



# Detection of femtogram amounts of biogenic amines using self-assembled DNA-protein nanostructures

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Chimera Biotec, founded in 2000 as a spin-off from academic research in Germany, has implemented its proprietary DNA-antibody conjugates within the highly innovative Imperacer product line, enabling the ultra-sensitive detection of proteins and other antigens. The ready-to-use, commercially available Imperacer kits offer a performance-enhancing tool to improve the limit of detection of conventional ELISA protocols by more than 1,000-fold. Hence, the detection and quantification of trace amounts of basically any antigen, ranging from large proteins to small molecules such as hormones or biogenic amines, can be accomplished with an Imperacer-boosted immunoassay.

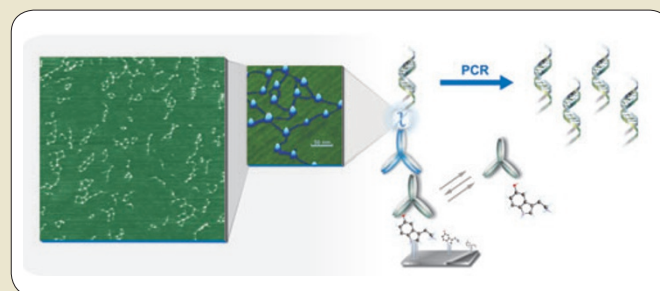
## Imperacer—detection of proteins by PCR

Our comprehension of the molecular basis of fundamental biological processes demands analytical techniques that allow for detailed studies of biomolecular interactions, preferably at the level of single cells or even single molecules. Analytical methods combining high sensitivity with fast, robust and cost-efficient protocols are therefore required to enable basic research and routine diagnostic applications. PCR is a prime example of such an analytical technique. Due to its almost exponential amplification efficiency, it allows the detection and quantification of small numbers of nucleic acid molecules with standard laboratory equipment. To extend the scope of PCR to the high-sensitivity detection of proteins, in 1992 Sano *et al.* established the immuno-PCR (IPCR) method<sup>1</sup>, which takes advantage of specific antibody-DNA conjugates. Since then, IPCR has evolved to become a well-respected research methodology, establishing its general applicability for the sensitive detection of numerous protein antigens. As a rule of thumb, IPCR usually brings a 100–10,000-fold improvement in the limit of detection (LOD) of the analogous conventional enzyme-linked immunosorbent assay (ELISA)<sup>2,3</sup>.

Based on the outcome of recent studies of self-assembled protein-DNA nanostructures, which have proven to be highly efficient reagents in IPCR applications<sup>4</sup>, Chimera Biotec has enabled IPCR to become a routine laboratory method by developing the Imperacer technology. The commercially available and ready-to-use Imperacer

kit opens up IPCR-based ultrasensitive detection of antigens to a broad range of users (Fig. 1).

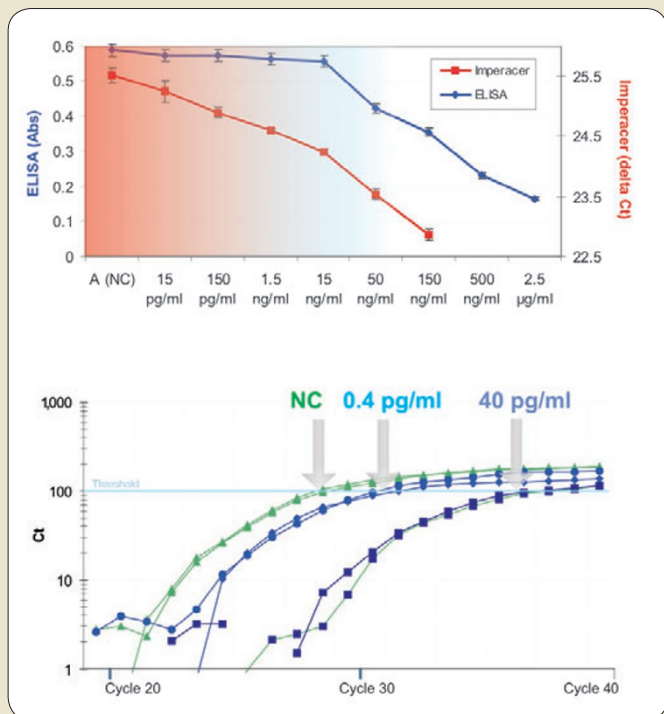
However, the biological world is not limited to nucleic acids and proteins. A large number of meaningful compounds—such as many hormones, messengers and transmitters—have a low molecular weight and are too small to be suitable for quantification via the typical ELISA setup, which uses two specific antibodies for capture and detection purposes. Ever since the development of the first radioimmunoassay in 1959, numerous competitive assays have been developed with the goal of sensitive detection of such analytes, and such an assay could potentially be indispensable for applications in basic research, clinical diagnostics and health care.



