An agglutination assay for ACPA-detection in rheumatoid arthritis patients

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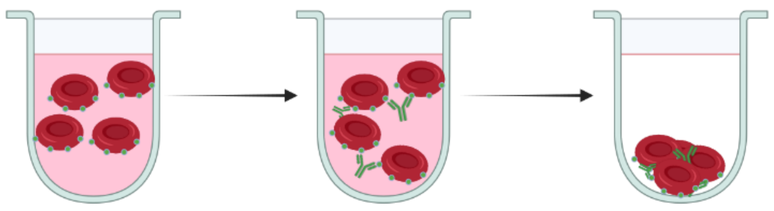
Introduction

Anti-citrullinated protein/peptide antibodies (ACPA) are the most specific serological biomarker of rheumatoid arthritis (RA). The presence of ACPA early in the disease, even before onset of clinical symptoms, facilitates early diagnosis and ACPA is among the most prominent classification criteria for RA. Importantly, early diagnosis and immediate start of treatment is strongly correlated with improved outcomes. Several ACPA-detection assays are available for clinical use, but most are based on the same principle: ELISA with cyclic citrullinated peptides or citrullinated proteins. While this technique can be automated in modern diagnostic laboratories, it is ill-suited for low volume laboratories or resource-poor environments.

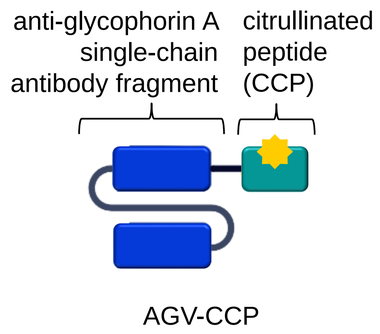
Here, we present the development of a single-step agglutination-based assay for ACPA detection in patient blood samples.

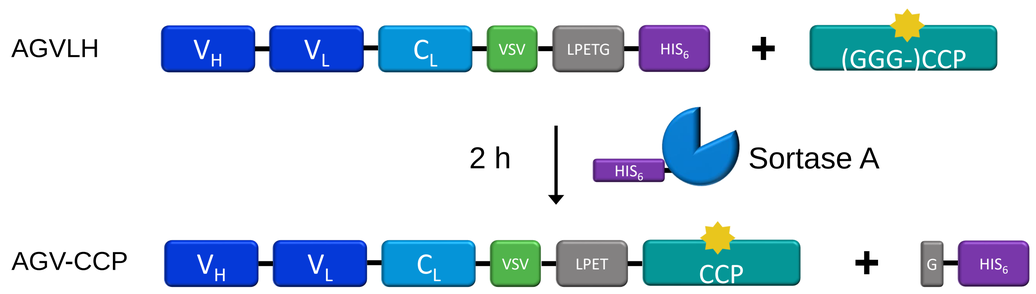


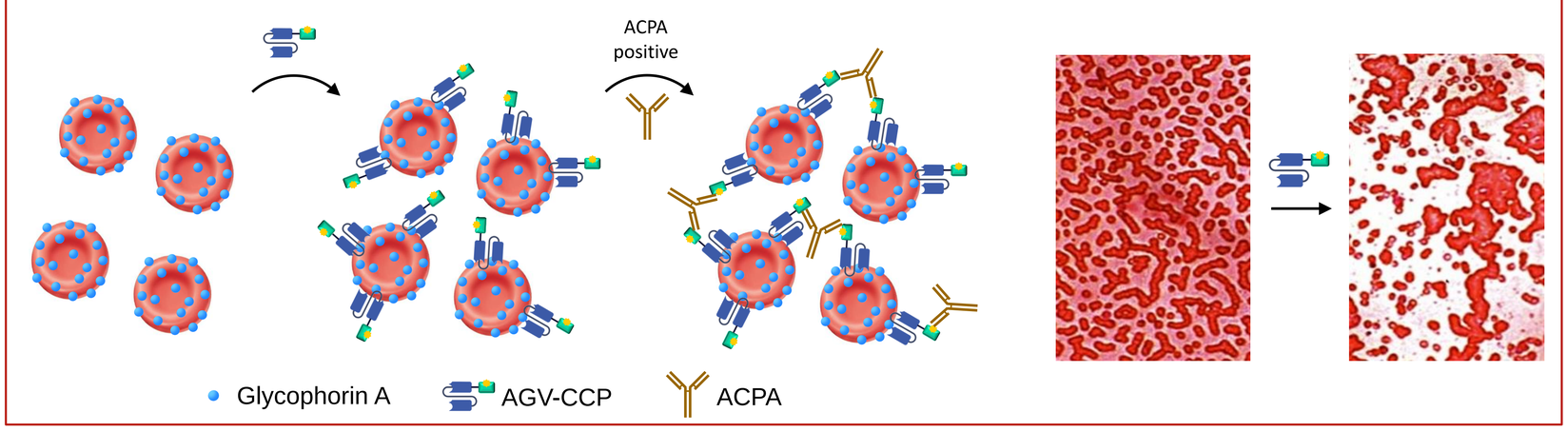
Hemagglutination

To achieve hemagglutination in the presence of ACPA, we have generated a mediating molecule by protein engineering. It is based on a single-chain antibody fragment that binds to the glycophorin A receptor, which is ubiquitous-ly present on the surface of erythrocytes, and is conjugated to a citrullinated peptide recognized by ACPA. As a result, when erythrocytes and ACPA-positive serum or a whole blood sample from an ACPA-positive patient are mixed with the mediator molecule (AGV-CCP), agglutin-ation of the erythrocytes is induced.

