# Heat-treated Bethesda assay in the monitoring of acquired haemophilia A

Bowyer AE, Shepherd MF, Titchard P, Lowe AE, Sampson BM, Brown PD, Leeson K, Kitchen S, Makris M

Coagulation Department, Royal Hallamshire Hospital, Sheffield, UK

## INTRODUCTION AND OBJECTIVES

- The Bethesda assay (BA) is used to quantitate inhibitory antibodies to Factor VIII (FVIII) in both congenital (HA) and acquired haemophilia A (AHA). The presence of measureable FVIII activity (FVIII:C) may affect the sensitivity of the BA to inhibitor detection and the assay is generally avoided once the FVIII:C is above 0.10 IU/ml.
- A variation of BA involves pre-incubation of patient plasma at high temperature (heat treatment, HT) to destroy FVIII and permit accurate inhibitor measurement [1, 2].
- This study compared the standard and HT BA in patients with congenital HA and AHA.

## METHODS AND MATERIALS

- 20 Severe HA (SHA) patients with no previous history of inhibitors, 14 samples from 10 severe HA with inhibitors and 46 samples from 23 patients with AHA were included in this study.
- Samples were tested using a standard Nijmegen BA with adjustment for presence of FVIII:C greater than 0.05 IU/ml or a HT version in which plasma was pre-incubated for 30 minutes at 58°C, centrifuged at 5000g for 2 minutes before use in a standard BA (see figure 1).
- Standard human plasma (SHP) was used as the source of FVIII. FVIII-deficient plasma (both Siemens, Germany) was included in the control mix. Dilutions were made in Imidazole buffer (0.05M, pH 7.3) and were identical in both standard and HT assays.
- One-stage clotting FVIII:C were performed using the above reagents and Actin FS (Siemens) with Sysmex CS5100i (Kobe, Japan) instrumentation to determine % residual FVIII
- Bethesda results of >5 BU/ml were considered high titre and 0.6-5 BU/ml as low titre. Very low titre inhibitors could not be excluded if the residual FVIII was between 75% and 80% so were resulted at <0.6 BU/ml.

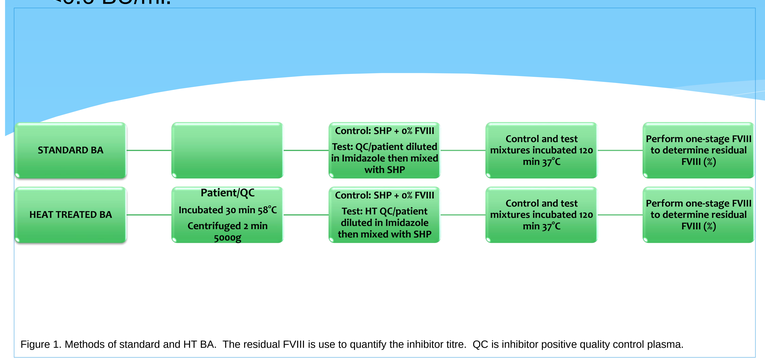


Figure 1. Methods of standard and HT BA. The residual FVIII is use to quantify the inhibitor titre. QC is inhibitor positive quality control plasma.

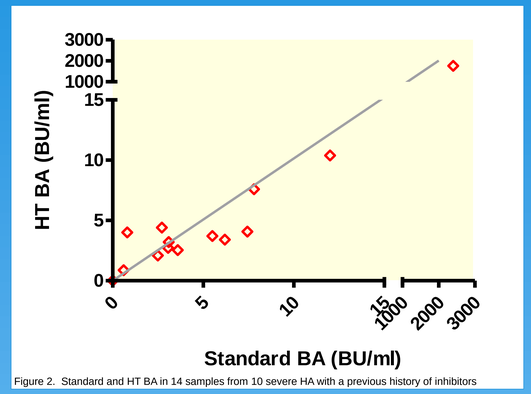


Figure 2. Standard and HT BA in 14 samples from 10 severe HA with a previous history of inhibitors

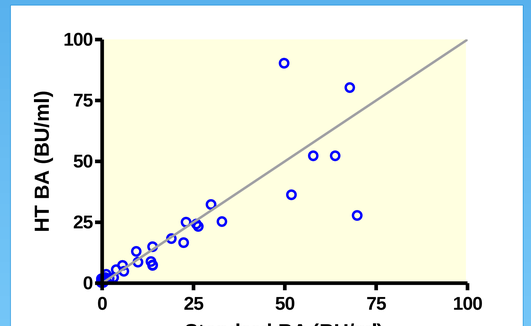


Figure 3. Standard and HT BA in 46 samples from 23 AHA. Results greater than 100 BU/ml not shown.