**Figure 1** | Chimera Biotec's Imperacer (a trademark of Chimera Biotec GmbH; registered trademark pending) technology uses conjugates of protein and DNA within ready-to-use reagent kits for the conduct of microplate immunoassays. Species-specific antibody-DNA conjugates are used as secondary reagents in an ELISA-based assay for serotonin: the binding of serotonin-specific antibodies (green) to the serotonin-tagged plate surface is inhibited by the availability of free analyte. The amount of antibody immobilized is measured by quantitative real-time PCR using a secondary antibody-DNA conjugate (blue). PCR amplification of the DNA tag generates a signal that is inversely proportional to the amount of free serotonin present in the sample.

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## APPLICATION NOTES



**Figure 2 | Imperacer detection of serotonin.** (a) 1,000-fold boost of the sensitivity of conventional ELISA technology by means of the Imperacer kit. Shown are representative results for the detection of serotonin using the conventional serotonin competitive ELISA (blue curve) and the improved Imperacer signal readout (red curve). Note the increase in sensitivity, the small error bars and the large dynamic range of the Imperacer detection. (b) Fast and precise signal detection in real-time with Imperacer. Using the serotonin ELISA as a platform for Imperacer signal amplification, 0.4 pg/ml serotonin can be reliably detected. For each concentration, a double determination is shown here. Note the high reproducibility of the signal curves, which underlines the robust sensitivity and precision of the Imperacer technology.

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### Imperacer—detection of biogenic amines

In response to the tremendous importance of small-molecule analytics in clinical research, Chimera Biotec took initial steps toward the development of a competitive IPCR assay for the detection of low-molecular-weight analytes. This was achieved by linking appropriate hapten groups to nanostructured DNA-protein conjugates<sup>5</sup>. Although this initial approach proved feasible, it required elaborate and sophisticated labeling for each individual antigen. In continuation of this work, Chimera Biotec has now adapted the Imperacer technology to the competitive immunoassay format, opening up the world of small molecules to high-sensitivity PCR-based detection.

Based on the commercially available ELISA kits of Labor Diagnostika Nord (LDN), a provider of a broad range of isotopic and non-isotopic immunoassays for the detection of biogenic amines and their metabolites<sup>6</sup>, Chimera has launched the first series of Imperacer kit systems for the high-sensitivity detection of biogenic amines, enabling a broad range of diagnostic tests. Among other applications, the detection of increased amounts of the neurotransmitter serotonin could provide a useful diagnostic tool for a number of associated diseases, such as malignant carcinomas, endogenous depression or schizophrenia.

The general principle of detecting biogenic amines with Imperacer technology is schematically depicted in **Figure 1**. As a first step, the experimental protocol of the Imperacer assay involves the acylation of the biological sample to enhance the binding of the primary detection antibody, which is specific for the biogenic amine<sup>7</sup>. The antibody is then added to the sample and the mixture is incubated in thermocycler-compatible microtiter plates containing surface-immobilized hapten groups. Following incubation and short washing steps to remove unbound materials, the specific Imperacer antibody-DNA conjugate is used as a secondary label. Subsequent to a final washing step, a PCR reagent mixture is added, containing specific TaqMan<sup>®</sup> probes that enable real-time PCR detection of the amplified DNA marker.