Design and generation of agglutination mediator

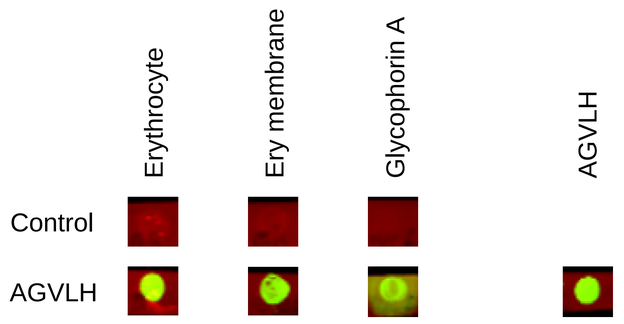




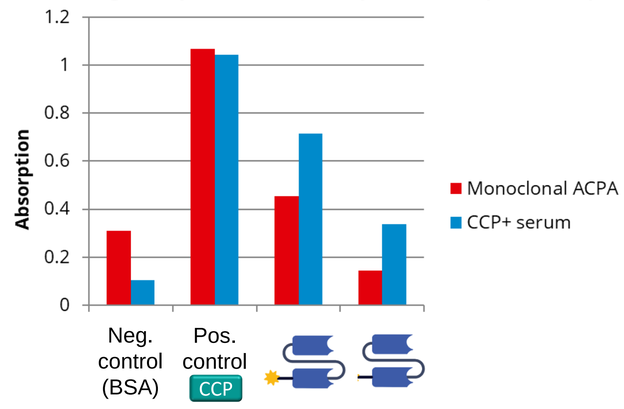


Characterization of agglutination mediator AGV-CCP

Binding to erythrocytes / glycophorin A (dot blot)

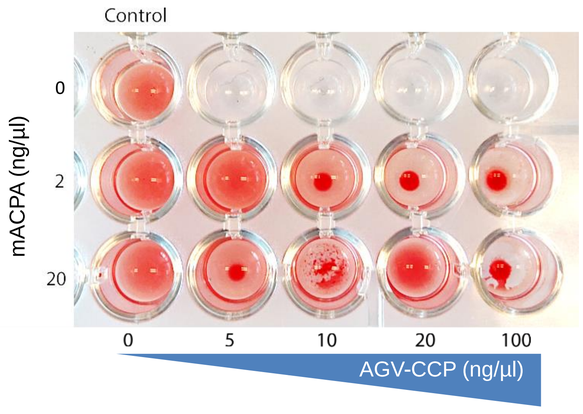


Binding to (monoclonal) ACPA (ELISA)

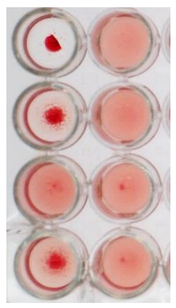


Optimization of agglutination assay

Agglutination with monoclonal ACPA



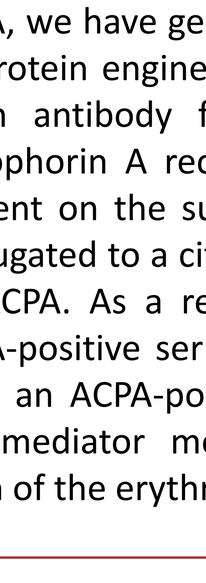
Optimization with patient blood



- Dilution blood sample
- Concentration mediator
- Addition of PEG
- Control without mediator

ACPA detection in patient blood

Score



ELISA	#	Anti-CCP2 positive
RA Cohort 1	103	67%
PsA Cohort 1	77	4%
RA Cohort 2	101	63%

Agglutination	#	*Score 2+3+4*	*Score 3+4*	*Score 4*
RA Cohort 1	103	61%	48%	23%
PsA Cohort 1	77	21%	9%	2.6%
RA Cohort 2	101	60%	51%	43%

Conclusions

The generation of an ACPA-dependent agglutination mediator allows the development of a hemagglutination assay for ACPA detection in blood samples.

- Haemagglutination is simple, fast, has a visual readout and does not require sophisticated equipment.
- The sensitivity of the ACPA agglutination test comes close to that of the golden standard CCP2 ELISA.
- Replacement of the CCP epitope by an-other autoantibody target may facilitate the detection of other autoimmune bio-markers in a similar assay.

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