1000-fold Enhanced Detection Limit of ELISA-Assays Using Immuno-PCR as a Routine Diagnostic Method

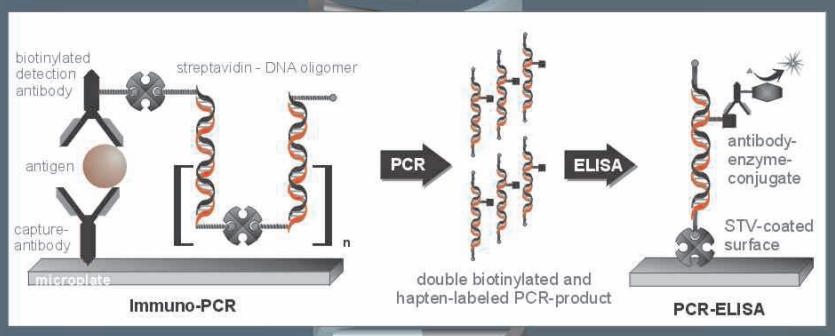
Michael Adler[1], Christof M. Niemeyer[1], Helle Knudsen[2]

1 **chimera biotec GmbH:** c/o Universität Bremen UFT, R.1600 Leobener Str., D-28359 Bremen
Phone: +49 421-218-7228/4911, e-mail: chimera@chimera-biotec.de

2 **Nunc A/S:** Kamstrupvej 90. P.O. Box 280, DK-4000 Roskilde, Denmark.
Phone: +45 46312000, Fax: +45 46312175, e-mail: hek@nunc.dk

Introduction: The Immuno-PCR Method

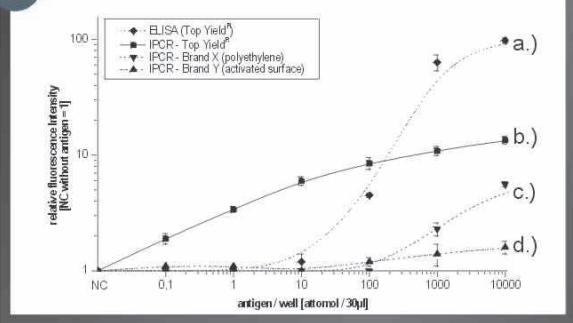
Immuno-PCR (IPCR) (Lit.1), a combination of the signal amplifying power of the Polymerase Chain Reaction (PCR) with the principle of Enzyme Linked Immuno Sorbent Assay (ELISA), allows to increase common ELISA sensitivity up to about 1000-fold (Lit. 2).



The use of reporter-DNA-labeled antibodies combines the signal amplifying power of the PCR with conventional ELISA-methods for antigen-detection. Pre-formed, self-assembled conjugates of bis-biotinylated DNA and the biotin-binding protein streptavidin (STV) were utilized for the coupling of the specific antibody and marker-DNA (Lit. 3).

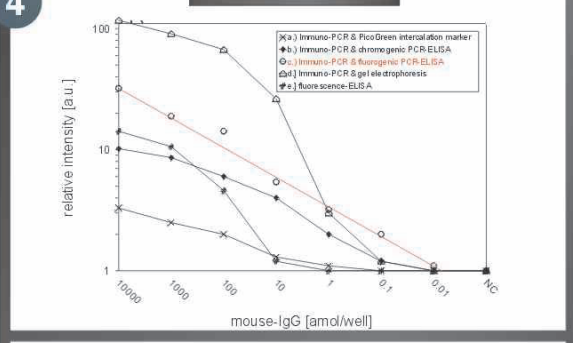
Materials and Methods

1 Reaction vessels



Influence of different MicroWell surfaces on immuno-PCR performance. TopYield strips (NUNC) are compared with two other temperature resistant reaction vessels. A sandwich immuno-PCR for the detection of mouse IgG was carried out, using TopYield strips (b) and polyethylene modules (c), coated with anti-mouse-IgG and a chemically activated surface with covalently bound anti-mouse-IgG (d). With TopYield (NUNC), a sensitivity of 0,1 amol antigen was found in the immuno-PCR, a 1000-fold enhancement of sensitivity compared to conventional ELISA (a) (Lit. 7).

