Dear Sir,

Thrombotic thrombocytopenic purpura (TTP) is a syndrome characterised by thrombocytopenia and microangiopathic haemolytic anaemia; it is often associated with neurological dysfunction, renal failure and fever (1). TTP is closely associated with a severe functional deficiency of ADAMTS13, the protease specifically cleaving large multimers of von Willebrand factor (VWF) in plasma (2). Both ADAMTS13 mutations (hereditary TTP) and auto-antibodies (acquired TTP) result in a severe functional ADAMTS13 deficiency in patients (3–5). As the clinical characteristics of TTP may be similar to those of other thrombotic microangiopathies (TMA) such as haemolytic uremic syndrome (HUS), an assay measuring ADAMTS13 activity would be a helpful tool for appropriate differential diagnosis and subsequent treatment. Many previous methods for the measurement of ADAMTS13 activity are not widely used at the clinical level because of technical complexity.

Recently, a VWF fragment (VWF73) comprising the 73 amino acid residues (aa 1596–1668) of VWF subunit was shown to be the minimal substrate for ADAMTS13. The enzyme specifically cleaves this fragment in non-denaturing conditions and in a very short incubation period at the Tyr-Met bond. This VWF73 fragment was thus considered as an alternative substrate to full-length VWF for measurement of ADAMTS13 activity (6). Taking advantage of this substrate, a rapid and high throughput fluorescent energy transfer (FRETS) assay has been developed in which a chemically synthesized fluorogenic fragment (FRETS-VWF73) was used as a substrate to measure ADAMTS13 activity in plasma samples (7). A comparison with multimeric assay was performed and found to be highly correlated (8).

The present study was designed to evaluate the FRETS-VWF73 fluorescence assay when compared to our immunoradiometric assay (IRMA) using full-length VWF for the measurement of ADAMTS13 activity in plasma samples from a cohort of TMA patients and from normal subjects. Plasma from 64 TMA patients including 41 patients with acquired acute TTP, three patients with inherited TTP and 20 patients with acute HUS were tested after obtaining appropriate consent in agreement with the institutional reviewing board of Assistance Publique – Hôpitaux de Paris and the declaration of Helsinki. Plasma of 10 healthy subjects was used as controls. ADAMTS13 activity of plasma samples was measured by a two-site IRMA using full-length WT recombinant VWF as substrate and selected monoclonal antibodies to VWF, as previously described (9). Simultaneously, all samples were tested by FRETS-VWF73 fluorescence assay. Briefly, pooled human plasma used for calibration
ADAMTS13 activity. The linear regression analysis showed the equation \( y = 0.94x + 1.71 \) with a correlation coefficient of 0.97. Tested by IRMA, normal subjects and most HUS patients showed normal ADAMTS13 activity (\( \geq 50\%) \) whereas all TTP patients had an ADAMTS13 activity <5%: in the healthy volunteers (n = 10), the values of ADAMTS13 activity ranged from 93% to 110%; out of the 64 patients with TMA, ADAMTS13 activity was normal in 18 HUS cases, decreased in two HUS patients (38% and 41%, respectively) and <5% in 44 TTP cases. Corresponding results from fluorescence assay showed an ADAMTS13 activity of 100% to 120% in healthy individuals, ≥ 50% in 17 HUS cases, moderately decreased in three HUS cases (30%, 33% and 38%, respectively) and <5% in 44 TTP cases. Although the relationship between ADAMTS13 activity and TTP is more complicated than originally thought (10), the management of patients with TTP, HUS, or other TMA may benefit from the laboratory measurement of this metalloprotease. Because of the numerous methods developed to determine ADAMTS13 activity (11), it is essential to evaluate their relative performance characteristics. We chose to compare a very recently developed fluorescence assay (7) to an IRMA previously set-up in our laboratory (9) for measurement of ADAMTS13 activity in normal, TTP or HUS plasma. The IRMA can be performed in the setting of a hospital laboratory but it still remains time-consuming because of the long hydrolysis step (48 hours). In contrast, using FRETS-VWF73 fluorescence assay, quantitative analysis is achieved within a one-hour period using 96-well format in commercial plate readers with common filters. The main characteristics of the two ADAMTS13 assays are summarized in Table 1. In the present study, the results of the FRETS-VWF73 fluorescence assay were in close agreement with the corresponding results of the IRMA. Similar observations have been recently reported by Groot et al., comparing the FRETS-VWF73 assay to the other existing methods for ADAMTS13 activity measurement including proteolytic multimer assay, immunoblotting assay and IRMA (8).

In conclusion, compared to our IRMA method, the FRETS-VWF73 fluorescence assay exhibited reliable results to detect low, moderately reduced as well as normal levels of ADAMTS13 activity. The assay was also found to be as sensitive as the IRMA method to identify undetectable levels of the enzyme in hereditary or acquired TTP. Accuracy and reproducibility of the assay combined with its short incubation time make the method highly attractive and appropriate for the clinical screening of patients with TMA.

Table 1: Main characteristics of the ADAMTS13 activity measurement methods.

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<th>Method</th>
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<th>Assay conditions</th>
<th>Incubation time</th>
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References