

BioTides™ as High Throughput Screening Tool for the Identification of Antibody Binding Sites

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BioTides™ are biotinylated unpurified peptides. They can be rapidly produced, in large numbers at a fraction of the cost of purified, resin-synthesized peptides (ResPeps). This makes them attractive for peptide-based screening studies using thousands of peptides, provided that their performance is comparable to high purity ResPeps. Our study showed excellent batch to batch reproducibility of BioTides™ and a strikingly high correlation between the analyzed BioTides™ and ResPeps.

Introduction

The identification of antibodies related to autoimmune diseases, cancer and infectious agents is fundamental to basic clinical research and important for the prediction, diagnosis and monitoring of disease. In recent years it has been demonstrated that circulating plasma autoantibodies in cancer patients react with tumor-associated antigens^{1,2}. Multiple detection systems and technologies employ hundreds of recombinant antigens to screen for autoantibodies in plasma samples. Because these immunoassays use recombinant proteins expressed in bacteria or eukaryotic cells high background and nonspecific binding often result in control groups. High throughput screening for linear antibody binding sites with huge libraries of synthetic peptides offers a new and more effective approach to current screening technologies. Therefore we tested JPT Peptide Technologies' BioTides™ for their application as a timesaving and cost-efficient screening tool compared to the "gold standard" resin-synthesized-peptides (purity >70%) (ResPeps).

Materials & Methods

20 biotinylated peptides of different proteins (e.g. EBV) with known reactivity to human plasma samples were synthesized as BioTides™. Each of the 20 peptides was synthesized 37 times in one BioTides™ lot with 3 replicates for each lot. Additionally, all 20 biotinylated peptides were ordered as two lots of ResPeps (purity >70%) for comparative analysis. Antibody reactivity of 36 human plasmas to peptides was measured by multiplexed Luminex® immunoassay analysis.

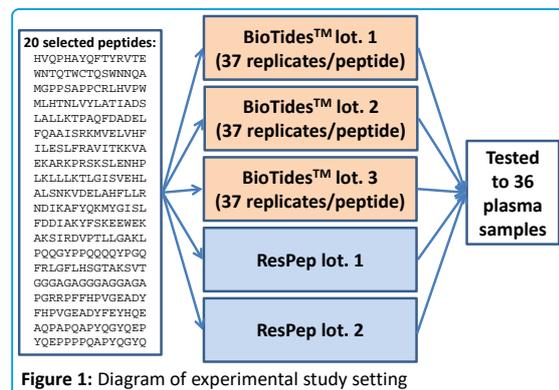


Figure 1: Diagram of experimental study setting

Solid-phase LumAvidin® beads with different-colored fluorophores were immobilized with different peptides and incubated with sera and phycoerythrin-labeled secondary antibody. Following the antigen identity of up to 100 beads the corresponding antibody titers were measured simultaneously on a BioRad Bio-Plex™200 system. Luminex® signal intensities (MFI) were normalized to the plasma specific background and a logarithmic transformation was applied.

For the statistical analysis Pearson's correlation coefficients and Spearman's correlation coefficients were calculated by averaging the peptide replicates. Pearson's correlation coefficients confidence intervals were estimated by bootstrapping.

Results

Peptide-based screening of plasma samples requires a highly reliable and robust method. For this purpose we first compared the reactivity of 36 human plasma samples with a set of 20 randomly selected peptides of two different ResPep lots.

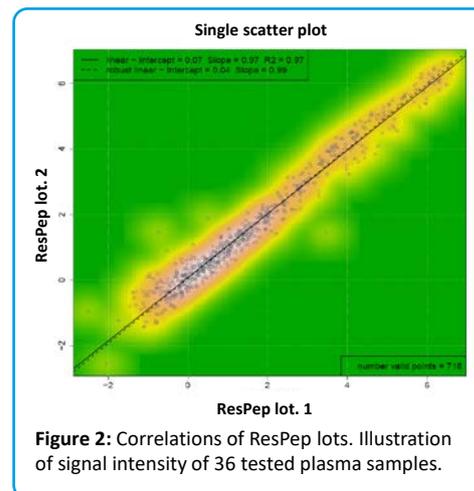


Figure 2: Correlations of ResPep lots. Illustration of signal intensity of 36 tested plasma samples.

The signal intensity of measured peptide-antibody binding showed an excellent correlation of R=0.98 between the two tested lots and a high reproducibility (R2= 0.97, Slope= 0.99, figure 2). We compared the quality of BioTides™ as a cost-efficient alternative to ResPep peptides. Replicates of all 20 peptides were synthesized in 3 synthetic batches (37 per synthesis lot, n=3) using JPTs high throughput synthesis platform. Figure 3A shows the intra-synthesis variability for 2 representative peptides. It was comparable to the inter-synthesis deviation calculated as an average of peptide replicates (see figure 3B). All three BioTides™ lots showed an excellent Pearson correlation coefficient ranging from R= 0.96 to R=0.98 and is equivalent to the explained variance between R2= 0.94 – 0.96. An important impact for the qualification of BioTides™ as a screening tool is a comparison to the "gold standard" ResPeps (see figure 3C). The Pearson correlation coefficient of the 3 BioTides™ lots to ResPeps was lower with R=0.88 – 0.93, corresponding to explained variance of R2= 0.77 – 0.87. Nevertheless, in this study we demonstrated for the first time a sufficiently high quality of BioTides™ when compared to ResPeps.

