greater instability of the former. Rapid freezing and lyophilization were procedures explored as alternative methods to prolong the shelf-life of protein-coupled beads, and both were procedures explored as alternative methods to improve the fluorescent output within the plateau of the trendline. However, antibody-conjugated beads showed diminished fluorescent emission (data not shown).

This study illustrated the complexity of quantifying target analytes within antigen arrays. Production of purified antibodies is laborious and expensive. Methods that can be used for antibody purification, e.g., affinity chromatography, could theoretically be used to obtain material comparable to the target analyte of an antigen array. However, consistent antibody purity is paramount for quantification.

Although this approach has broad application for the comparison of any IgG, it will not measure absolute concentrations of target analyte. This is largely because of the presence of factors (e.g., soluble receptors, heterophilic antibodies, serum binding proteins, hemoglobin, and lipids) in sera that can interfere with antibody-based immunoassays (18). Nonetheless, this method has reduced intraassay variability and enables interassay comparisons for a wide range of antigen arrays.

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Detection of LAs is of major importance in patients with these conditions (1, 2). LAs are diagnosed according to the criteria proposed by the Scientific and Standardization Committee on LAS of the International Society of Thrombosis and Hemostasis (3). According to these criteria, a diagnosis of LA should follow a 4-step procedure respecting the following principles: (a) prolongation of 1 (or more) PL-dependent clotting test (screening test); (b) evidence of inhibition demonstrated after mixing equal amounts of patient and normal plasma (mixing test); (c) evidence that the inhibitor is PL dependent, as demonstrated by correction of the clotting defect in the presence of excessively high PL concentrations (confirmatory test); and (d) lack of specific inhibition of any coagulation factor (distinction from other coagulopathies by specific factor assays).

Despite these criteria, diagnosis of LA remains a problem for the clinical laboratory. Contributing to these problems are the marked differences in sensitivity and specificity for the various LA screening assays that have been proposed, the lack of a universally accepted definition of a positive mixing test, technical variables affecting the various assays for LA, the difficulty with result interpretation, and the heterogeneous nature of LA itself (4, 5). At present, the most used screening tests for detecting LA are a dilute activated partial thromboplastin time, kaolin clotting time, and the diluted Russell venom time. Silica clotting time (SCT) has been described as a specific and sensitive alternative to kaolin clotting time for detecting LA (6–9). We have evaluated a new automated “SCT screening and confirmatory” assay (not commercially available at this time) that has been proposed for the detection of LA.

The SCT Screen (HemosIL; IL) was run on an ACL 9000 automated coagulometer (IL). Prolongation of the SCT Screen tests was expressed as the ratio of patient coagulation time to the clotting time of the control (normal pool). Mixing studies were carried out on 1:1 and 4:1 mixtures of patient and normal plasmas on all samples that had prolonged SCT Screen times; failure to correct the clotting time was considered evidence of an inhibitor. For a confirmatory test of SCT, we used this new commercial assay (SCT Confirm, HemosIL; IL). LA was diagnosed when the confirmatory procedure was positive. Whereas the results of SCT Screen coagulation tests were expressed as the (sample clotting time/normal pool clotting time) ratio, the results of SCT Confirm tests were expressed as a “normalized LA ratio”: the ratio result from the LA screen test divided by the ratio result from the LA confirmation test (patient confirm clotting time/normal pool confirm clotting time).

Mean (SD) and the parametric 95th percentile of clotting time ratios in 30 healthy controls (voluntary blood donors; 15 males and 15 females; age range, 16–81 years) were 1.04 (0.12) and 1.21 for SCT Screen and 1.02 (0.10) and 1.22 for the SCT Confirm test. Confirm ratios above the 95th percentile were regarded as positive.

For quality control, we used a normal pool and a LA-positive sample (LA+). Within-run imprecisions (CVs; n = 10) for the SCT Screen were 1.0% for the normal pool and 3.1% for the LA+ sample; for the SCT Confirm, the CVs were 2.0% for the normal pool and 2.6% for the LA+ sample. The CVs over 10 separate days for the SCT Screen were 3.9% for the normal pool and 5.2% for the LA+ sample; for the SCT confirm, the CVs were 4.8% for the normal pool and 5.7% for the LA+ sample.

We investigated the diagnostic specificity in 41 patients with known coagulation abnormalities: 12 patients on low–molecular-weight heparin therapy (LMWH), 23 patients on oral anticoagulant therapy [OAT LA+ (n = 7) and OAT LA- (n = 16)], 3 patients with factor deficiencies of the intrinsic coagulation system (1 with a factor VIII:C activity of 37%, 1 with a factor IX:C activity of 39%, and 1 with a factor XI:C activity of 41%), and 3 patients with type 1 von Willebrand disease (vWD; Table 1). Six of 12 patients on LMHW, all of the patients with defects of intrinsic coagulation factors, and 1 of 3 patients with vWD had prolonged SCT Screen times, but all of them were identified as LA-negative by the SCT Confirm assay. Nineteen of 23 patients on OAT had prolonged SCT Screen times: 7 of these 19 previously identified as having a LA were confirmed as LA-positive by the SCT Confirm assay, whereas the other 12 were identified as LA-negative (see Fig. 1).

