

Network: Infection and Inflammation: from Pathogen-induced Signatures to Therapeutic Target Genes

Project: Development of New Diagnostic Tools for Rheumatoid Arthritis

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Summary

The aim of the project is to develop tools for the improved diagnosis of RA and associated autoimmune disorders (AI). A reliable, diagnostic test strip for Rheumatoid arthritis will be developed by in.vent Diagnostica GmbH in collaboration with other applicants. Also, a multiparameter test strip for overlapping features of autoimmune disorders (RA-AI-DiagStrip) will be produced. Both strips are of great clinical value, because so far there is no easy to use, non-laboratory based RA-specific assay available which can be used by family doctors or which serves as assay of associated autoimmune diseases. Early diagnosis of RA is crucial, since long-term RA is leading to severe problems of life quality. Hence, only early intervention can reduce irreversible pathological processes. The development of the assays will not only be based on already published/unpublished results of the applicants (e.g. Skriner *et al.*, 1997) but will be based on the newly discovered autoantigens by the applied high-throughput autoantigen selection system. This information on autoantigenic peptides and proteins is indispensable for the design of a RA-DiagStrip. In.vent will place the RA-DiagStrip and the RA-AI-DiagStrip on the market for diagnostic assays. The different product lines will allow an applicable monitoring of the disease and lead to major advantages for the patient. The laboratory-based RA-Diag array will be launched during the time course of a consecutive project.

Development of two unique test strips

Diagnosis of Rheumatoid Arthritis

The Goal of this project is to develop a fast and reliable test strip for the family doctor. The strip will contain some of the already known antigens such as RF, CCP, RA33 and immunogenic proteins and peptides found in NGFN1. Based on that knowledge, recombinant fusion proteins will be generated by cloning techniques. These newly designed molecules will be expressed, purified and then tested in immunoblotting and ELISA with RA patient sera by our partner. These investigations are accompanied by the development of a test strip. The test strip is based on a system in which the antigens are bound to a solid-phase and then used in conjunction with the serum associated with a gold conjugated anti-human antibody. Using capillary force, this complex is able to bind to the antigen forming a sandwich complex, which turns red in a colour reaction. In a qualitative test, this colour reaction could indicate an early RA situation. The strip will be covered in the first phase by the available RA antigens, peptides and evaluated. Three different recombinant fusion proteins and one peptide were generated at the Charité. Purified recombinant proteins were lyophilised by in.vent for further tests on Ra-Diag Strip. In the second phase additional available autoantigens of overlap diseases will be applied. Moreover, a semi-quantitative test system has to be developed allowing the detection of low antibody titers in patients that is usable in the monitoring of treatment.

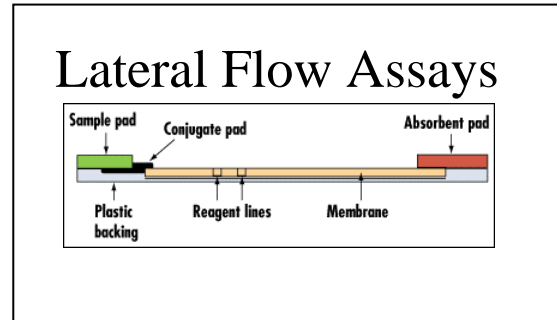


Fig 1: Lateral Flow Assay- Design

RA-DiagStrip and RA-Auto-DiagStrip generation

The advantage of a strip test is its fastness, easiness of handling and of low cost in production. The strip test combines the simple technical usage with the analytical sensitivity of commonly used ELISA tests. Test strips are based on solid phase modified membranes which allow different marker proteins or peptides to be applied in parallel. Membranes are fixed to a plastic matrix to introduce rigidity. The possible number of antigens is limited by the solution of protein in the matrix and the space for interpretation, mainly the ability of the human eye to distinguish two close lines. As a maximum 20 lines are technically reasonable. Antibody-antigen complexes are detected analogous to the ELISA technique, using enzyme-bound anti-human antibodies. Additionally, a system where antigens are bound to a solid-phase which is then used in conjunction with serum associated with a gold conjugated anti-human antibody is used. Using capillary force, this complex is then able to bind to the antigen forming a sandwich and – in the case of positivity – the complex turns red in a colour reaction. The strip will be covered in the first phase by the available RA antigens, peptides and evaluated. Recombinant fusion proteins will be generated in collaboration with the Charité. In the second phase additional available autoantigens of overlap diseases will be applied. The outcome of the reactivity patterns with the strips will be compared with ELISA and Immunoblot data generated at the Charité with respect to sensitivity and specificity. Depending on the sensitivity and specificity of each antigen during the test, it will be decided which antigen is used for diagnosis on a strip or better in ELISA or immunoblot.

Lit.: 1. Skriner K, Sommergruber WH, Tremmel V, et al. Anti-A2/RA33 autoantibodies are directed to the RNA binding region of the A2 protein of the heterogeneous nuclear ribonucleoprotein complex. Differential epitope recognition in rheumatoid arthritis, systemic lupus erythematosus, and mixed connective tissue disease Journal of Clinical Investigation 100 (1997), 127-135.