

IKDT Laboratory

IKDT as Service Lab (CRO) for Molecular Diagnostics

IKDT lab offer is complete diagnostic service to all external customers. We could perform as well single procedures or complex examinations of few samples as expanded studies. Sample processing follows internal quality management system as diagnostic specimen or we perform examinations as CRO corresponding to client specific requirements. Thereby applied diagnostic methods could be adapted quickly for requested diagnostics. All examinations will be performed and recorded in accordance with common GLP/GCP guidelines. Applied methods for comprehensive diagnostics of endomyocardial biopsies could be transferred and adapted easily for other diagnostic clinical topics or requirements of biomedical research.

The laboratory equipment is on highest international level. Only some diagnostic laboratories are able to offer histological, immunological and molecular biological examinations and to perform comprehensive diagnostics of rare patient samples following GLP/GCP guidelines. The staff is well-educated and consists of physicians, bioscientists and medical technical assistants.

A. Histological examinations

All histological examinations are performed by a surgical pathologist. Standard staining of all paraffin sections are HE, PAS and Elastica v. Giesson (EvG). In clinically suspected cases will be added special staining for amyloid (Congo red), acid mucosubstances (Alcian Blue), calcium (v. Kossa) and on iron (Prussian Blue stain). During microscopic examination results are fixed as paperwork and subsequently transferred in the computer database. The microscopic analysis is exemplary documented by digitally printed microphotographs. Morphologic characteristics of stained endomyocardial tissue (size, quality, fibrosis, fatty tissue, capillaries and so on) are rated by numeric scaling and the corresponding values are fixed on written examination protocols and in the electronic IKDT database. These data improve histological reports and allow further interpretation of morphological parameters in context with clinical outcome in retrospective analysis or in clinical studies.

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Service offers Histology:

- Fixation (e.g. formalin, RNAlater) and storage of tissue samples
- Embedding of tissue in paraffin blocks
- Preparation of serial paraffin sections by rotation microtom
- Staining of tissues on request (manual, automatic)
- Fluorescence and light microscopy
- Digital measurement and evaluation of morphological structures
- Histological assessment of stained sections
- Digital photographs of representative aspects
- Transfer of data, also via internet
- Archiving of reports, electronic data, paraffin blocks, stained slides according to GLP/GCP

B. Immunohistochemistry

Immunohistochemical examinations will be applied for evaluation of inflammatory processes by detection of infiltrating leukocytic cells in tissue on cryo sections with specific antibodies. Multiple diagnostic sets (viral proteins, inflammation, remodelling of extracellular matrix, cytokines, soluble adhesion molecules) are available for various diagnostical problems.

Service offers immunohistochemistry:

- Fixation (e.g. TissueTek, RNAlater) and storage of tissue samples
- Embedding of tissue in cryo blocks
- Preparation of serial cryo sections by cryomicrotom
- Antibody stainings will be tested and performed on request (tumors, virus)
- Fluorescence and light microscopy
- Digital measurement and evaluation of stained immunospots and tissue sections
- assessment of stained sections including numeric values
- Digital photographs of representative aspects
- Transfer of data, also via internet
- Archiving of reports, electronic data, cryo blocks, stained slides according to GLP/GCP

C. Molecular biology

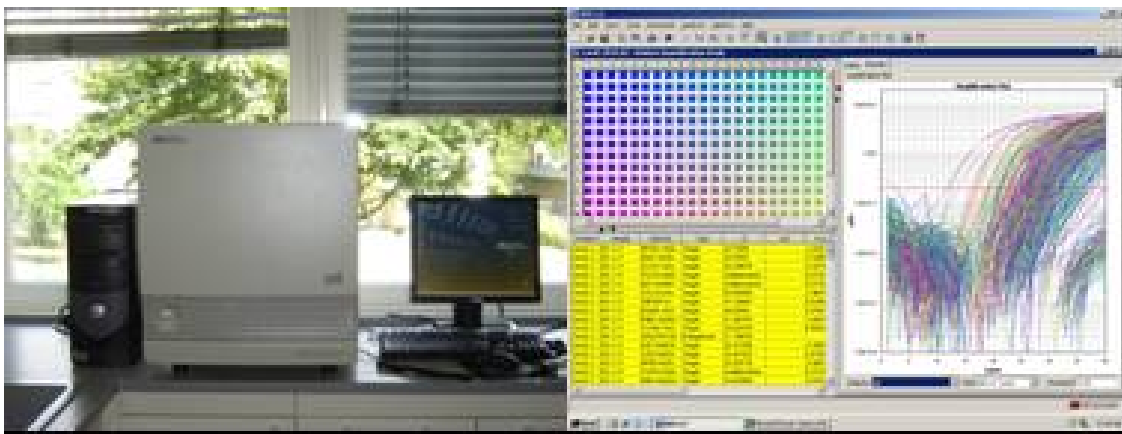
Detection of most frequent cardiotropic viruses (Adenovirus, Coxsackievirus, Epstein-Barr-Virus, humanes Herpesvirus 6, Parvovirus B19) is performed routinely by nested and quantitative PCR including sequencing for estimation of virus subtyp or individual virus variants.