### 10,000-fold improved sensitivity

With the Imperacer's digital data output it is now possible to automate all DNA quantification steps during PCR, thereby minimizing the method's error<sup>3</sup>. The conventional serotonin competitive ELISA, which has a detection limit of 15 ng/ml, was improved to an LOD of 0.4 pg/ml when quantification was performed using the serotonin Imperacer kit (**Fig. 2**). This corresponds to an enhancement in sensitivity of more than five orders of magnitude. Further optimization of the serotonin assay even made it possible to achieve an LOD of 3 fg of serotonin, a content of only ten platelets. The blood circulating platelets, which are derived from megakaryocytes in the marrow, are the largest single source of vasoactive amines in the body.

The impressive gain in sensitivity of the Imperacer assay is coupled to a very small error in double determinations, with an average standard deviation below 3% and a broad dynamic range, spanning at least five orders of magnitude. In addition to the increased sensitivity and precision of the Imperacer assay, it should be emphasized that the completion of the entire assay requires only 3 hours, with just 30 minutes of hands-on time, and can be performed in the standardized, automation-compatible ELISA-microplate format.

Comparable results were also achieved with Imperacer kits designed for the detection of noradrenaline and dopamine. The sensitivity of ELISA assays for dopamine and noradrenaline were enhanced 100-fold, simply by boosting the ELISA's performance with the appropriate Imperacer kit. Specifically, respective detection limits with ELISA and Imperacer were found at 150 pg/ml and 1.5 pg/ml for dopamine, and 4 ng/ml and 0.04 ng/ml for noradrenaline. These results, obtained without optimization of the ELISA steps of the protocol, underline the power of Imperacer kits for routine applications.

### Imperacer technology opens up new fields of research

Due to the enormous sensitivity of PCR-based analyses, the minimization of nonspecific reagent binding is essential to avoid background signal in the assay. Chimera's proven Imperacer technology, combined with LDN's innovative serotonin-EIA protocol and supplemented with the superior performance of real-time PCR detection, solves this general problem. The combined efforts of Chimera Biotec and LDN facilitate the use of these well-proven platform technologies as a routine analytical tool for the highly sensitive detection of small molecules.

The scope of applications of the ELISA-analog Imperacer immunoassay is broad. From an economical point of view, for instance, there is an increasing demand for noninvasive methods of biological sample acquisition that can be carried out by nonacademic medical

personnel. Therefore, the analysis of saliva or urine is considered a promising alternative to the analysis of blood serum samples. As the former matrices contain only low concentrations of many analytes, Imperacer technology opens up new diagnostic approaches with consistent and even increased sensitivity. This is of particular value for the analysis of neurotransmitters and hormones, targets of increasing interest in numerous applications, ranging from neuroscience to the field of sports medicine and the development of ultrasensitive doping tests.

Furthermore, the dramatic increase in sensitivity provided by Imperacer will allow for earlier detection of tumor markers, such as serotonin, and therefore a better chance for successful therapy of cancer. As the analysis of a single tumor marker is usually insufficient for the diagnostics and personalized treatment of diseases, the option of performing panels of highly sensitive immunoassays on a single microplate will permit access to a new area of biomolecular diagnostics, facilitating the needs of our growing comprehension of the molecular basis of biological processes.

### Imperacer—a universal approach to ultrasensitive immuno-analytcs

In addition to the detection of small molecules, a wide range of standardized, ready-to-use Imperacer kits are available for high-sensitivity detection of proteins. For instance, universal Imperacer toolkits for the detection of IgG from various species, such as mouse, goat or human, allow for the adaptation of nearly any given immunoassay. Application of these species-specific Imperacer kits provides a strong increase in sensitivity along with a reduction of assay time, independent from the origin of the primary antibody specific for a chosen target.

Because of its flexibility and modularity, Imperacer technology can easily be adapted to other challenges of routine analytical practice. In addition to the standard species-specific Imperacer kits, available now as the core component for the adaptation of Imperacer to a number of conventional ELISA protocols, Chimera Biotec offers the synthesis of custom-tailored reagents, that is the synthesis of specific DNA conjugates from any binding protein of the customer's interest. Moreover, to provide the high-sensitivity Imperacer technology for customers without access to real-time PCR cycling equipment, Imperacer kits can be conveniently combined with Chimera's microplate-based PCR-ELISA detection system.

This application note illustrates how biomolecular nanostructures can add a new level of performance to well-established methodologies of biomedical diagnostics. Chimera's Imperacer technology will open up a broad range of new applications and make ultrasensitive analyses accessible to every laboratory.

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