

IKDT Laboratory

*Your Partner for Molecular Diagnostics in
Primary and Secondary Cardiomyopathies*

IKDT Manual 2011

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Summary

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1. General

In order to define the current role of endomyocardial biopsy (EMB) in the management of cardiovascular disease, a multidisciplinary group of experts in cardiomyopathies and cardiovascular pathology were convened by the American Heart Association (AHA), the American College of Cardiology (ACC), and the European Society of Cardiology (ESC). In form of 14 clinical scenarios were published recommendations for taking EMBs and the requested examinations in October 2007 (Circulation 2007;116;2216-2233).

The IKDT Institut Kardiale Diagnostik und Therapie GmbH is performing routine and specialized diagnostics of cardiologic diseases in close cooperation with Department of Cardiology and Pulmonology, Medical Clinic II in Berlin. IKDT is performing nearly all requested examinations on EMBs following the international recommendations.

It was founded in August 2002 and started its laboratory work in January 2003. IKDT lab is performing diagnostic examination of endomyocardial biopsies. Its service is offered to all cardiological hospital departments in Germany and Europe.

Today IKDT is one of the leading laboratories on viral infections of heart muscle tissue. Molecular diagnostics of patient samples and also research samples is ensured by sufficient and well-educated and highly motivated technicians. All necessary analytical devices are existing in IKDT lab. New diagnostic tests will be adapted from currently applied methods.

IKDT lab is the only clinical laboratory in Germany which is accredited by College of American Pathologists (CAP) to perform extended diagnostics of endomyocardial biopsies. CAP is the only organization which is approved by US Food and Drug Administration (FDA) for accreditation of diagnostic labs outside of the US. The Laboratory Accreditation Program (LAP) includes a regular, biannual peer-review of laboratory and intermediate self-inspection by laboratory director. Implemented QM systems is following the GLP/GCP guidelines and Clinical Laboratory Improvement Amendments of 1988 (CLIA-88).

From 2003 to 2006, IKDT was the core lab for molecular diagnostics of endomyocardial biopsies during the European trial on treatment of chronic cardiomyopathies by Beta-interferon, which was organized by Department of Cardiology and Pulmonology, Medical Clinic II of Charite University Hospital Benjamin Franklin (CBF) in collaboration with Schering AG Berlin.

2. Performance

IKDT is performing diagnostics on endomyocardial biopsies on requests of hospital-affiliated institutions or private doctor offices. For routine diagnostics will be covered three main topics: 1. Histology, 2. Immunohistochemistry and 3. Molecular Virology. Four endomyocardial biopsies at minimum are required for whole routine procedure. Increased number (6-8) will be beneficial for diagnostic accuracy. Sampling error is not negligible for detection of cardiotropic viruses.

In the currently finalized European clinical trial on Beta-Interferon treatment of chronic cardiomyopathy (BICC) were included about 400 patients, 9 myocardial biopsies from each patient, which have to be analyzed by three different methods: PCR for viral genomes, surgical histology and immuno-histochemistry.

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The histological/immunohistochemical examination is performed within 2 working days, whereas routine diagnostics of EMBs is performed completely within 5 days.

Additionally IKDT is offering testing on circulating antibodies to heart proteins in patient. For these assays, based on reactivity of patient antibodies against myocardial structure components like nucleus, basal membran, mitochondria, striated muscles, are requested fresh serum or plasma.

3. Quality Assurance - Quality Management

The institute disposes over a management with a rigid organization which does not allow any undefined deviations. The laboratory director of IKDT and representatives of consulting CBF board are responsible for the assurance of the quality of examination results. The QM system is covering internationally accepted GLP/GCP CLIA-88 guidelines.

From the beginning in-house established QM system was focused on Laboratory Accreditation Program of College of American Pathologists (CAP). Their Laboratory Accreditation Program (LAP) was established in 1961. In 1995 CAP received approval as an accrediting organization under the Clinical Laboratory Improvement Amendments of 1988 by the Centers for Medicare and Medicaid Services (CMS), an agency within the U.S. Department of Health and Human Services. In 2001, this approval was extended for an additional six years, through September 2007.

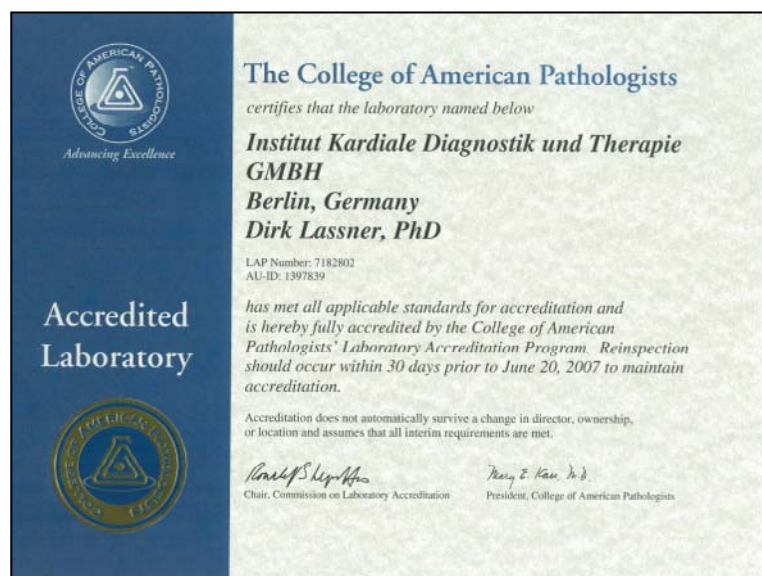


Figure 1: Certificate of IKDT lab accreditation by College of American Pathologists (CAP)