Virus infection or inflammation processes are inducing differential gene expression in effected tissues. IKDT lab is involved in a genome wide screening project (30.000 genes) by microarray technology. The differentially expressed genes will be confirmed by single gene tests and then validated as diagnostic biomarkers.

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A diagnostic set of confirmed candidate genes will be included in a *TaqMan Low Density Array (TLDA, Fa. Applied Biosystems)*. TLDA's provide a combination of efficiency and high throughput suitable for complex gene expression studies. These TaqMan QPCR low-density-arrays allow the simultaneous quantification of 12 to 384 human genes in one patient sample. cDNA synthesis in IKDT is adapted to perform such screening tests if required.

Genetic variations in candidate genes could determine their pre-disposition of individual patients to various diseases (tumor, autoimmunity). The determination of known point mutations (SNP) in human genes is possible by direct sequencing of genome regions or specific SNP arrays. IKDT has set up both technologies in the lab and is now transferring SNP test for apoptotic genes. Genomic DNA from peripheral blood is applicable for this tests.



ABI Prism 7900 HT and amplification plot of 384 gene measured by TaqMan Low-Density-Array

Detection of infectious agents

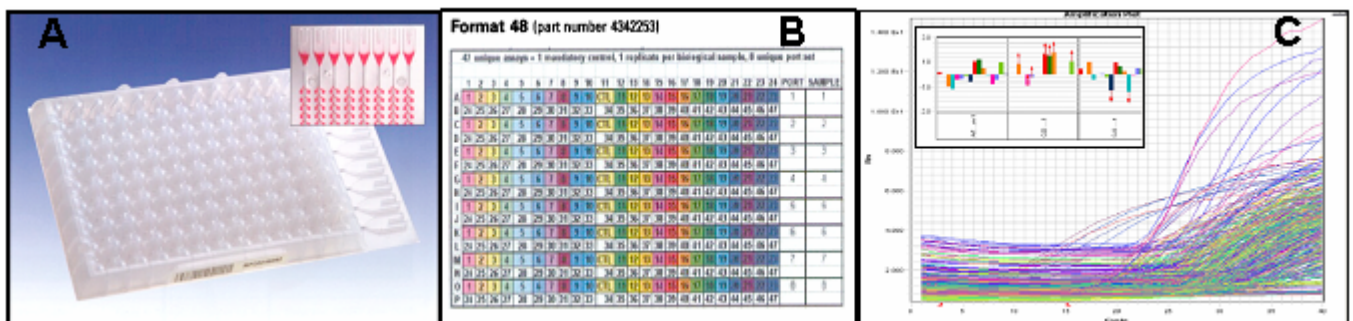
For cardiology, direct detection of cardiotropic viruses (ADV, Coxsackie, HHV6, PVB19) in myocardial tissue is performed by nested-PCR. Therefore DNA and RNA will be isolated individually from different biopsies or peripheral blood cells. Virus-positive patient samples will be sequenced and quantified for estimation of viral load by quantitative PCR (viral genomes per myocardial tissue). This procedure is applicable to all other clinical requests. The isolation techniques for nucleic acids will be adapted to corresponding patient material (blood, cerebrospinal fluid, urine, tissue from various organs). Down-stream applications of PCR, gelelectrophoresis are similar to temporarily established methods in IKDT lab.

Sequencing of generated PCR products is performed for quality control, infectious agent identification, mutation screening etc. This should lead to a specialized therapy in estimated disease situation. Quantification of genes or genomes will be performed by real-time-QPCR (TaqMan, Fa. Applied Biosystems) for measurement of gene load in analyzed tissue and for monitoring of applied therapy. A differential treatment of examined patients is possible in correspondence to detected agents in the patient sample.

