

# EUROPLUS™ ANCA BIOCHIP Mosaic: MPO and PR3 antigen dots improve the detection of ANCA by indirect immunofluorescence

J. Damoiseaux<sup>1</sup>, M. Buschtez<sup>2</sup>, U. Steller<sup>2</sup>, B. Zerbe<sup>2</sup>, A. Rosemann<sup>2</sup>,  
K. Fechner<sup>2</sup>, W. Schlumberger<sup>2</sup>, J.W. Cohen Tervaert<sup>1</sup>, W. Stöcker<sup>2</sup>

<sup>1</sup>Department of Clinical and Experimental Immunology, University Hospital Maastricht, the Netherlands

<sup>2</sup>Institute of Experimental Immunology, affiliated to EUROIMMUN AG, Luebeck, Germany

<sup>3</sup>Clinic of Rheumatology Bad Bramstedt, Germany

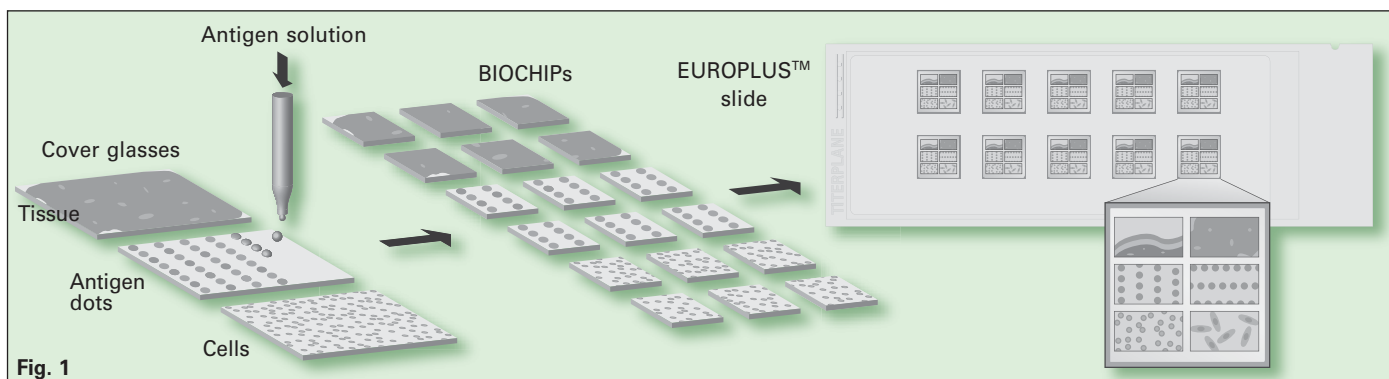


Fig. 1

## Introduction

The indirect immunofluorescence test (IIFT) is considered the gold standard for the detection of antibodies against neutrophil granulocytes (ANCA). The EUROPLUS™ system enables to combine cells or tissues with substrates of single antigen microdrops, e.g. granulocytes with MPO and PR3 dots in one incubation field. In the present study we evaluated the diagnostic applicability of this new system to ANCA-associated vasculitis (AAV).

## Methods

An AAV cohort consisting of 248 cases (114 biopsy-proven AAV patients, 74 AAV patients in our outpatient clinic, and 60 Wegener's granulomatosis patients) as well as 112 control samples (other forms of vasculitis n=55, rheumatoid arthritis n=30, healthy controls n=27) were analysed by IIFT using the EUROPLUS™ BIOCHIP Mosaic 23 (see Fig. 2). Samples were only classified as pANCA positive if a positive result was obtained for both ethanol and formalin fixed granulocytes. The reference tests were Anti-PR3-hn-hr and Anti-MPO ELISA (EUROIMMUN AG).