Since 2003 IKDT lab is accredited by CAP to perform endomyocardial biopsy diagnostics under certified conditions. CAP accreditation is the only certification process outside of USA which is accepted by US Food and Drug administration (FDA).

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The accreditation programs examine pre-analytical, analytical and post-analytical aspects of quality management (QM) in the laboratory. This includes the performance and monitoring of general quality control (QC), test methodologies and specifications, reagents, controls and media, equipment, specimen handling, test reporting and internal performance assessment, and external proficiency testing. In addition, personnel requirements, safety, document management and other management practices are included in the inspection process. Laboratories that meet accreditation requirements distinguish themselves as quality laboratories (Fig.1).

The objective is efficient sample processing in a short time respecting a high quality standard of data acquisition by latest state of the art modern diagnostic methods and following supplement of submitting institution with detailed data report.

IKDT disposes over sufficient and suitable rooms as well as modern facilities with new equipment and devices which are regularly checked for proper functioning based on servicing contracts. A sufficient number of employees is available to carry out the necessary work. Explicit standard operating instructions (SOPs) are available for all equipment and procedure.

The personnel is qualified correspondingly to all areas and will also in future attend courses for continued medical education (CME).



Figure 2: Certificates of successful participation at national INSTAND NAT survey

PCR results most critical for resulting treatment of patients in hospitals or by drug therapy. Main focus is set on the permanent control of achieved PCR result. Detection of virus infection in endomyocardial biopsies is a multi-step-procedure, whereas each step is essential for final result.

IKDT is participating three times per year at national surveys on nucleic acid amplification techniques (NAT) for virus detection organized by INSTAND e.V., Düsseldorf. Through this program, the INSTAND provides individual laboratories with unknown specimens for testing. The participants analyze the specimens and return the results to the INSTAND for evaluation. In turn, each participating laboratory receives a report of their performance and a certificate of successful

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participation as well as a report summarizing the results of all participating laboratories. IKDT use these survey for evaluation of test and its accuracy. IKDT passed the existing surveys for all corresponding viruses successfully (Fig. 2).

4. The IKDT Staff

The managing and laboratory director Dr. Dirk Lassner is a biochemist with long time experience in molecular biology and clinical chemistry. Prof. Dr. Ulrich Gross, former director and vice-director of Institute of Pathology at CBF, is responsible as medical director for whole laboratory and for histological examination of endomyocardial biopsies.

Beside medical and managing directors there are currently 3 medical technical laboratory assistants, one biologist and one secretary. Physicians of the consulting board of the Department of Cardiology and Pulmonology, Medical Clinic II of Charite University Hospital Benjamin Franklin (CBF) support IKDT lab in validation of diagnostic findings, formulation of diagnostic reports and in clinical recommendations for submitting physicians.

5. Pre-analytical treatment of patient material

The IKDT performs diagnostic tests for detection of inflammatory processes or viral infection of human myocard biopsies under stringent compliance of FDA guidelines. The diagnostic spectrum includes histology, immunohistochemical analysis and PCR assays for detection of viral genomes. Myocardial biopsies in native or fixed form are the preferred sample materials for these tests. For suitable assays and results it is important that the submitted material undergoes a well defined pre-analytic treatment (Tab.1).

For endomyocardial biopsies should be used a novel reagent for conservation by ambient temperature. ***RNAlater*** is an aqueous, non-toxic tissue storage reagent that stabilizes and protects cellular RNA in intact, unfrozen tissue samples. ***RNAlater*** eliminates the need to immediately process tissue samples or to freeze samples in liquid nitrogen for later processing. ***RNAlater*** is also suitable for preparation for genomic DNA, histological examination and immunohistochemistry. Tissue pieces can be harvested and submerged in ***RNAlater*** for storage without jeopardizing the quality or quantity of RNA obtained after subsequent RNA isolation. ***RNAlater*** can be added to cell pellets and even cells in medium. The samples can then be stored frozen or unfrozen.