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Expression profiling for human genes

TaqMan Low Density Arrays (TLDA) allow the simultaneous quantification of 12 to 384 human genes in single patient sample (Applied Biosystems, Forster City, USA). These quantitative TLDA's will be pre-designed for evaluation of disease situation (tumor, infection, apoptosis). Pre-designed TaqMan QPCR systems which could be combined for different expression panels are available for any human gene. The set-up of gene profile is customized for the individual problem (tumors, infection, inflammation).



Micro Fluidic Card (A) with various array formats (B) and mRNA expression profiles (C)

Human MicroRNA profiling by TaqMan Micro Fluidic Cards

MicroRNAs (miRNAs) are a class of naturally occurring noncoding RNAs that play a key role in gene regulation. These transcripts are highly conserved, single-stranded RNAs (~22 nucleotides) that are cleaved from larger hairpin precursor transcripts.

Applying TLDA technology a comprehensive coverage of Sanger miRBase v10 is enabled across a two-card set of TaqMan® MicroRNA Arrays (Arrays A and B) for a total of 667 unique assays specific to human miRNAs (Applied Biosystems, Forster City, USA). This approach enables the customer to estimate quantitatively microRNA profiles in human samples.

Downstream measurement of selected miRNAs could be performed by individual, pre-designed miRNA TaqMan Assay.

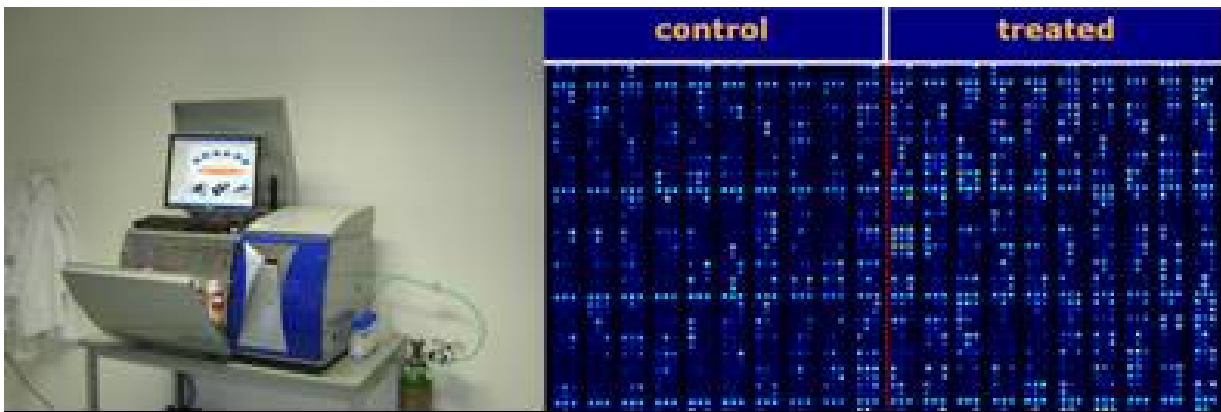
Customized genechips for any gene and any species

IKDT lab is offering gene profiling of all published gene sequences in customized or pre-designed gene chips on the *GENIOM RT Analyzer* (febit biomed, Heidelberg, Germany). Only flexible GENIOM platform allows the customer to design individual arrays for simultaneous detection of gene sequences from different species (human, mouse, virus, bacteria) in one assay.

A GENIOM gene chip contains 8 arrays with currently up to 15624 features each, in near future up to 40000 features. The 8 arrays on the Custom Geniom Biochip can be synthesized with identical or different content depending on your amount of samples and experiment setup. Pre-designed probe set for all human genes and for many different species are currently available.

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Customer has to submit its biological samples. The labeling, hybridisation and measurement of gene arrays will be performed in IKDT lab.



GENIOM RT Analyzer (febit biomed) and gene array plot of 6000 customized gene probes

Additionally gene chips for the detection of miRNA are immediately updated following new Sanger miRBase releases to offer you the most comprehensive and curated panel of miRNA. Human microRNA chip contains currently probes for all published 835 microRNAs.

