

A flow cytometric immunoassay for the detection of antibodies specific for (r)bST in serum of dairy cows

Nathalie G.E. Smits¹, Maria G.E.G. Bremer^{1,*}, Susann Ludwig¹, Michel W.F. Nielen^{1,2}

Aim

To develop a screening method for tracking down rbST abuse in herds of cattle. To overcome non-specific binding in the microsphere-based immunoassay (MIA) for detection of (r)bST specific antibodies (α -(r)bST), different kind of pretreatments were analyzed.

Method

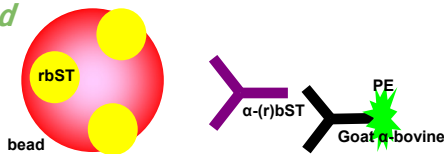


Figure 1. α -(r)bST MIA using the xMAP™ technology (Luminex).

- A direct MIA was used for α -(r)bST detection (Fig. 1).
- As a start, serum samples of 27 untreated and 4 treated cows were diluted 100x in PBST and measured in MIA.
- Based on the obtained responses, six serum samples with different characteristics were selected:
 - 2 from treated cows (1 high and 1 low responder)
 - 4 from untreated cows (2 high responders)
- Responses of these samples were compared for the reduction of non-specific binding after: dilution, size exclusion (100 kDa), octanoic acid precipitation, protein G purification and glycine-SDS pretreatment

Results

Dilution of serum samples resulted in (Fig. 2):

- Big differences in responses untreated cows \rightarrow SD 69%
- One false positive result \rightarrow non specific binding
- False negative responses in 50% of the treated cows
- An indecisive assay for tracking down rbST abuse

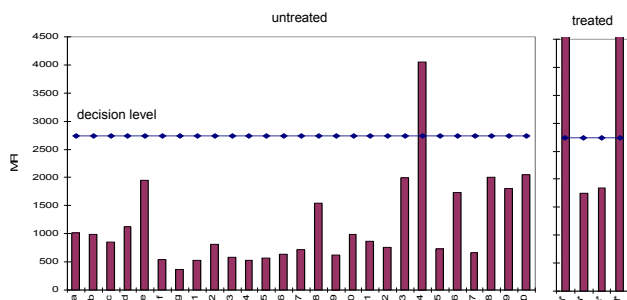


Figure 2. Responses of 100 times diluted serum samples of untreated and treated cows, decision level: Average untreated response + 2SD.

Comparing all sample preparations (Fig. 3) best results were obtained with the glycine-SDS pretreatment, as it:

- showed the ability to reduce non-specific binding

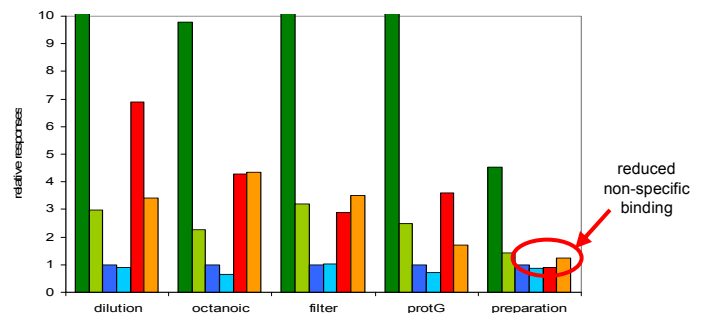


Figure 3. Comparison of sample preparation techniques on six selected serum samples, two positives (■), two negatives (■) and two false positives (■).

Glycine-SDS pretreated serum samples from 27 untreated and 4 treated cows resulted in (Fig. 4):

- Relatively small differences in responses of untreated cows \rightarrow SD 21%
- No false positive results
- 75% of treated cows could be identified, even 4 weeks after the last injection (results not shown).

Conclusion

We were able to pinpoint (r)bST abuse in 75% of the treated cows with α -(r)bST detection after glycine-SDS sample pretreatment. A screening method with high potential for tracking down (r)bST abuse in herds of cattle is developed.

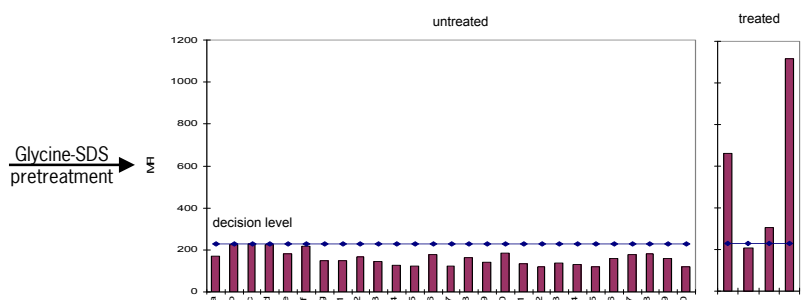


Figure 4. Responses of glycine-SDS pretreated serum samples of untreated and treated cows, decision level: Average untreated response + 2SD.

¹ RIKILT – Institute of Food Safety

P.O. Box 230, NL-6700 AE
Wageningen, The Netherlands
Phone: +31 317 48 02 56
Internet: www.rikilt.wur.nl

* Corresponding author: monique.bremer@wur.nl

² Wageningen University

Laboratory of Organic Chemistry
Dreiijenplein 8, NL-6703 HB
Wageningen, The Netherlands

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