The endomyocardial biopsies (less than 0.5 cm in any one dimension) are simply submerged in approximately 0.5 ml of ***RNAlater*** at room temperature. Please submerge biopsies by inverting tube 5 times. The solution permeates the cells, stabilizing the RNA. Preferably, samples should be sent directly at **ambient temperature** to IKDT lab or stored at **+4°C before transport..** The transport could be performed in a padded envelope by conventional mail. After reception in IKDT lab, the sample will be stored at **+4°C for one night** and then can be stored at 4°C for up to a month or at 25°C for up to a week or at -20 to -80°C indefinitely (the tissue does not freeze).

Never freeze or treat the biopsies by other fixatives before *RNAlater* fixation!

RNAlater is only suitable for very small tissue samples (biopsies). Larger piece (explanted heart samples) has to be divided rapidly in small aliquots or should be cryo-conserved in liquid nitrogen.

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Table 1: Proposed pre-analytical treatment of endomyocardial biopsies for diagnostics in IKDT lab

Submitted material	Pre-analytic treatment	Detection method	Native/ fixed	Shipment
Myocardial biopsies	<i>RNAlater</i>	PCR & Histology & Immunohistochemistry	Fixed in <i>RNAlater</i>	Ambient temp
<i>Alternative pre-treatment</i>				
Myocardial biopsies	frozen in liquid nitrogen	PCR & Histology & Immunohistochemistry	native	on dry ice
Myocardial biopsies	fixed in 4-5% buffered formalin	Only for histology !	fixed in formalin	Ambient temp, Do not freeze!

IKDT lab is providing submitting institutions by screw-cup tubes filled with *RNAlater* for immediate use. Taken biopsies fixed in *RNAlater* should be transferred to IKDT for diagnostic procedure.

Additional analysis

Detection of systemic viral infections or cytokine profiles is performed by analysis of peripheral blood fractions. DNA or RNA from peripheral blood cells is examined by nested- and QPCR on presence of viral genomes for exclusion or confirmation of systemic infection. EDTA-blood is requested for detection of systemic viral infection (Tab. 2).

Predominating immune response in patient is evaluated by quantification of different sets of human chemokines or cytokines in plasma or sera by ELISA tests. Plasma for immunological tests should be collected with additives EDTA, aliquoted immediately in Eppendorf vials (0.2-0.5 ml per tube) and stored till use at minimum at -20°C or preferable colder (-80°C). Serum for immunological tests should be collected without any additives. Transfer to IKDT lab should be performed on dry ice. Cytokines are very thermosensitive and will be destroyed after second thawing cycle (table 2).

Table 2: Proposed pre-analytical treatment of peripheral blood fractions for diagnostics in IKDT lab

Submitted material	Pre-analytic treatment	Detection method	Native/ fixed	Shipment
Blood	EDTA-tubes	PCR	native	+4°C or ambient
Serum/Plasma	EDTA-tubes	Immunology	native	frozen, on dry ice below -20°C

6. Histology

For histological examinations 4-5µm thick sections are prepared of paraffin-embedded biopsies by cutting with the rotary microtome. For each staining procedure on one slide are placed between 3-8 serial sections. Routine diagnostics includes always HE, PAS and Elastica v. Weigert staining. The staining of specimens, with the exception of special stainings, is always carried out in the staining machines (Fig. 3).

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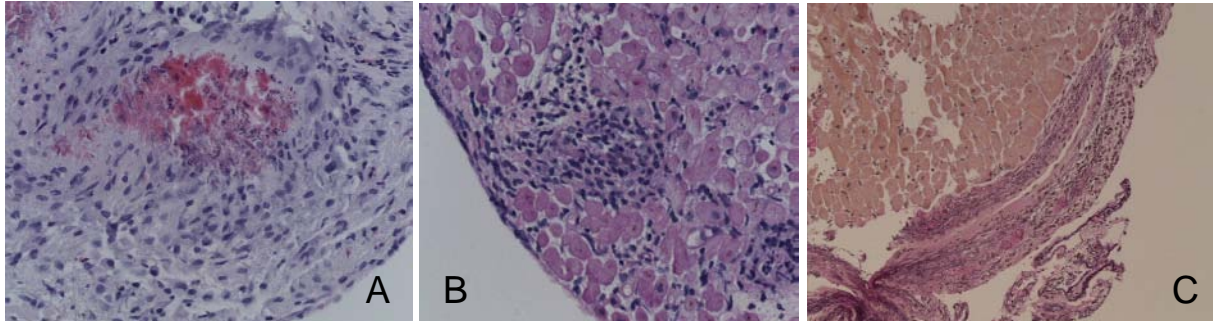


Figure 3. Histological examination of Löffler's endo and myocarditis (A), acute myocarditis with necrosis (B) and endocarditis (C)

Special staining for amyloid (Congo red), calcium (v. Kossa), acid mucosubstances (Alcian Blue) and iron (Prussian Blue reaction) will be added in clinically suspected cases or on request of submitting physician (Fig. 4).

