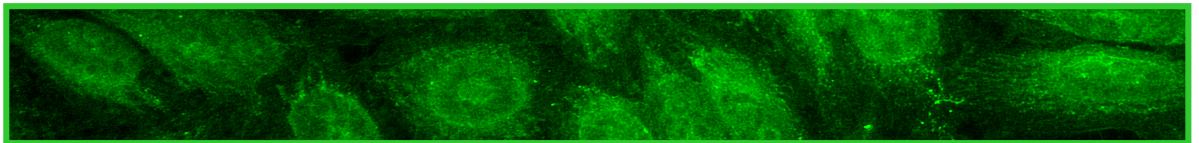




## Fully Automated System for Evaluation of Cell-based Immunofluorescence Tests



AKLIDES® performs:

- Fully automated reading
- Titer prediction without dilution series
- Archiving of results

**Modular assembly of the system:**

- Microscope unit
- Motorized scan stage
- CCD camera
- Software for automated reading and evaluation

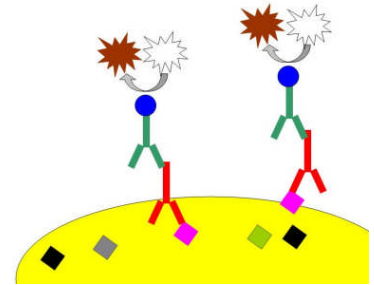
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# Cell-based Immuno- fluorescence Tests

Immunofluorescence (IF) is an immunohistochemical method for biomolecule detection using antigens or antibodies labeled by fluorescent dyes.

Indirect immunofluorescence (IIF) can be used for the determination of circulating autoantibodies (AAb) which may be associated with autoimmune diseases. For that purpose a serum sample is contacted with tissue sections or cells (cell-based IF) immobilised on a solid phase. Autoantibodies from serum interact specifically with target antigens within tissue or cells. A secondary fluorochrome-labeled antibody binds to the AAb subsequently. Excitation energy provided by a light source generates a fluorescence signal which visualizes the AAb indirectly on the target tissue or cell and creates a specific fluorescence pattern (Figure 1).



**Figure 1:** Schematic picture of indirect immunofluorescence test

## Substrates of cell-based IIF

Immunofluorescence tests employing cells as a substrate are routine techniques in autoimmune diagnostics.

Three different cell substrates are routinely used:

- 1) HEp-2 cells are specific cells for the detection of anti-nuclear antibodies (**ANA**) - general markers of rheumatic diseases.
- 2) The protozoa *Crithidia luciliae* is used for the detection of AAb against dsDNA which are associated with Systemic Lupus erythematoses (**SLE**).
- 3) Human granulocytes are used for the detection of anti-neutrophilic cytoplasmic antibodies (**ANCA**) which are associated with vasculitides.

## Advantages of cell-based IIF

- ▶ Easy and short test procedure
- ▶ Low costs of reagents and material
- ▶ Multiparametric assay

## Disadvantages of cell-based IF

- ▶ Subjective analysis
- ▶ No possibility of full automatization
- ▶ Insufficient standardisation

## AKLIDES® eliminates disadvantages by providing:

- ▶ Fully automated analysis of immunofluorescence assays
- ▶ Standardisation of ANA pattern recognition
- ▶ Archiving and innovative data processing

# Hardware components



**AKLIDES®**

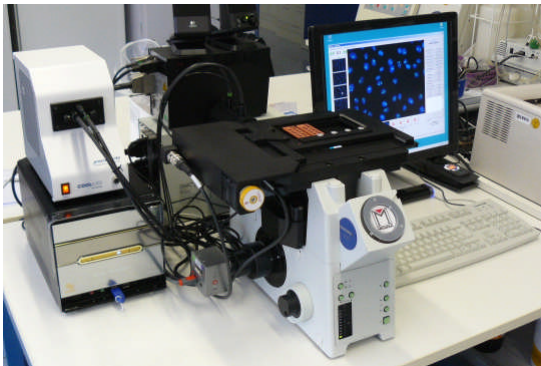


Figure 2:  
Hardware components comprise fluorescence microscope, scan stage, LED unit providing the excitation energy, gray-scale camera and PC with the AKLIDES® Software.

Figure 2: AKLIDES® □□□□□□

## Microscope unit

Figure 3:  
The microscope unit supports focussing (z-axis) and change of objective lenses. These mechanics are controlled by AKLIDES® Software.

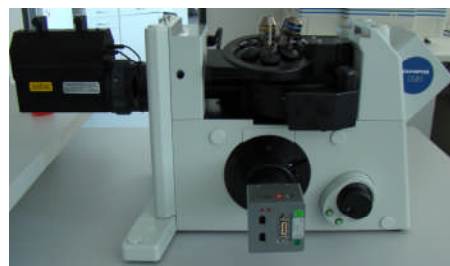


Figure 3: Fluorescence microscope unit of AKLIDES® System

## Scan stage

Figure 4:  
Scan positions (x- and y-direction) are controlled by AKLIDES® Software. Up to 4 slides or 1 microwell plate can be loaded.



Figure 4: Sample deck of AKLIDES® System

## LED unit

Figure 5:  
The LED generates light of defined different wavelengths, thus several excitation signals can be provided. LEDs have a superior life time of more than 10000 hours.