To evaluate the screening performance (sensitivity and specificity) of the SCT Screen and Confirm assays, we collected and studied consecutive plasmas from 136 “anticoagulant-free” patients (54 males and 82 females; age range, 16–84 years) for whom a LA determination was requested by the Department of Thrombosis and Hemorrhagic Diseases. All 136 plasma samples were further analyzed for the presence of LA by our laboratory’s routine LA (SCT in-house method) screening and confirmation tests (Fig. 1). Forty-six of 136 patients were identified as LA-positive by our routine LA tests. Of these samples, a prolonged SCT Screen test was found in 40. The inhibitor activity observed in SCT Screen-positive patients was confirmed to be of the LA type by the SCT Confirm assay. Six of 46 samples identified as having a LA were SCT-negative; in these patients, the only test positive was the diluted Russell venom time. Six of 91 LA-negative patients were positive by SCT Confirm assay. Of these patients, 2 were positive for anti-cardiolipin IgM (18.0 and 12.0 MPL, respectively; normal values <7.0 kMPL/L), 1 for anti-prothrombin IgG (12.5 kIU/L; normal values <9.0 kIU/L), 1 for anti-protein S IgM (21.0 kIU/L; normal

| Table 1. Mean (SD) SCT Screen and SCT Confirm clotting time ratios in patients during LMWH or OAT therapy and in patients with factor deficiencies or vWD. |
|----------------|----------------|----------------|----------------|----------------|
|                | Mean (SD) ratio | SCT Screen     | SCT Confirm     |
| LMWH (n = 12)  | 1.29 (0.13)     | 1.04 (0.08)    |
| OAT (n = 23)   | 2.12 (1.32)     | 1.41 (0.80)    |
| Factor deficiencies (n = 3) | 1.31 (0.19) | 1.07 (0.05) |
| vWD (n = 3)    | 1.21 (0.17)     | 1.03 (0.04)    |
values <15.0 kIU/L, and 1 for anti-protein C IgM (28.4 kIU/L; normal values <18 kIU/L) autoantibodies, and 2 were negative for all anti-phospholipid antibodies investigated by the ELISA method. Thus, the new SCT assay was positive in 87% of those who had a positive result by our LA test and was negative in 93% of those whose results were negative by our LA assay.

All of these patients had histories of thromboembolic disease (4 with venous thrombosis and 2 with arterial thrombosis). These findings can suggest, at least in some patients, a role of SCT as an independent risk factor for thrombosis. Furthermore, the presence of SCT positivity in patients with thromboembolic events reduces, at least in part, the number of patients who are otherwise seronegative for anti-phospholipid autoantibodies. More data and prospective studies are needed to confirm this hypothesis.

Immuochemical Quantification of Free Light Chains in Urine, Ileana Herzum,* Harald Renz, and Hans Günter Wahl

(1) Quantitative measurements of plasma and urinary para
protein concentrations play a major role in the monitoring of patients with multiple myeloma. The concentrations are routinely estimated from the size of the M-spike on protein electrophoresis (PEL) or by automated immunologic assays for IgG, IgA, IgM, IgE, or IgD. In the case of light chain myeloma and intact immunoglobulin myeloma with predominant light chain production, light chain concentrations could, until recently, be measured only by the size of the urinary light chain M-spike on PEL or by the measurement of the total (free and bound) light chain concentrations.

A latex-enhanced assay (Freelite; The Binding Site, Ltd.) measuring free light chains (FLCs) in serum and urine has recently become available for the BNII (Dade Behring) analyzer. The Myeloma Management Guidelines (1) recommend the Freelite test for serial monitoring of the FLCs in serum, but periodic 24-h urine collection is still required for Bence Jones proteinuria (BJP) and total urinary protein (TUP) quantification. Depending on the glomerular and tubular function, serum and urine FLC concentrations may not change to the same degree (2), so that monitoring of serum FLC alone is questionable for revealing the actual degree of disease in patients with BJP and tubular dysfunction.

We evaluated the analytical performance of the immunochemical test for serum and urine with the BNII analyzer. The test uses antibodies that specifically recognize an epitope of the common region of κ and λ light chains that is “hidden” when the light chains are attached to the immunoglobulin heavy chain (3).

Intra- (within) and interassay (day-to-day total) imprecision (CV) was determined with control material and with patient samples containing high and low concentrations of polyclonal or monoclonal FLCs (Table I). The high CV observed for the serum sample with a high monoclonal λ FLC concentration may reflect the variable

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