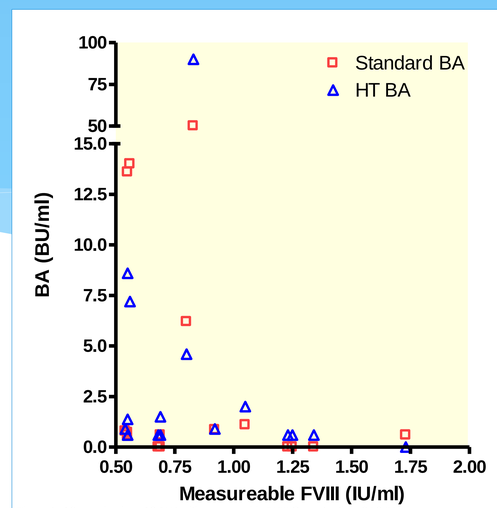


Figure 4. 16 samples from 10 AHA with measureable FVIII:C greater than 0.50 IU/ml.

## RESULTS: CONGENITAL HA

- The BA (standard and HT) of patients with congenital HA and no inhibitors were negative (residual FVIII greater than 80%). FVIII:C ranged from <0.01-1.70 IU/ml, mean 0.29 IU/ml (data not shown).
- The median standard BA in patients with previous inhibitors was 3.35 BU/ml (range 0-2400 BU/ml). The median HT BA was 3.5 BU/ml (range 0-1760 BU/ml). See figure 2.
- No statistically significant difference was observed between BA ($p>0.05$ by paired t-test). Pearson correlation coefficient $r=1.0$.

## RESULTS: ACQUIRED HA

- In AHA the mean measureable FVIII:C was 0.43 IU/ml (range <0.01-1.73 IU/ml).
- The average standard BA was 50.3 BU/ml (median 7.9 BU/ml) and average HT BA was 46.2 BU/ml (median 7.0 BU/ml). See figure 3.
- 27/46 samples demonstrated both non-linear standard and HT BA due to type 2 kinetics.
- Good correlation ($r=0.84$) and no statistically significant difference (paired t-test $p>0.05$) was demonstrated between BA assays.
- A positive or possible inhibitor (standard or HT BA) was observed in 16 samples from 10 patients with measureable FVIII:C above 0.50 IU/ml (average 0.91 IU/ml). See figure 4.
- The standard BA was positive in 11/16 samples with an average of 5.4 BU/ml (median 0.66 BU/ml). 2 samples from different patients had a result of <0.6 BU/ml (residual FVIII 75-80%). The HT BA was positive in 15/16 samples with an average of 8.0 BU/ml (median 0.9 BU/ml). 5 samples from 3 patients had a result of <0.6 BU/ml (residual FVIII 75-80%).

## CONCLUSIONS

- The presence of antibodies to FVIII can influence the treatment of congenital HA.
- Little difference in titre was observed between the standard and HT BA in congenital or acquired HA.
- Low and high titre inhibitors were detectable in some Acquired HA patients despite measureable FVIII:C within the normal range.
- In AHA, the standard or HT BA, should be performed even in the presence of normal FVIII:C.
- The heat treated BA is a suitable assay for the assessment of specific inhibitors to FVIII when the measureable FVIII:C is greater than 0.10 IU/ml.

## REFERENCES

1. Verbruggan B. Diagnosis and quantification of factor VIII inhibitors. Haemophilia (2010), 16, 20-24
2. Miller CM, Platt SJ, Rice AS, Soucie JM. Validation of Nijmegen-Bethesda assay modifications to allow inhibitor measurement during replacement therapy and facilitate inhibitor surveillance. JTH (2012), 10, 1055-61

Annette.Bowyer@sth.nhs.uk

Sheffield Teaching Hospitals NHS
NHS Foundation Trust