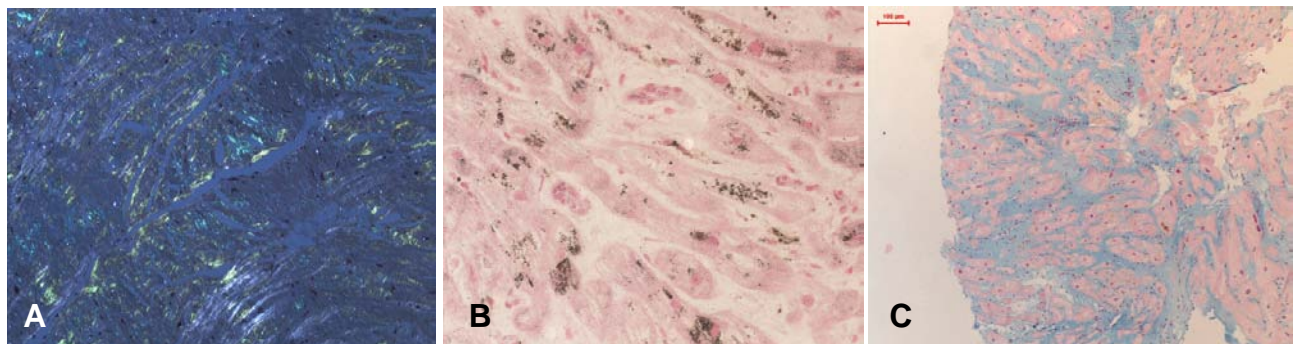


Figure 4. Special stains of amyloid with Congo Red visualized in polarized light (A), von Kossa stain for calcium (B) and Alcian Blue for acid mucosubstances (C)

All histological examinations are performed by medical director Prof. Gross, which was over many years Director of Institute of Pathology of CBF. Histological examination in IKDT lab follows Dallas criteria for exclusion of acute or active myocarditis in examined biopsy sample. Main focus is oriented on detection of myocytolysis in combination with leukocytic infiltrates.

Observations are fixed as paperwork and as digitally printed colour-photographs which are saved and stored on the data server as TIF-files or JPG-Files. Morphologic characteristics of stained endomyocardial tissue (e.g. diameter of cardiomyocytes, size and quality of biopsy, fibrosis, fatty tissue, capillaries) are rated by numeric scaling and the corresponding values are fixed on written examination protocol and in the electronic IKDT database.

7. Immunohistochemistry

Immunohistological diagnostics are based on application of specific primary antibodies on cryo-fixed tissue section and following detection of coupled primary antibody by secondary antibody. Secondary antibody is conjugated with enzyme complex which could produce a precipitating coloured complex after use of staining solution.

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For immuno-histochemical examinations sections are prepared of cryo-embedded biopsies by use of cryostat microtome. Therefore endomyocardial biopsy will be placed on pre-cooled (-20°C) metallic tray and covered completely by plastination glue Tissue-Tek. Tissue-Tek is freezing down immediately and preserve a hard consistence of embedded tissue.

Generally, cutting is performed for 3-5 slides for each antibody (about 20 cryo sections per patient) before immuno-staining is started. Then separated areas are processed with different antibodies accompanied with appropriate blocking and incubation steps and finally stained by an enzymatic conversion of dye AEC for producing red-colored immunospots for following microscopic examination. Second antibody and the colorimetric substrate are pre-mixed and well optimized for following digital image analysis. The final counterstaining of cryo sections is always carried out in staining machine (HE staining).

One microscopic slide is finally containing separated areas for 2 different antibodies. Hereby each following layer of cryo sections is placed in the field for the next antibody detection, i.e. in any area there are about 6 to 8 serial cryo sections. This cutting procedure ensure the more detailed analysis by simultane staining of different levels of biopsy by various antibodies.

Five sets of immunohistochemical staining are offered for specialized diagnostics of heart muscle tissue, whereas only the sets *IC1-Heart muscle inflammation* is proposed to perform in routine diagnostics procedure. Set *IC2-Activation marker/Viral proteins* is recommended in clinically suspected cases with high viral load of corresponding cardiotropic virus. Set *IC3-ARVD Diagnostics* is recommended in clinically suspected cases of ARVD to detected disrupted gap junctions of cardiomyocytes.

IC1: Heart muscle inflammation (CD3, CD11a, CD11b, Perforin, HLA class 1, CD54)

IC2: Activation marker/Viral proteins (CD45R0, 27E10, CD69, CD106, EV-VP1, PVB19, HHV6)

IC3: ARVD Diagnostics (*Plakoglobin, Connexin 43, N-Cadherin*):

IC4: Collagen Expression / Fibrosis (*Collagen 1 und 3 incl. Sirius-Red staining*):

IC5: Endothel Activation (*CD31, VCAM1*)

