Rational antigen design for cell based immunological assay systems in different immunological subjects

Kevin Strecker[1] Nicole Ettischer-Schmid[1] Rosemarie Preyer[1]
1 Genome Identification Diagnostics GmbH (GenID), Straßberg, Germany

Introduction

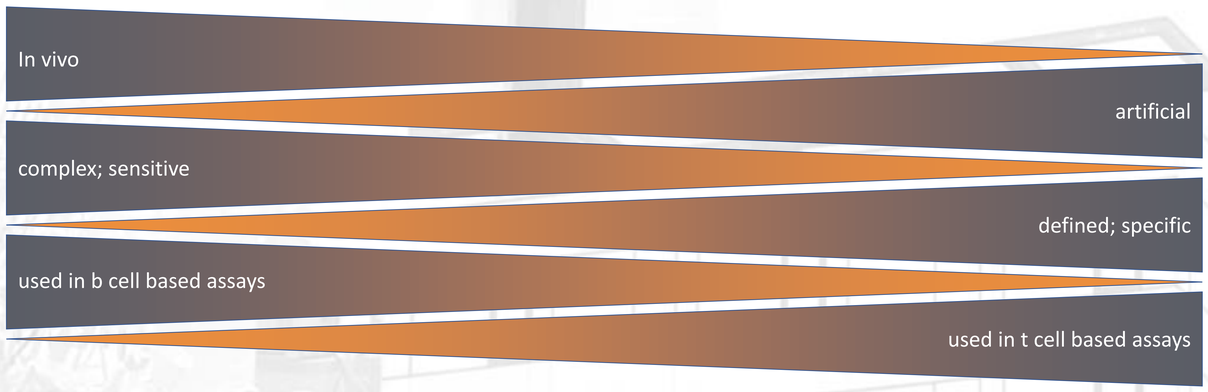
Cell based immunological assay systems are widely used in research as well as routine diagnostics in these days to gain better and more detailed information compared to classical assay formats. Despite the less varying framework of well-established standard assay formats like ELISA, Flow Cytometry or EliSpot Assays, the antigens used for stimulation are undergoing dynamic changes as new applications are needed to manage complex emerging immunological challenges.

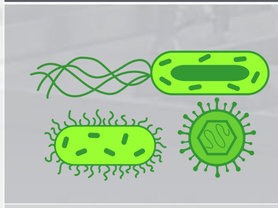
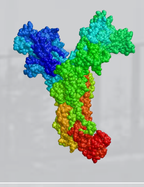
Methods

Consequently, the most important component in such assay systems are the antigens used for stimulation. There are several methods to generate different types of stimulants which all have their own inherent advantages and disadvantages depending on their intended use and immunological problem being addressed. It is mandatory to ensure that the strategy used for the choice of a suitable antigen type is compatible with the used assay format and cell type analysed. Furthermore, the quality and design of those antigens are main factors influencing a clinically sufficient sensitivity and specificity of the final assay system. Depending on the desired clinical outcome of a given assay system, not all types of antigens fulfil the necessary requirements to gain sufficient results.

Conclusion

Here we want to give a comprehensive overview of the most common available methods in antigen design and give advice when to use which kind of antigen type depending on the specific application. We will show the general tasks to accomplish during the process of designing new antigens used as stimuli in cell based immunological assay systems.



whole cell lysate	Viral-like particles / structural elements	recombinant proteins	synthetic peptides
			
• screening assays • linear and conformational epitopes (quaternary structure)	• screening and confirmatory assays • Linear and conformational epitopes (quaternary structure)	• screening and confirmatory assays • Linear and conformational epitopes (tertiary structure)	• confirmatory assays • linear epitopes
• mixture of all components including various antigens • closed to in vivo situation • repetitive epitopes • high BSL for infectious agents • high percentage of cross-reactive epitopes possible • less research needed in advance • varying specificity • high requirements concerning purification • poor lot to lot reproducibility	• non-infectious artificial virions • complete outer surface possible • repetitive epitopes • lower BSL than for infectious agents • cross-reactive epitopes possible • combined advantages and disadvantages of whole cell lysate and recombinant proteins	• whole protein with possible posttranslational modifications • low lot to lot variation • high AG yield • different proteins can be used to gain more information • takeover from expression system possible • cross-reactive epitopes possible • structure may differ due to expression system • tags used for purification may alter structure	• chemically defined short chains of amino acids • very low lot to lot variation • high purity • maximal specificity • monitoring • peptide pools can be designed in order to address special problems in more detail • peptide scans of whole proteins may cause disadvantages of other AG types • limited length of peptide chains • extensive research in advance needed

Tab. 1: Comprehensive overview of the most common available methods in antigen design.