4 Read-out



Different detection methods for immuno-PCR amplification products of mouse-IgG detection. Curve (a): direct stainig with PicoGreen, a ds-DNA selective intercalating dye, curve (b): PCR-ELISA using chromogenic pNPP as the substrate for alkaline phosphatase, curve (c): PCR-ELISA using fluorescent AttoPhos substrate and FluoroNunc black maxisorp microtiter modules (NUNC), curve (d): ethidium bromide-stained agarose gel electrophoresis. Compared with conventional ELISA of mouse-IgG (e) allowed the combination of immuno-PCR and method (c) an 100-fold increase in sensitivity and a linear signal to antigen dependence over more than 6 orders of magnitude (Lit. 6).

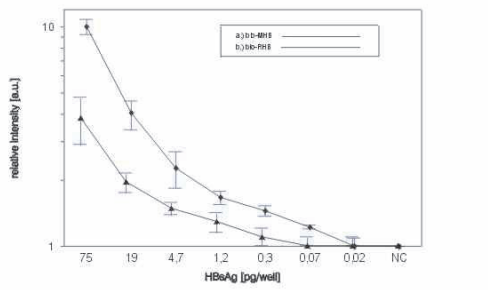
The following steps are essential to carry out the highly sensitive IPCR as a routine method:

1. **Reaction vessel** The microplate has to offer a material wich combines high affinity to immobilize antibodies, low background after blocking and compatibility with PCR-thermocylers.
2. **High quality antibodies:** Immunoglobulins with high affinity and specificity
3. **Efficient coupling:** antibodies have to be coupled with marker-DNA under mild and efficient coupling conditions
4. **High sensitivity read-out** of the IPCR-amplification products.

Immuno-PCR allows the reproduceable ultrasensitive microplate analysis of proteins even in serum samples, providing a suitable assay for the detection of highly potent medical drugs applied in amounts not accessible with conventional ELISA.
The method has been proven to be convenient for routine analysis, such as studies of pharmacokinetics.

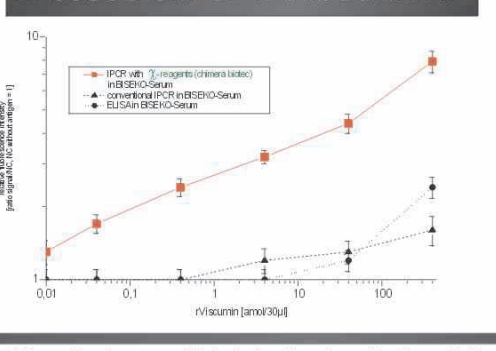
Applications and Results

2 Detection of Hepatitis B



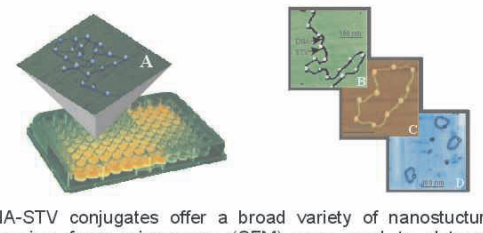
Routine assays for the detection of Hepatits B (HBV) include Radioimmunassays (RIA) or ELISA of the HBV surface antigen (HBsAg). For patients with low viral transcription, the detection limit of conventional immunoassays of approx. 50 pg could be too high. Immuno-PCR allows an approx. 700fold increase of sensitivity and the detection of 70 fg (approx. 2 attomol) rec. HBsAg. Best results were obtained using the polyclonal rabbit-anti-HBsAg antibody (RHB) as capture antibody and biotinylarted RHB (bio-RHB) as detection antibody (curve b). The monoclonal biotinylated mouse-anti-HBsAg antibody (bio-MHB) allowed only the detection of 1,2 pg and lower signal-to-background ratios (curve a).

3 Detection of rViscumin



The IPCR method was validated for the investigation of the pharmacokinetics of the immuno-modulating and antitumor drug substance rViscumin (Lit. 4), active in concentrations not detectable by conventional ELISA techniques. The use of the novel χ IPCR-reagents (chimera biotec) enhances IPCR-sensitivity 100-fold and allows one to analyze less than 0.1 attomol of the protein in blood serum samples with an routine microplate assay. IPCR could also detect rViscumin in samples of dog-sera after the application of 1 ng/kg body weight. Comparison of ELISA and Immuno-PCR showed an about 1000-fold increase of sensitivity. 200 fg/ml rViscumin equals 0.1 attomol (approx. 60000 molecules) in 30µl sample volume (BISEKO).

Reagents



DNA-STV conjugates offer a broad variety of nanostuctures. Scanning force microscopy (SFM) was used to determine structural properties of the conjugates and develop optimized reagents (χ- Reagents, chimera biotec) for IPCR (Lit 3, 5).

Literature

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