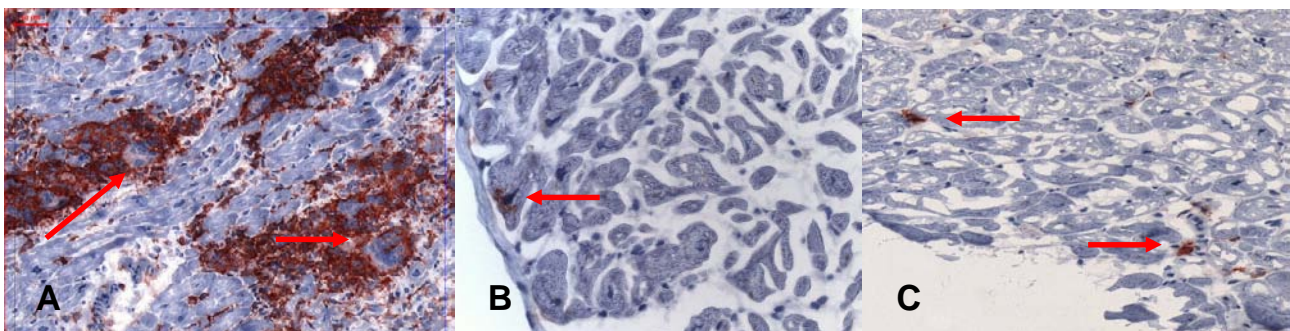


Figure 5: Immunostaining of giant cell myocarditis with CD3 (A), detection of PVB19 positive myocytes by VP2 antibody (B) and HHV6 infected cells (C) (see arrows)

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Coloured immunospots are counted digitally by application of in-house established digital imaging analysis software for calculating area fractions, numbers of immuno-spots and area of myocardial tissue (routine diagnostics). Values for counting are fixed by inclusion of digitally produced values in electronic database for in the final report. These report contains also numeric values for morphological characteristics like biopsy size, quality, fibrosis etc.

Physician in IKDT controls the digital results by additional examination and prepares the report for immunohistochemistry including laser print of colour photograph for the submitting physician.

In general, immunohistochemical staining is performed on frozen sections of a second biopsy, not identical to histological examination. This procedure is beneficial to reduce mis-interpretation by evaluation of only one biopsy.

8. Molecular Diagnostics

The molecular diagnostic approach of EMBs are based on detection, quantification and sequencing of viral genomes. With permantly increasing number of virus tests IKDT is focused on common cardiotropic viruses which are described as responsible triggers of heart failure problems. Established virus PCR detection methods of IKDT lab are listed in Table 3.

Test on cardiotropic viruses are based on qualitative detection of virus by nested-PCR and quantification of virus load by quantitative TaqMan PCR. Depending on the 2 types of viral nucleic acids we perform the isolation of DNA or RNA in separate extraction procedures. The transcriptional activity of virus in myocardial tissue or peripheral blood cells will be determined for the two most frequent cardiotropic viruses –Erythrovirus and HHV6 by nested-RT-PCR and QPCR.

Table 3: Established tests for cardiotropic viruses in IKDT lab

Virus	Nucleic Acid	nested-PCR	TaqMan	Subtypes / variants	Sequencing of positive PCR	Determination of virus subtyp by
Parvovirus B19	DNA RNA	X	X	G1, G2	yes	sequencing
Adenovirus	DNA	X	X	52	yes	sequencing
Human Herpesvirus 6	DNA, RNA	X	X	A and B	yes	sequencing
Cytomegalovirus	DNA	X		no	yes	
Epstein-Barr-Virus	DNA	X	X	no	yes	
Herpes simplex virus 1 and 2	DNA		X	1 and 2		TaqMan
Coxsackievirus	RNA	X	X	various	yes	sequencing
Influenza	RNA		X	A and B		TaqMan
Measles	RNA		X	no		

In order to calculate and standardize the estimation the virus load in small EMBs (viral genomes per μg human) IKDT lab apply the most accurate QUANTIFILER TaqMan test (Applied Biosystems, USA), which was primary developed for forensics to detect minute traces of DNA.

All amplified virus genomes were sequenced for determination of existing virus subtype or infectious variants. We apply double strand sequencing and subsequent manual alignment against in-house reference files and international NCBI database (Fig. 8).

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Isolation of DNA or RNA is performed from different endomyocardial biopsies in a parallel manner. During isolation procedure each staff member has to care for nuclease-free working conditions (sterile tips, DEPC-treated water, often change of gloves etc.). After DNA extraction the amount of isolated DNA has to be measured by special TaqMan assay. RNA is completely transcribed into cDNA after DNase digestion. Finally both nucleic acid fractions are existing as DNA molecules and by this way better conserved from RNase / DNase digestion.

Detection of viral genomes by nested-PCR

Polymerase chain reaction (PCR) is an artificial method for selective amplification of any desired genome fragment in a very short time by use of a thermal cyclor and thermostabile Taq DNA polymerase. We apply this method for detection of Adenovirus (ADV)-, Coxsackievirus (CVB), Epstein-Barr-Virus (EBV), ParvoB19-Virus (PVB) and human Herpesvirus 6 (HHV6) gene sequences in endomyocardial biopsies.

