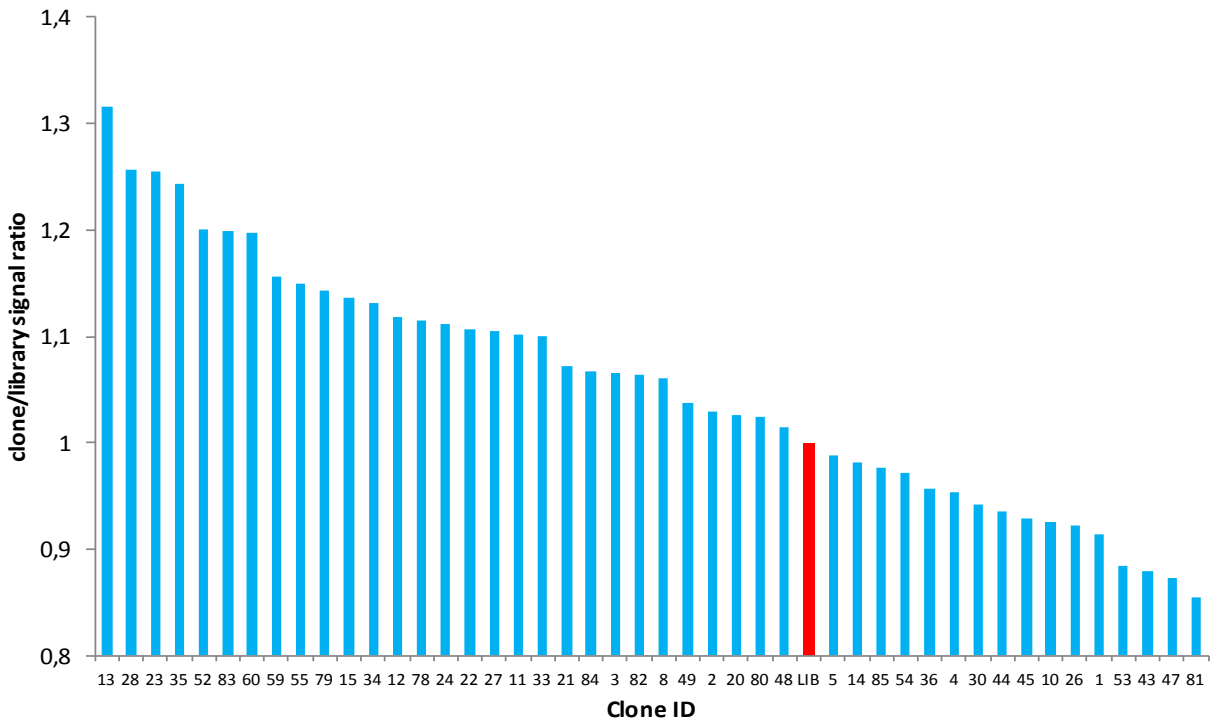

Figure 5 – Affinity assessment of VHH clones. X axis – VHH clone ID, Y axis - signal ratio between clone's and reference control (CD44/BSA).

For reference control, we used total VHH library generated after 6-th round of selection. HRP activity was measured for each clone and total library in BSA-coated and target protein (CD44) coated wells. We calculated target-to-control values (signal with CD44 / signal with BSA), and then compared each clone to reference control (total VHH library). Resulting ratios are presented in figure 5.

Analysis of 46 individual clones revealed substantial variations in their binding affinity. Around 40% performed worse than total VHH library, among others, 7 had over 20% higher binding properties than reference, and only 1 had 30% higher performance.

The developed screening method was not previously described, aim of the study was to search for individual affinity variants of VHH antibodies. Previously, when working with the phage library of VHH antibodies, screening was carried out either on monoclonal phages presenting antibodies on their surface or soluble antibody fragments expressed in bacteria. The latter is especially difficult in the case of screening large amounts of clones. Screening performed on soluble VHH fragments generated in vitro allows it to be carried out immediately after selection and is compatible with libraries selected by both phage and ribosome display.

CONCLUSION

In this work, we developed and tested rapid method of affinity testing of individual VHH fragments from ribosome display library which was obtained after six round of panning on CD44 protein.

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