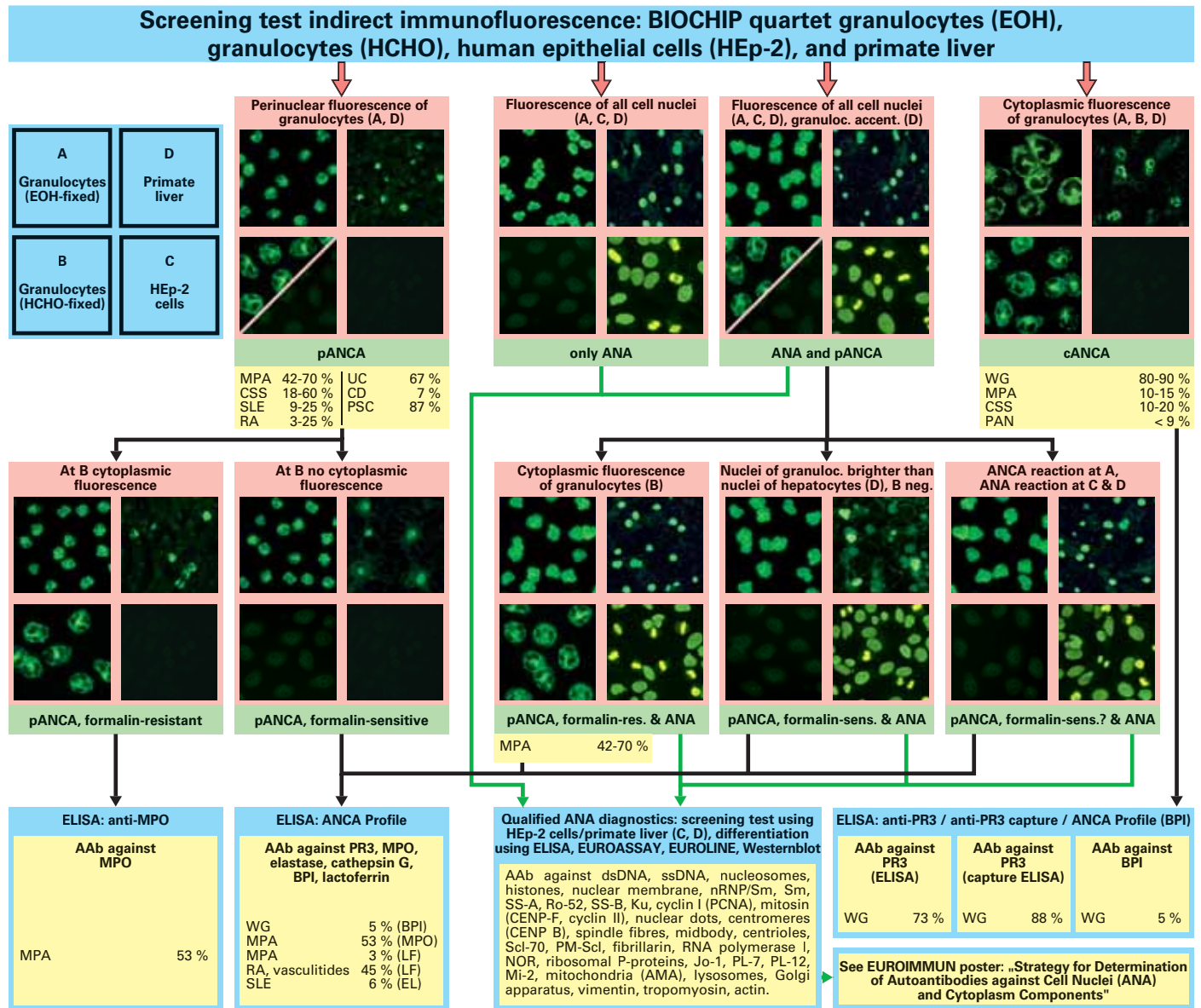




# Cost-effective Strategy for the Detection of Autoantibodies against Granulocyte Cytoplasm (ANCA)



The highest diagnostic sensitivity in the determination of autoantibodies against neutrophil granulocytes (ANCA) is achieved by using indirect immunofluorescence and enzyme immunoassays with defined target antigens (particularly PR3 and MPO) simultaneously at the start. However, under the pressure of cost optimisation, an immunofluorescence test may be performed on its own and then followed up by specific ELISA tests only if the result is positive.

Ethanol-fixed human granulocytes are the standard substrate for indirect immunofluorescence. With this substrate two relevant fluorescence patterns can be differentiated: the cytoplasmic type (cANCA) associated with Wegener's granulomatosis and the perinuclear type (pANCA), which indicates a range of various diseases. The differentiation of pANCA from antibodies against cell nuclei (ANA) is often difficult. Therefore, HEp-2 cells are used as an additional substrate (possibly with sedimented granulocytes). Primate liver is also suitable: the cell nuclei of hepatocytes and granulocytes present in the sinusoids are in the same field of view. If ANA and pANCA occur together, the granulocytes show a much brighter fluorescence than the hepatocyte nuclei.

Thanks to EUROIMMUN BIOCHIPs it is not necessary to incubate human epithelial cells on a second slide in parallel for the exclusion of cell nucleus antibodies, since all substrates are present in one and the same test field. A fourth BIOCHIP with formalin-fixed human granulocytes detects a large proportion of the diagnostically relevant antibodies against myeloperoxidase, whereas other pANCA (which are particularly important in gastroenterology) and almost all antibodies against cell nuclei (whose differentiation is a separate chapter in autoantibody diagnostics) are generally completely suppressed.

With the initial dilution of 1:10 recommended by EUROIMMUN for the indirect immunofluorescence test, some antibodies with a low titre, predominantly against PR3, are not detected. Thus, the prevalence for patients with Wegener's granulomatosis in IFT is between 80 and 90% (cANCA). With the EUROIMMUN Anti-PR3 Capture ELISA the prevalence can be increased to 92%, detecting Wegener's granulomatosis in many individuals in whom the disease had not yet established itself at the time of examination or where it is currently showing a low clinical activity. However, due to the fact that such findings mostly have no therapeutic significance for these patients, the cost-effective strategy presented in the above scheme is acceptable in general laboratory diagnostics. But when clinical symptoms and laboratory results are contradictory, it is always advisable to use all available methods simultaneously.

ANA: anti-nuclear antibodies BPI: bactericidal permeability increasing protein cANCA: anti-neutrophil cytoplasmic antibodies, cytoplasmic type CD: Crohn's disease CSS: Churg-Strauss syndrome EL: elastase EOH: ethanol HCHO: formalin HEp-2: human epithelial cells IFT: immunofluorescence test LF: lactoferrin MPA: microscopic polyangiitis MPO: myeloperoxidase PAN: polyarteritis nodosa pANCA: anti-neutrophil cytoplasmic antibodies, perinuclear type PR3: proteinase 3 PSC: primary sclerosing cholangitis RA: rheumatoid arthritis SLE: systemic lupus erythematosus UC: ulcerative colitis WG: Wegener's granulomatosis