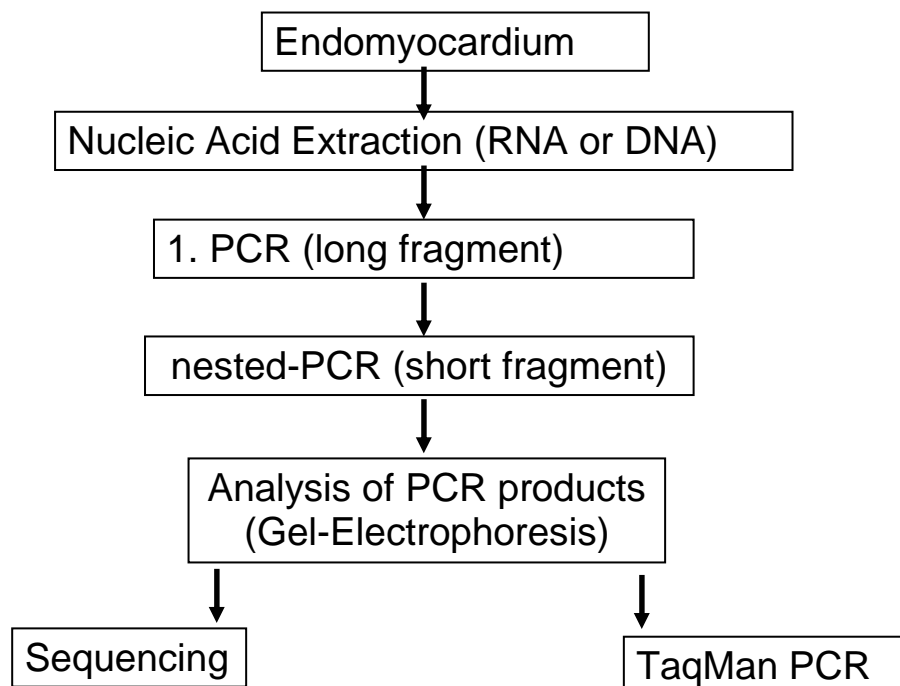


Figure 6: Flowchart of diagnostic procedure for detection of cardiotropic viruses

For all routinely analyzed cardiotropic viruses are used nested-PCR protocols consisting of two sequentially performed PCR assays, where the amplicon of first assay is the template for second reaction. This procedure is highly sensitive and enables us to detect very low copy numbers of viral genes (ultrasensitive). As amplification control there are simultaneously processed serial dilutions of a corresponding DNA-standard for checking PCR process.

Amplified PCR reactions are separated on agarose gel electrophoresis in ethidium bromide containing buffer. This allows the subsequent visualisation of generated PCR product by UV fluorescence. Amplicons with the same length size as the co-amplified standards are estimated as positive signals and correspond to existing viral infection of myocardium (Fig. 7).

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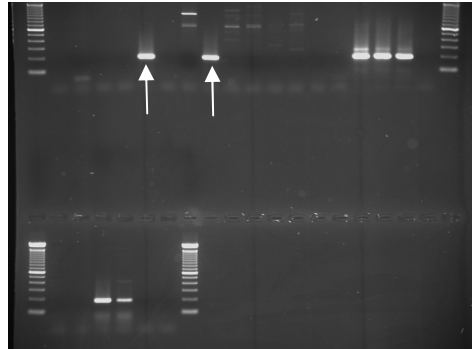


Figure 7: Gel electrophoresis of Coxsackievirus-nested-PCR (*arrows – infected patients*)

After nested-PCR aliquots of PCR samples are analysed by agarose gel electrophoresis. To the gel is added ethidiumbromide for following staining in UV light (Fig. 7).

Documentation of PCR results is done by photographs made with digital camera in the gel documentation system and a printout with a thermal printer. These printouts were ticked to corresponding lab book for long-term documentation.

QPCR and sequencing data are generated by certified, electronic software. All raw data and export files are stored on CD or streamer cassette for long-term storage following GLP/GCP guidelines.

Sequencing of viral genomes

All positive PCR reactions are sequenced as quality control of preceding nested-PCR and for detection of amplified virus subtype. The generated sequences are checked by manual alignment with PHYDE software (Institute of Botany, Bonn/Dresden) and online with NCBI database for confirmation of corresponding virus strain and / or estimation of specific virus subtypes or variants (Fig. 8).

