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# Evaluation of a sensitive detection method for peptide arrays prepared by SPOT synthesis

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**Abstract**

The growing range of applications for peptide arrays prepared by SPOT synthesis confirms that they are a powerful proteomics technique to study numerous aspects of molecular interaction events. The most frequent application for peptide arrays prepared by SPOT synthesis is the identification of linear epitopes that are recognized by antibodies. In the conventional format using secondary antibodies for detection unspecific binding and high background have been observed. This leads to difficulties in evaluation of developed membranes. Especially for application with combinatorial libraries false positive results are to be avoided. To circumvent this issue, we directly labeled compounds of interest with biotin and detected binding by incubation with streptavidin–horseradish peroxidase via chemiluminescence. Optimization of method conditions led to a very sensitive detection technique with no or low number of unspecific spots, which is superior to conventional detection with secondary antibodies. As one consequence, evaluation of competitive assays got more reliable.


*Keywords:* Biotin labeling; Epitope mapping; Peptide arrays; SPOT synthesis; Detection; Chemiluminescence; EpCAM

*Abbreviations:* BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; EpCAM, epithelian cell adhesion molecule; FVIII, (blood coagulation) factor VIII; HSA, human serum albumin; HRP, horseradish peroxidase; LC, long chain; NHS, *N*-hydroxysuccinimide; PBS, phosphate buffered saline; PVP, polyvinylpyrolidone; rHSA, recombinant human serum albumin; SC, short chain.

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## 1. Introduction

In 1990 a technique for simultaneous parallel chemical synthesis on membrane support, so called SPOT synthesis, was presented by Frank et al. and offered opportunity to synthesize and subsequently screen large numbers of synthetic peptides as well as other organic compounds arrayed on planar cellulose membranes [1]. After introduction of a semi-automated SPOT synthesizer in 1993 by the company ABIMED (Langenfeld, Germany), this technique combines the advantage of a reliable and easy experimental procedure with inexpensive equipment needs and high flexibility according to the easy adaptability to a wide range of bioassays and screening methods. However, the most frequent application of peptide arrays prepared by SPOT synthesis is identification of linear peptide epitopes using overlapping peptides derived from an appropriate protein sequence [2–7]. Detection of bound molecules can be achieved by conventional procedures known from ELISA or Western blot, including labeling techniques such as chemiluminescence or radioisotopes [8]. All of these techniques are very sensitive, but the high probability of their cross-reaction with presented peptides often causes high background signals on blank membranes. Therefore, identification of positive spots can solely be done by calculation of differences in signal intensity. At best it should be possible to determine positive spots only after incubation with their specific partner, but not with detection reagent only. This would lead to clear identification. The goal of this study was to establish a detection method which would allow to minimize unspecific binding and optimize identification of positive spots.

To circumvent the use of secondary antibodies, we directly labeled compounds of interest with biotin and detected binding by incubation with streptavidin - horesradish peroxidase (streptavidin–HRP) via chemiluminescence. Interestingly, the very simple and often used technique of biotin labeling has not been applied for SPOT analysis so far, at least there is no request in the public domain. For evaluation of this detection method different concentrations of streptavidin–HRP, as well as different providers were checked. Furthermore different salt concentration of buffer solutions, and influence of spacer used for labeling were investigated. In order to investigate a broad range of applications, first experiments were carried out using membranes representing peptides from different conceptual formulations (FVIII peptides, hexapeptides selected from combinatorial library screening, EpCAM peptides, combinatorial libraries consisting of hexa- and decapeptides). Further experiments for evaluation of this method were carried using only one model system exemplarily, namely binding of serum IgG to linear peptides of a transmembrane protein, namely the epithelian cell adhesion molecule (EpCAM). Firstly, influence of ligand and ligate density was investigated. Secondly, application of different blocking reagents, such as bovine serum albumin (BSA), casein and polyvinylpyrolidone (PVP) was evaluated. Thirdly, results achieved with the presented method were compared to results obtained after detection with secondary antibodies. After all, optimization of this detection method conditions led to a very sensitive detection technique with no or low number of unspecific spots which is superior to conventional detection methods with secondary antibodies.

## 2. Materials and methods

### *2.1. SPOT synthesis*

Cellulose bound peptide libraries were semi-automatically prepared according to the method first published by Frank et al. [1] slightly modified as described by Pflegerl et al. [9]. Briefly,