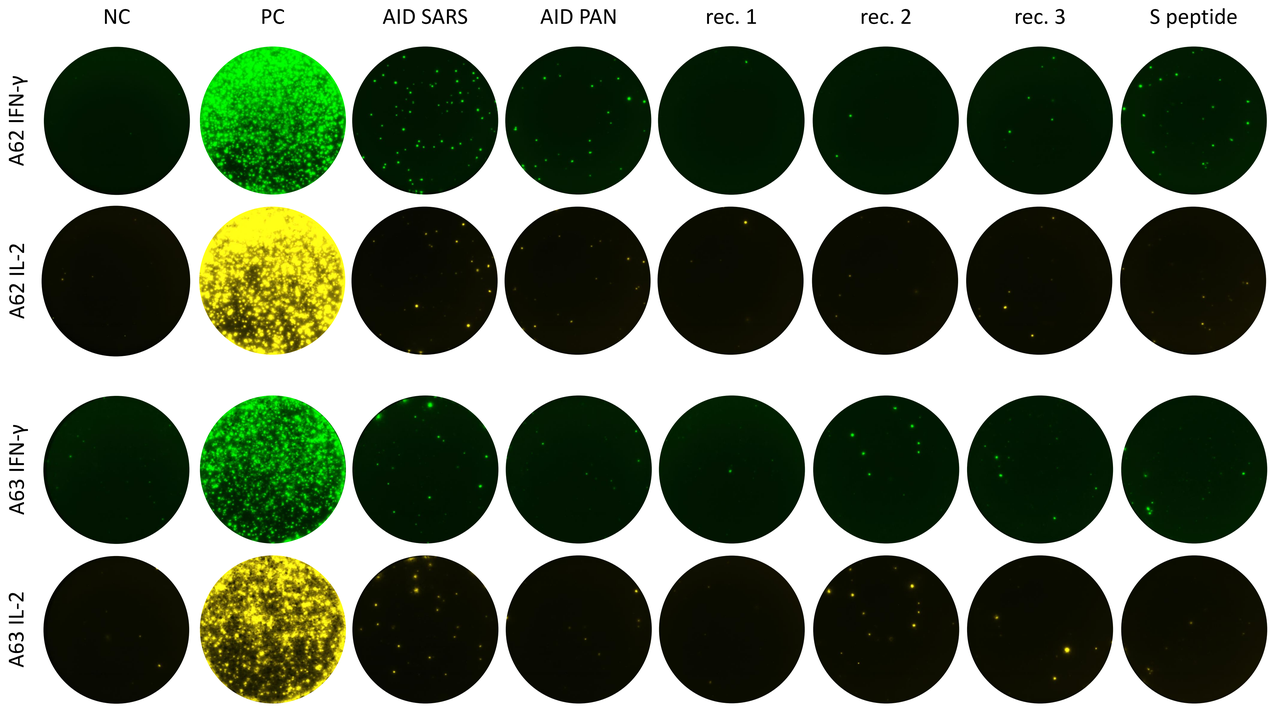


Fig. 1: Exemplary EliSpot results generated by different strategies in synthetic peptide design. Comparison of the AID COVID-19 peptide pools SARS-CoV-2 and PAN Corona with two commercially available recombinant SARS-CoV-2 S-Proteins (rec. 1 and 2), a university produced (rec. 3) and a commercially available peptide pool.
PC: positive control; NC: negative control; rec.: recombinant; IFN-γ: Interferon gamma; IL-2: Interleukin-2

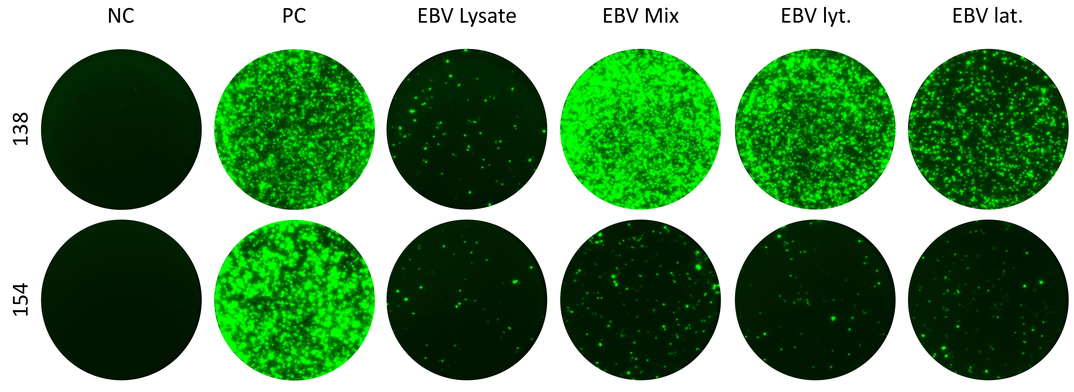


Fig. 2: Exemplary EliSpot results generated by different types of antigens. Sample 138 and 154 were stimulated with the AID EBV viral lysate, the antigen specific peptide pool EBV Mix (pool of lytic and latent peptide mix), EBV lytic-Mix and EBV latent-Mix.
PC: positive control; NC: negative control; rec.: recombinant; IFN-γ: Interferon gamma