The effect of M-proteins on the erythrocyte sedimentation rate; a comparison between the StarrSed and TEST 1 analyzers

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Introduction
Measurement of the erythrocyte sedimentation rate (ESR) is an easy and inexpensive laboratory technique that has been used as a non-specific screening procedure to assess the acute phase response for many years (1). The International Council for Standardization in Haematology has recommended the original method described by Westergren as the gold standard (1). The Westergren method measures the plasma column after one hour of spontaneous sedimentation and is thus a representation of the physical process of erythrocyte sedimentation. Any condition associated with increased levels of positively charged molecules will lead to an elevated ESR. Especially, M-proteins that can be found in multiple myeloma or Waldenstrom’s macroglobinemia will enhance rouleaux formation, thereby resulting in greatly elevated LSBR readings (2, 3).

A couple of years ago the TEST 1 (Alifax, Padovo, Italy) was marketed. TEST 1 measures the ESR using a quantitative capillary photometry-based technology in which data are converted into Westergen values (4). Previous studies have shown a good correlation between ESR readings by TEST 1 and Westergren based methods (5, 6). However, these studies have not taken into account the influence of the presence of a M-protein on ESR readings. As the TEST 1 measures the ESR in the initial lag-phase, we hypothesized that this method is less influenced by the presence of a M-protein compared to other methods based on the Westergren principle. The aim of this study was, therefore, to investigate the effect of M-proteins on the ESR measured by the TEST 1 analyzer in comparison to the ESR measured by the StarrSed analyzer which is Westergren based.

Materials and Methods
142 consecutive patients, known with a M-protein, visiting the laboratory for routine monitoring of M-protein levels, were asked to participate in the study. Informed consent was obtained from each patient and one additional tube containing K$_3$-EDTA was collected. ESR was simultaneously assessed using the TEST 1 and the StarrSed (Goffin Meyvis, Etten-Leur, The Netherlands), a Westergren-based method. In each patient type and concentration of the M-protein was determined by electrophoresis followed by immunofixation on the Sebia Hydrasys electroophoresis system using agarose gels and the Hydragel 6/12 IF Pentakit (SEBIA Benelux NV., Issy-les-Moulineaux, France). Data from a validation protocol (102 consecutive patients) served as a control population. In protocol LSBR was similarly measured using the same techniques as in the present study.

Data were statistically evaluated using MS Excel 2003 software (Microsoft, Redmond, WA, USA) and Analyse-It version 1.72 (Analyse-It Software Ltd., Leeds, UK). Passing-Bablok analysis was used to compare ESR values and Bland-Altman analysis was used to evaluate bias and limits of agreement. Linear regression was used to investigate the effect of M-protein level on LSBR. For linear regression analysis samples of which M-protein level could not be determined quantitatively from the electrophoresis pattern have been excluded. Values of $p < 0.05$ were considered to be statistically significant.

Results
In a random hospital population good agreement between the StarrSed and the TEST 1 was found with a slope of the Passing-Bablok method comparison of 0.96 (95% CI 0.85-1.05), see figure 1. In contrast, the presence of a M-protein (concentration up to 55 g/L) resulted in a poor agreement between these methods with a slope of the Passing-Bablok curve of 0.67 (95% CI 0.57-0.77). From the Bland-Altman plots it becomes clear that this arises from the large divergence when higher ESR values are measured. ESR readings of >40mm/h show not only a larger difference between methods, but individual ESR readings by the StarrSed are also higher than those measured by the TEST 1, resulting in a larger mean bias in patients with a M-protein.