## Results

In a mixed AAV cohort, the observed cANCA and pANCA patterns were supported by a positive reaction of the PR3 or MPO dots, respectively, in 128 (97%) and 63 (93%) of the cases. Of 116 cANCA negative and 180 pANCA negative cases, 115 (99%) and 178 (99%) were confirmed by the dots not to contain anti-PR3 or anti-MPO autoantibodies. The negative dot results of the 4 cANCA positive cases were confirmed by ELISA. One case was PR3 dot positive and cANCA negative: the positive dot result

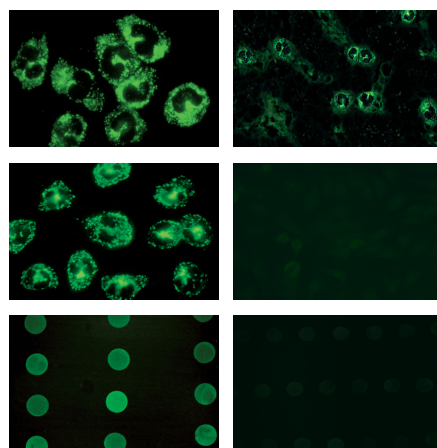


Fig. 2a: EUROPLUS™ Granulocyte Mosaic (granulocytes EOH, primate liver, granulocytes HCHO, HEp-2, PR3, MPO): cANCA, PR3-positive.

AAV cohort, n = 248	IIFT, cANCA	
	positive	negative
EUROPLUS PR3 dot	128	1
	negative	4
		115

AAV cohort, n = 248	IIFT, pANCA	
	positive	negative
EUROPLUS MPO dot	63	2
	negative	5
		178

was in agreement with ELISA. Four of the 5 MPO dot negative cases that were pANCA positive were also negative with the Anti-MPO ELISA. In one of the 2 pANCA negative but MPO dot positive cases the presence of anti-MPO antibodies was confirmed by ELISA. For all 109 control samples that showed neither a cANCA nor a pANCA pattern the absence of PR3-ANCA and MPO-ANCA was confirmed by the antigen dots. The antigen dots also confirmed the absence of anti-PR3 and anti-MPO antibodies in agreement with the ELISA results in all difficult cases of both cohorts that showed an atypical ANCA pattern (about 12%).

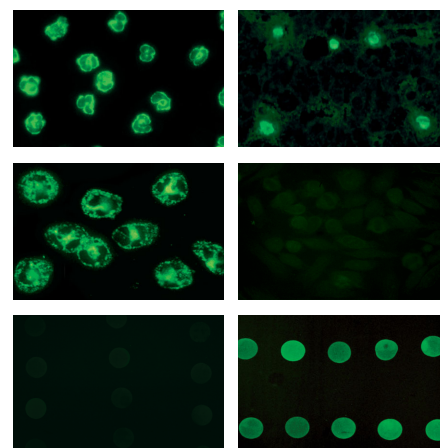


Fig. 2b: EUROPLUS™ Granulocyte Mosaic (granulocytes EOH, primate liver, granulocytes HCHO, HEp-2, PR3, MPO): pANCA, MPO-positive.

## Discussion

EUROPLUS™ BIOCHIPS significantly facilitate the interpretation of IIFT results. In the vast majority of cases the easily interpretable results of the PR3 and MPO antigen dots support the ANCA pattern observed on the conventional cell substrates. In the discrepant cases, the dots provide additional diagnostic information: Negative results for the antigen dots help to identify cases where cANCA and pANCA are directed against antigens other than PR3 and MPO. Alternatively, the dots help to identify anti-PR3 and anti-MPO antibody positive samples that would have been missed by interpretation of the ANCA pattern only, e.g. because both, anti-PR3 and anti-MPO antibodies are present in the sample. The final diagnosis of specific autoantibodies can be obtained after just one incubation since the EUROPLUS™ dots already lead to antigen specific results that correlate very well with other monospecific tests. However, for quantitative results, a corresponding ELISA has to be performed.

Scientific presentation at the 8<sup>th</sup> Dresden Symposium on Autoantibodies (Dresden, Germany, September 2007)