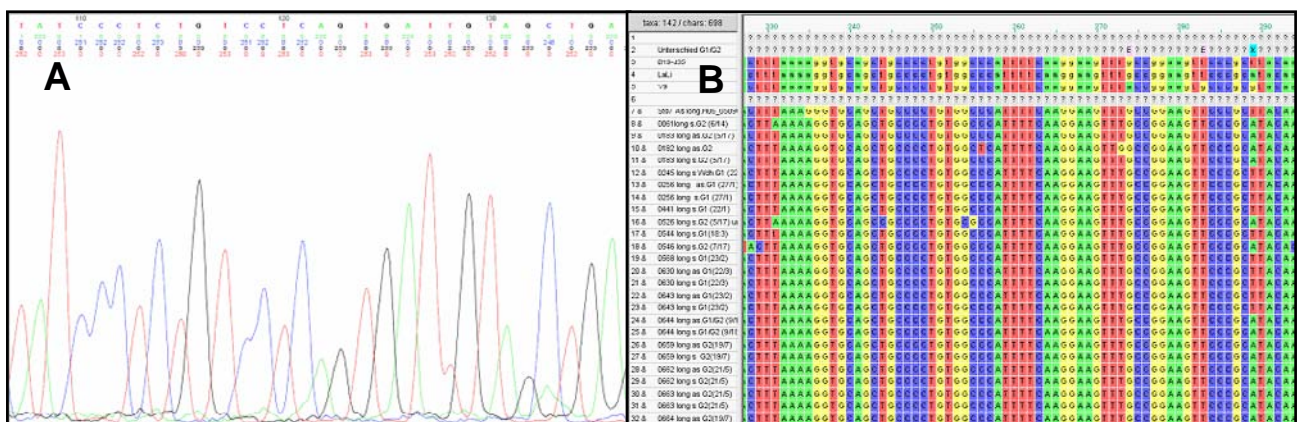


Figure 8: Sequence fragment of Coxsackievirus (A) and alignment of PVB19 genotypes (B)

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Quantification of viral load

Monitoring of successful treatment or therapy of infected patients has to be accompanied by estimation of viral load in EMBs at different time points. Viral load is the ratio of viral genome copies to associated amount of extracted myocardial tissue. In IKDT lab human genomic DNA, as counterpart of extracted biopsy, was measured by quantitative TaqMan assay, which is recommended for analysis of DNA amount in forensic traces by FDA.

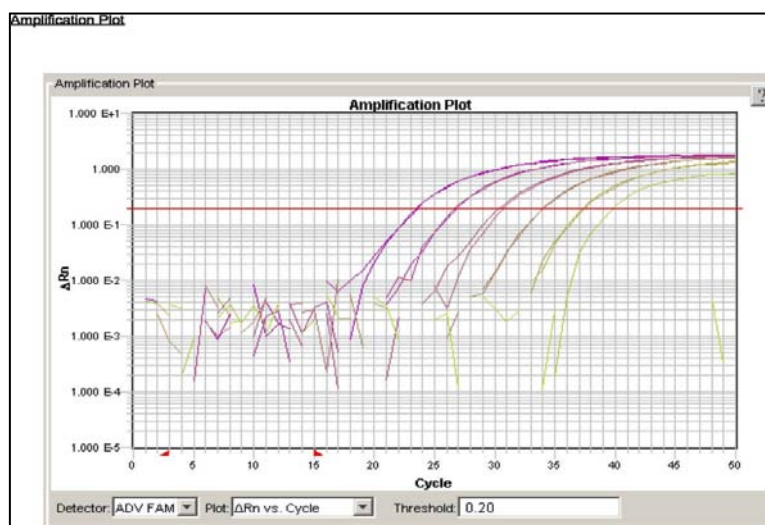


Figure 9: Standard curve for HHV6 TaqMan-QPCR for quantification of viral genomes

Quantitative determination of viral genomes by real-time PCR is based on additional use of a fluorescent probes in a PCR assay. By simultaneous measurement of a calibration curve based on a serial dilution of a plasmid standard the number of viral gene copies is detected during amplification process (Fig. 9).

This extremely sensitive and highly optimized method is unique for estimation of viral loads for DNA viruses in human tissues and was also applied in the European BICC trial.

9. Autoimmunity Testing

Autoimmune cardiomyopathy is an immune-mediated chronic inflammation of the myocardial tissue. Induction of autoantibodies could be performed by infection of heart muscle by viruses or by intramyocardial inflammation. In order to diagnose autoimmunity in patients with cardiac problems 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.

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Ultrathin glass slides covered with heart muscle tissue sections of monkey 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.

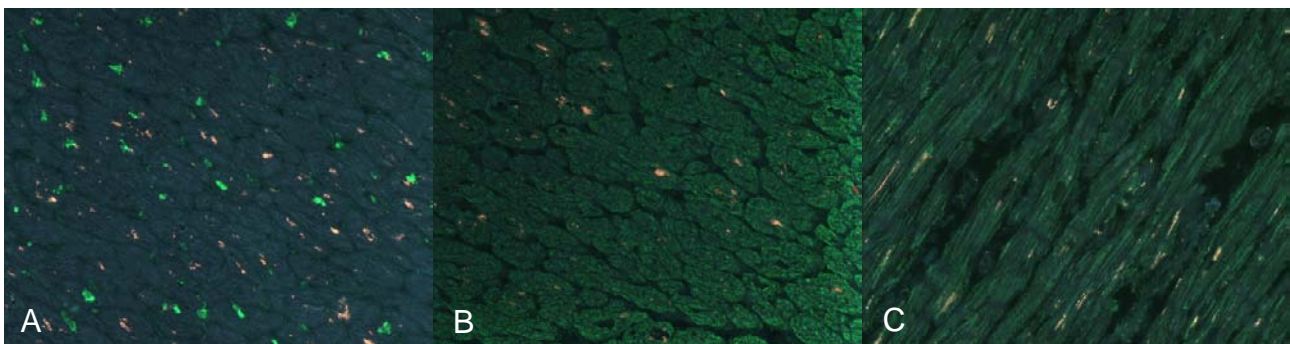


Figure 10: Indirect immunofluorescence detection of autoantibodies against nucleus (ANA) (A), cross-striated (B) and along-striated muscles (C) on monkey heart tissue by dual green-red fluorescence microscopy (red: fusicyclin in cardiomyocytes)