Service offers Molecular Biology:

- Fixation (e.g. RNAlater) and storage of tissue samples
- Isolation of DNA and RNA from various tissues or blood
- Reverse Transcription of mRNA in cDNA
- Quantification of nucleic acids by TaqMan QPCR
- Detection of viral genomes (nested-PCR, QPCR, sequencing)
- Measurement of gene expression of human, murine or rat genes by TaqMan QPCR
- **TaqMan Low-Density-Arrays - 12 to 384 genes in one sample (human, murine, rat)**
- **MicroRNA Profiling on TaqMan Low-Density-Arrays (660 human microRNAs)**
- Estimation of quantitative gene profiles on *TaqMan Micro-Fluidic Cards*
- Gene profiles are determined by customer (e.g. tumors, inflammation, diabetes)
- **Customized gene arrays of 15.000 probes for mRNA and microRNA (GENIOM)**
- Sequencing of PCR products or plasmids
- Detection of polymorphisms or gene variations by fragment length analysis
- Detection of disease relevant single nucleotide polymorphisms (SNPs)
- **Identity testing by STR analysis**
- Set-up and performance of novel PCR systems (virus, bacteria)
- Transfer of data, also via internet
- Archiving of reports, electronic data, tissues and remaining nucleic acids according to GLP/GCP

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D. Autoimmunity Testing

In order to diagnose autoimmunity in patients IKDT apply indirect immunofluorescence assays based on BIOCHIP technology of EUROIMMUN (Germany).

The indirect immunofluorescence test is the analytical method of choice for screening on different autoantibodies or when it would be too difficult or too complicated to prepare the test antigens individually for enzyme immunoassays. For the determination of autoantibodies or antibodies against infectious agents, cells, tissue sections or purified, biochemically characterized substances are used as antigen substrates.

Ultrathin glass slides covered with different tissue sections will be incubated with patient sera. If the sample is positive, specific antibodies in the diluted serum sample attach to the antigens coupled to a solid phase. In a second step, the attached antibodies are stained with fluorescein-labelled anti-human antibodies and visualized with the fluorescence microscope.

High specificity: positive and negative samples produce a large difference in signal strength. Each bound antibody shows a typical fluorescence pattern depending on the location of the individual antigens. Immunofluorescence enables simultaneous detection of antibodies against several biochemically different antigens on one single biological substrate. Positive samples can be titrated in steps.

Using several BIOCHIPS coated with different substrates side by side on one and the same reaction field, antibodies against various organs or infectious agents can be investigated simultaneously. Detailed antibody profiles can thus be established with comparatively little effort, allowing the reciprocal determination of the results on different substrates.

Service offers Autoimmunity Testing:

- Detection of autoantibodies by fluorescence and light microscopy
- Simultane investigation of antibodies against various organs or infectious agents
- Transfer of data, also via internet
- Archiving of reports, electronic data and patient sera according to GLP/GCP

E. Immunology

Predominant immune response in patients is decisive for induction of acute phase, viral persistence or no significant reaction after viral infection. Different phases of infection are characterized by changing cytokine profile. Simultane measurement of upto 27 human chemokines and cytokines by bead-based multiplex-ELISA (Luminex technology) allows immunological classification of examined patients. This improves evaluation of clinical course of patients and supports indication for a specialized therapy approach in context with parallel determination of infections or inflammatory processes.

Therefore, IKDT lab is offering measurement of single cytokine by conventional ELISA on microplates (pipetting robot) or estimation of cytokine profiles by multiplex-ELISA for simultane measurement of upto 50 cytokines for clinical follow-up studies.

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One strategic objective of IKDT is early detection of auto-antibodies, differential diagnosis and risk assessment of induced autoimmunity in patients. Detection of auto-antibodies in sera of patients will be performed by use of commercially available microscopic slides spotted with different tissues which react as antigens. Processed reaction between sera and specific tissue will be visualized by application of a second fluorescence labelled antibody.

Service offers Immunology:

- Extraction and storage of patient samples (sera, tissues)
- Performance of ELISA tests (single parameter, Multiplex)
- Estimation of phosphorylation stage of proteins
- Detection of autoantibodies by fluorescence and light microscopy
- Design or performance of individual ELISA tests or bead-based immunoassays
- Transfer of data, also via internet
- Archiving of reports, electronic data and patient sera according to GLP/GCP

IKDT Labor offers its complete diagnostic service in histology, immunology, immuno-histochemistry und molecular biology for different requests in biomedicine and research. Performance of examinations or studies will be adapted according to individual requirements of clients.

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