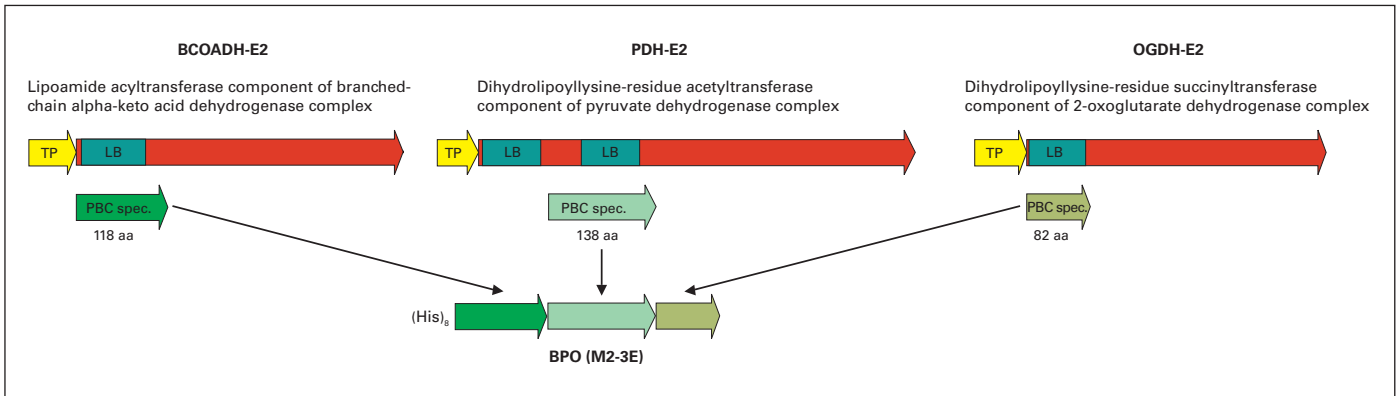


# Detection of primary biliary liver cirrhosis-associated anti-mitochondrial antibodies using an improved test system: Anti-M2-3E ELISA

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**Schematic illustration of BPO (synonym: M2-3E):** The recombinant polypeptide His-BPO consists of the immunogenic lipoyl binding domains of the E2 subunit of branched-chain 2-oxoacid dehydrogenase (BCOADH), the E2 subunit of pyruvate dehydrogenase (PDH), the E2 subunit of 2-oxoglutarate dehydrogenase (OGDH) and a N-terminal His-Tag. TP: transit peptide, LB: lipoyl binding, PBC spec.: primary biliary liver cirrhosis specific (autoantibody binding), aa: amino acids, 3E: 3 enzymes.

## Introduction

Determination of anti-mitochondrial antibodies (AMA) is of particular significance for the diagnosis of primary biliary liver cirrhosis (PBC), an immune-mediated chronic inflammatory cholestatic liver disease of unknown aetiology. The most specific and sensitive diagnostic markers are antibodies against the M2 antigen. The molecular targets of these autoantibodies have been identified as members of the 2-oxoacid dehydrogenase complex family of enzymes within the mitochondrial respiratory chain. Among these enzyme components, the lipoyl binding domains (E2) are the major autoantigens in PBC.

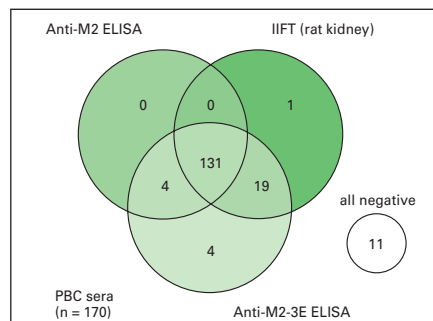
## Methods

This study reports the development of a new ELISA using an antigenic preparation based on a mixture of native M2 with a recombinant fusion protein consisting of the lipoyl domains of BCOADC-E2, PDC-E2 and OGDC-E2 (BPO, synonym: M2-3E). The new assay called Anti-M2-3E ELISA was compared with a commercially available conventional ELISA using native PDC alone, or an ELISA based on BPO alone or an indirect immunofluorescence test (IIFT) using a BIOCHIP Mosaic™ of rat kidney/stomach/liver tissue and HEP-2010 cells as substrate. Tests were performed on serum samples obtained from patients with PBC (n=170),

autoimmune hepatitis (AIH, n=46), PBC/AIH type 1 overlap syndrome (n=3), chronic viral hepatitis (n=200), and from 400 healthy blood donors (HBD).

## Results

At a comparable specificity of approximately 100%, use of the recombinant fusion protein BPO increased the sensitivity of the ELISA to 90.2%. By applying a mixture of native M2 and the recombinant antigen as target structures, the sensitivity of the ELISA system could be further increased to 93.1%. 99% of the samples that tested



Test system	Sensitivity	Specificity
Anti-M2-3E ELISA	93.1%	99.6%
Anti-BPO ELISA	90.2%	98.8%
Anti-M2 ELISA	79.8%	100.0%
IIFT AMA kidney (rat)	89.0%	98.8%

## Discussion

positive in IIFT were also identified by the novel Anti-M2-3E ELISA, whereas 5% of all AMA positive samples were exclusively detected by ELISA.

The special configuration of the BPO (M2-3E) antigen ensures simultaneous presentation of all three major antigens of the mitochondrial respiratory chain. Combined use of the recombinant polypeptide BPO with native M2 as substrates in one ELISA resulted in an increase in sensitivity of 14% compared to the classic anti-M2 ELISA, while preserving a specificity of 99.6%. The new Anti-M2-3E ELISA may be used for the detection of AMA as an alternative to IIF. Moreover, it offers the potential to identify AMA in patients with suspected PBC but negative results in IIF.