Applying BIOCHIP with monkey heart muscles tissue allows simultaneous detection of autoantibodies against nucleus (ANA), mitochondria (AMA), intercalating discs, basal membrane, endothelium and striated muscles (Fig. 10).

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.

10. Immunoassays

The immune system protects the body from infection by creating and maintaining barriers that prevent bacteria and viruses from entering the body. If a pathogen breaches the barriers, and gets into the body, the innate immune system is equipped with specialized cells that detect, and often eliminate, the invader before it is able to reproduce, potentially causing serious injury to the host. The innate immune system protects the host by establishing humoral, chemical and cellular barriers to infection. Inflammation is produced by chemical factors; including specialized chemical mediators, called cytokines, and serves as a protective barrier. Cytokine levels in peripheral blood correspond to systemic situation in patients initiated by various, but often global factors like infections or inflammatory processes. Cytokines or chemokines are estimated in blood serum for characterization of present immune response.

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The diagnostic goal of immunology is non-invasive measuring of biomarkers like cytokines and auto-antibodies characterizing different states of DCM in body fluids (blood), which allow the monitoring of disease progress or outcome of applied therapy. IKDT offers Multiplex-ELISAs for measurement of cytokine profiles of DCM patients (Fig. 11).

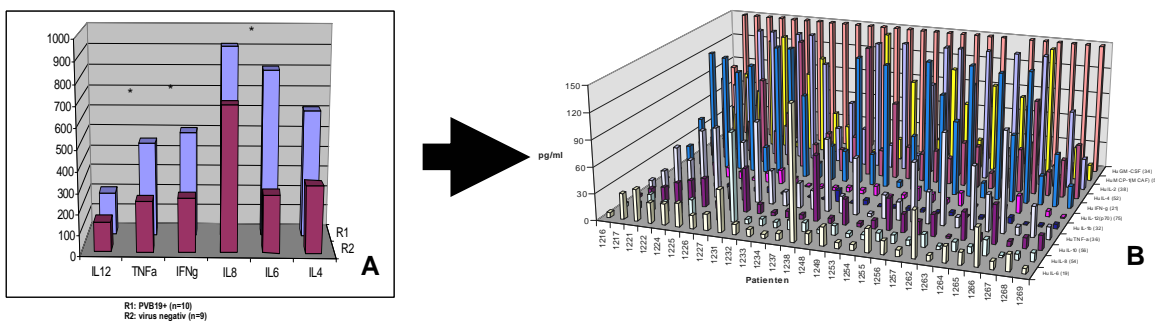


Figure 11: Cytokine profiling by single parameters (A) or Multiplex-ELISA of 17 cytokines (B)

The samples for the immunoassay department are mainly patient sera or whole blood. Separation of sera from EDTA-blood is performed by centrifugation. ELISA are processed immediately after centrifugation step or aliquots (200 µl) of sera were stored in a freezer (-20°C) until use. Quantitative detection of cytokines is performed by commercially available immunoassays on full-automated ELISA system DSX (Fa. ThermoLabsystems, Fig. 11A) or by multiplex ELISA (Fa. Bio-Rad, Fig. 11B). All standards and controls are included in corresponding kits. All data for ELISA tests are reported as data files and printed reports.

Cardiomyopathy is a poorly understood disease because it progresses through stages with distinctly different mechanisms and manifestations finally leading to dilated cardiomyopathy and chronic heart failure. Most cases of myocarditis result from a viral infection, which may progress to an autoimmune phase followed by progressive cardiac dilatation. One strategic objective of IKDT is early detection of auto-antibodies, differential diagnosis and risk assessment of post-viral autoimmunity in patients suffering from cardiomyopathies.

Detection of auto-antibodies in sera of patients with cardiomyopathies 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. Auto-immunoassays were documented by digital photographs after fluorescence microscopy.

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Summary

IKDT lab offers the most comprehensive approach to analyze myocardial tissue (EMBs) on morphological abnormalities, viral infections and inflammatory processes as causive reasons for heart failure problems. The current routine protocol will be expanded continuously by new methods or biomarkers. IKDT is providing submitting institution by diagnostic parameters of examined patient and also provide you the possiblity to perform clinical trials or be included in running research project.

Dirk Lassner, PhD
-Laboratory Director-

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-Medical Director-