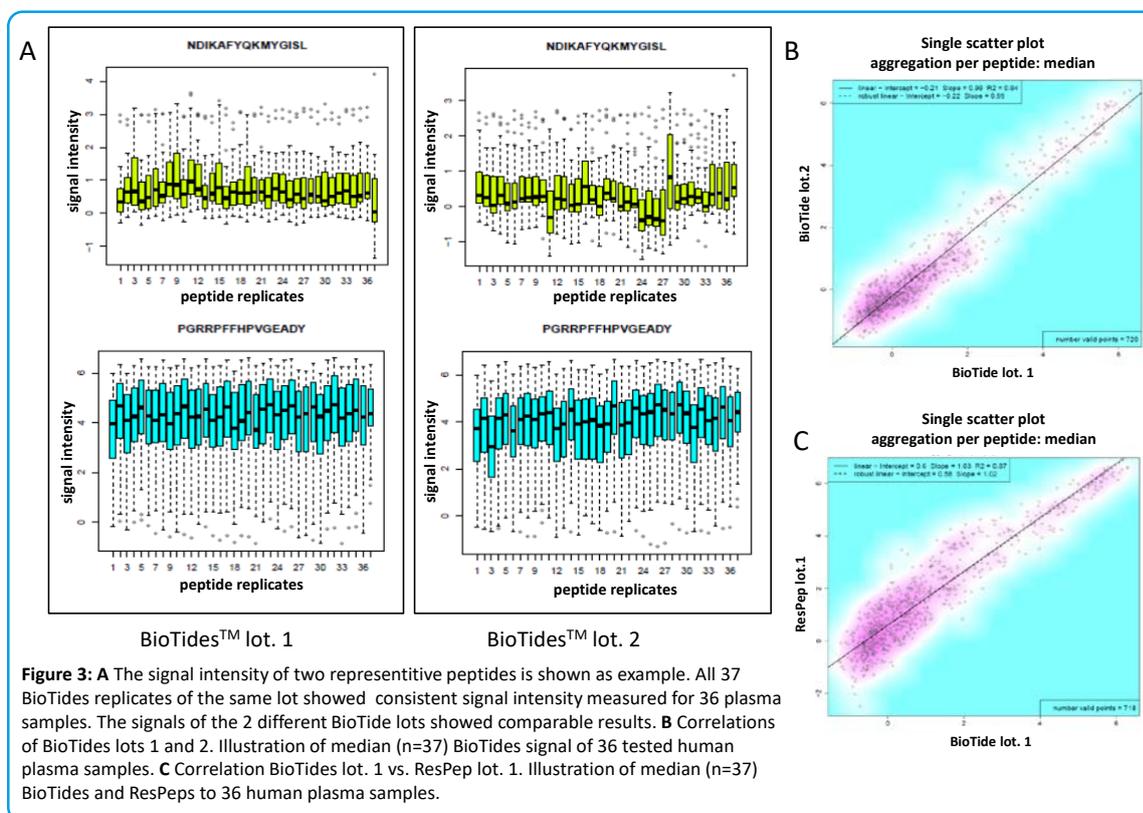


Figure 3: **A** The signal intensity of two representative peptides is shown as example. All 37 BioTides replicates of the same lot showed consistent signal intensity measured for 36 plasma samples. The signals of the 2 different BioTide lots showed comparable results. **B** Correlations of BioTides lots 1 and 2. Illustration of median (n=37) BioTides signal of 36 tested human plasma samples. **C** Correlation BioTides lot. 1 vs. ResPep lot. 1. Illustration of median (n=37) BioTides and ResPeps to 36 human plasma samples.

Discussion & Conclusions

This immunoassay demonstrated that BioTides™, crude, intact biotinylated peptides are well suited for an Avidin-Biotin assay. Taken together, we showed that the reproducibility of different BioTides™ syntheses itself and the correlation to ResPeps qualify BioTides™ as a basis for high-throughput screening. Low-cost BioTides™ are synthesized by JPT's SPOT synthesis technology with additional capping after each synthesis step. As a consequence only full-length target peptides will be biotinylated and capped truncation sequences are removed during immobilization onto the Avidin-surface. This eliminates the need of additional purification steps, resulting in product qualities comparable to purified peptides.

References

1. "Tumor-associated Antigen Arrays for the Serological Diagnosis of Cancer"; Casiano et al., Mol & Cell Proteomics (2006)
2. "Autoantibody signatures: progress and perspectives for early cancer detection"; Desmetz et al., J Cell Mo IMed (2011)

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The Company

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