INTRODUCTION
Thrombotic Thrombocytopenic Purpura (TTP) is a life-threatening disease characterised by microvascular platelet deposition and thrombus formation in selected organs with resulting microangiopathic haemolytic anaemia, renal failure, neurological symptoms and thrombocytopenia. Typically a very rare disorder, TTP is being seen with increased frequency in patients infected with the human immunodeficiency virus (HIV). Deficiency of the Von Willebrand factor cleavage protease, also known as ADAMTS13, has been implicated as a major aetiological factor in TTP. However, before studying the role of ADAMTS13 in HIV related TTP, it is necessary to know the normal values of ADAMTS13 levels in the population group in question because lower ADAMTS13 levels had been reported in other populations such as the Chinese. It is also necessary to know whether HIV infection in the absence of TTP has any effect on ADAMTS13 levels.

AIM
The aim of the study was threefold:
1. To compare the ADAMTS13 levels in the local Caucasian and African populations;
2. To study the effect of HIV infection on ADAMTS13 levels by correlating CD4 counts and viral loads with ADAMTS13 levels in HIV positive patients without TTP; and
3. To measure ADAMTS13 levels in patients with HIV associated TTP.

METHODS
Ethical Approval:
Ethical approval was obtained from the Ethics Committee of the Faculty of Health Sciences, University of Free State.

Study populations:
Group 1 (n=40): Normal group - subdivided into Caucasian (n=19) and African (n=21) participants respectively. No history of chronic disease or use of chronic medication; no symptom(s) of disease and a normal full blood count.
Group 2 (n=38): HIV positive patients from a local HIV clinic were randomly selected on a number of clinic days (patients were aware of their HIV status). Three Sub-groups: CD4>200 not on HAART; CD4<100 not on HAART; patients with a viral load of 3400 to 700 000.
Group 3 (n=20): Patients with HIV associated TTP were also tested.

Sample collection and preparation:
EDTA and citrated venous blood were collected, processed and analysed. Plasma was aliquoted and stored at -80°C until ADAMTS13 & viral load assays were done. CD4 counts were determined with a Beckmann Coulter flow cytometer (Epics XL.MCL) and viral loads with a Roche Amplicor HIV-1 (RT-PCR) kit according to the manufacturer’s instructions.

RESULTS (Fig. 1)

ADAMTS13 levels in healthy, normal subjects:
HIV+: CD4 < 200
They postulated two explanations:
HIV+: CD4 < 100
ADAMTS13 & viral load assays were done. CD4 counts were determined and viral loads were measured in 20 HIV associated TTP patients. The values were all low (144 ± 109 ng/ml) when determined with a Beckman Coulter flow cytometer (Epics XL.MCL) and viral loads with a Roche Amplicor HIV-1 (RT-PCR) kit according to the manufacturer’s instructions.

ELISA for ADAMTS13-antigen levels:
An ELISA was done using three antibodies 20A5, 5C11 and 13F7 that were received from the laboratory of Prof Deckmyn, Belgium. A 96-well ELISA plate was coated overnight with the 20A5 anti-ADAMTS13 monoclonal antibody. After washing with PBS containing 0.1 % Tween-20, the plate was blocked with 4% skimmed milk in PBS for 2 hours at room temperature. Plasma samples of each normal subject or patient were diluted in [1:10 and 1:20 dilutions] and added in duplicate. Normal pooled plasma was calibrated against a standard plasma form American Diagnostica and added in serial dilutions, i.e. 100ng/ml, 50, 25, 12.5, 6.25, 3.125 and 1.56 ng/ml. A blank and a plasma control with a known ADAMTS13 level (American Diagnostica, USA) were added to the plate and incubated for 1.5 hours at 37°C. The 2 biotinylated anti-ADAMTS13 antibodies 5C11and 13F7 were added and incubated after which peroxidase-conjugated streptavidine was added. The colour was developed and read at 490 nm with the SYNERGY HT microplate bio-kinetics reader (BioTek Instruments, Vermont, USA). An eight-point standard curve was drawn with the known ADAMTS13 levels on the X-axis and the optical density values on the Y-axis. The ADAMTS13 level value for each subject/patient was read off the standard curve.

ADAMTS13 levels in HIV associated TTP group:
Deficiency in ADAMTS13 is a tool in the setting of HIV positive patients with TTP. It is necessary to know whether the severity of HIV infection as reflected by the CD4 counts and viral loads has any effect on ADAMTS13 levels. Viral loads varying from 3 400 to 700 000 had no effect on ADAMTS13 levels.

CONCLUSION
1. We have standardised an ELISA for measuring ADAMTS13-antigen levels and established reference ranges for the local Caucasian and African population groups. We found no statistical difference in the ADAMTS13-antigen levels between Caucasians and Africans. The reference range for ADAMTS13-antigen levels in our laboratory is 585 – 893 ng/ml

2. We could also show that neither HIV infection, nor the severity of the disease as reflected by CD4 count and viral load had any effect on ADAMTS13 levels, making it a useful diagnostic tool in the setting of HIV positive patients with TTP.

3. We found low ADAMTS13 levels in all 20 patients with HIV associated TTP patients (244 ± 121 ng/ml).

REFERENCES