Due to the molecular structure of the M-protein subtypes, it may be expected that subtypes behave differently in ESR measurement. In a subgroup analysis based on M-protein subclassification we found that M-proteins of subclass IgG and IgA show poor agreement between the two methods with a slope of the Passing-Bablok curve that was similar for both M-protein subclasses. The agreement between methods seemed to be even worse for M-proteins of subclass IgM as the slope of the Passing-Bablok curve was lower, but with an overlap of the 95% confidence intervals (data not shown).
The clinical impact of the use of both methods was investigated by comparing the effect on clinical interpretation (i.e., ESR below or above reference level for both methods). Although TEST 1 ESR readings are lower than those of the StarrSed, clinical decision making is hardly influenced by the method used for ESR measurement. Of the overall population under study the data from 128 (90%) subject showed concordance with respect to clinical interpretation. When subdivided into M-protein subclasses this was 88%, 100% and 89% for M-protein of subclass IgG, IgA and IgM, respectively. These findings suggest that the presence of a M-protein still results in an elevated ESR reading when using the TEST 1, but that the elevation is less pronounced in comparison to ESR readings by the StarrSed.

We hypothesized that the observed differences were dependent on the amount of M-protein present. When the relationship between M-protein concentration and height of ESR was investigated, we found that both methods were influenced by the concentration of M-protein present (table 1). However, the influence of M-proteins on the ESR reading by the StarrSed were statistically higher than those measured by the TEST 1. The slope of the linear regression was 2.6-times higher (2.4 versus 0.9) and the 95% confidence intervals did not overlap. For the StarrSed a statistical significant linear relationship was found for the presence of a M-protein of either subclass with the largest effect found for subclass IgM. Surprisingly, only a statistically significant correlation was found between the ESR reading by TEST 1 and M-proteins of subclass IgG.

Discussion
In this study we have shown that for patients with a M-protein there is no difference in clinical interpretation when ESR is measured with either the StarrSed or the TEST 1. However, the presence of a M-protein has dif-

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**Figure 1.** Comparison of ESR readings measured with the StarrSedd and TEST 1 analyzers in a random hospital population (A/C) and patients with a M-protein (B/D). A and B: Passing-Bablok plots. Regression equations are shown in the graph. C and D: Bias plots. Horizontal lines (— • —) denotes limits of agreement (C: -15.8 to 14.1 mm/h and D: -65.1 to 40.1 mm/h).
Different impact on the ESR reading. Especially for ESR readings >40mm/h a large difference between both methods exists. This can most likely be explained by the M-protein dependency of the ESR reading which is more pronounced in the StarrSed in comparison to TEST 1.

This is the second study known to us that has investigated the influence of M-proteins measured by TEST 1 in comparison to a Westergren-based method. In a previous study, Ajubi et al. (7) have shown similar findings for the whole population. However, their findings in a subgroup of patients with a M-protein of subclass IgM are in contradiction to our findings that could possibly be explained by the differences in composition of the investigated groups. Whereas the study of Ajuby et al. consisted of few subjects with M-protein of subclass IgM (n=9) with levels up to 20 g/L, our study was larger (n = 28) with M-protein levels up to 43 g/L.

In addition, we have also investigated the relationship between M-protein levels of all three subclasses for both the TEST 1 and the StarrSed and found that ESR values measured by the StarrSed are M-protein concentration dependent, whereas those measured by TEST 1 are not, with the exception of M-protein of subclass IgG. The different influence of M-proteins on ESR readings in both methods might be explained by the dissimilarities in method principles. In TEST 1 the ESR reading takes place in the lag-phase when only small changes in erythrocyte distribution occur. As this process is most probably not influenced by the presence of a M-protein, there will only be a minimal effect on the ESR reading. In contrast, in Westergren-based methods the ESR reading is largely influenced by the physical interactions during the sedimentation phase of the process. As M-proteins of subclass IgM have the greatest effect, the interaction of immunoglobulins with the negatively charged erythrocytes is most likely size-dependent. Moreover, as a pentamer IgM has not only the best capability to shield the negative charges on the surface of an erythrocyte, but its size will also enhance rouleaux formation by coupling individual erythrocytes. This also explains why different slopes were found for M-protein of subclasses when linear regression was performed on the three individual subclasses.

In conclusion, this study has shown the validity of TEST 1 for ESR measurement in patients with a M-protein. ESR readings by TEST 1 result in similar clinical interpretation, but are less elevated by the presence of a M-protein, as found with Westergren-based methods. TEST 1 is, therefore, not a good indicator for the detection of patients with a M-protein.

References