Figure 5: LED unit of AKLIDES® System

## Camera

Figure 6:  
The camera unit of AKLIDES® System is a highly sensitive grayscale CCD camera controlled by **AKLIDES® Software.**

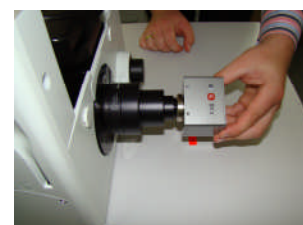


Figure 6: Camera of AKLIDES® System



# AKLIDES<sup>®</sup> Software

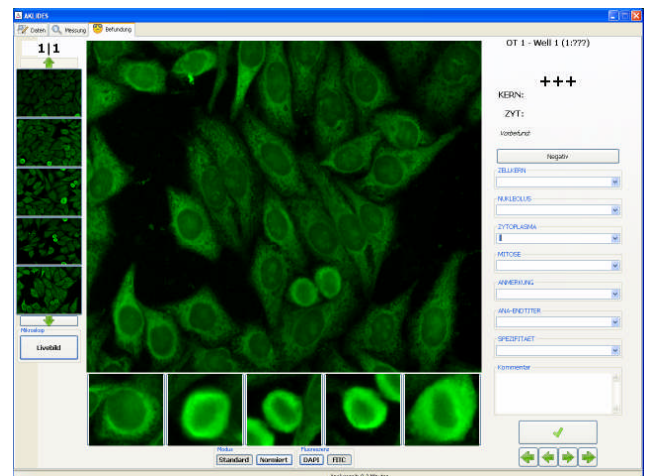
AKLIDES<sup>®</sup> Software is the key component of the system. The software guarantees optimal image processing and fully automated analysis of immunofluorescence signals. Innovative data storage provides the basis for archiving and generation of a data base.

## Image generation

AKLIDES<sup>®</sup> Software automatically reads out images by controlling scan positions (x- and y-direction), focussing (z-direction), calibration and recording of the fluorescence signals.

## Image processing

AKLIDES<sup>®</sup> Software reads the fluorescence pattern from the location of the signal inside the cell and guarantees objective pattern recognition. The concentration (titer) of the antibody is calculated from the intensity of the fluorescence signal.



Therefore, **standardisation** of immunofluorescence technique is possible for the first time. This paves the way for an increase of quality and comparability of results between laboratories.

## Image storage

AKLIDES<sup>®</sup> Software automatically stores the fluorescence image and defined pattern of each sample. This allows digital archiving of results in an internal database.

## Image and pattern export

AKLIDES<sup>®</sup> Software can be connected to LIMS for import and export of patient's data and results.

## Kit components

- ▶ 480 determinations
- ▶ AKLIDES<sup>®</sup> specific reagents
  - FITC conjugate
  - Positive and negative control
  - Mounting medium
- ▶ AKLIDES<sup>®</sup> specific slides
  - 6, 12, or 16 wells

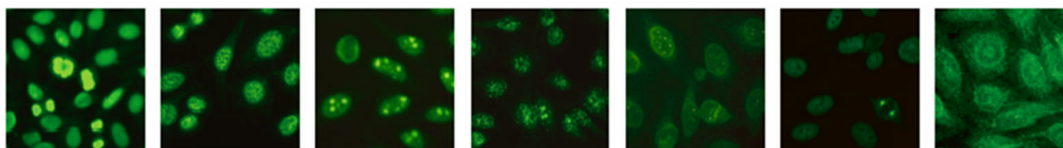
## Test procedure

- ▶ Conform with EASI guidelines
- ▶ 25 µl sample volume
- ▶ Incubation 2 x 30 minutes
- ▶ Screening dilution 1:80 and 1:320
- ▶ No dilution series for titer determination

## Analysis

AKLIDES<sup>®</sup> Software automatically evaluates intensity and distribution of the fluorescence signal.

Positive-negative differentiation is provided and the fluorescence image is evaluated and assigned to 7 patterns:



homogeneous speckled nucleolar centromere dots mitotic cytoplasmic

End titer (antibody concentration) is predicted without a dilution series.  
Time of analysis is less than 1 minute per well.



# Further KLIDES<sup>®</sup> Assays

## In evaluation:

 KLIDES<sup>®</sup> ANCA

 KLIDES<sup>®</sup> Anti-nDNA

## My notes:

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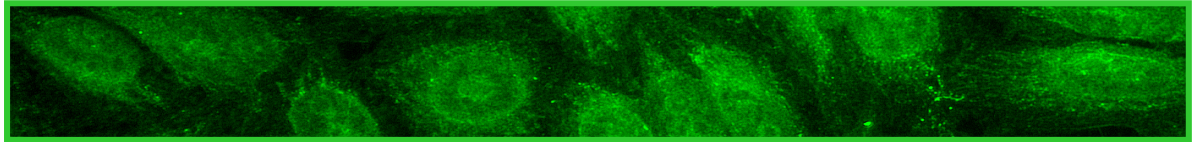
# AKLIDES<sup>®</sup> at a glance



- ▶ Fully automated screening
- ▶ Titer prediction without dilution series
- ▶ Fully automated analysis in less than one minute per well
- ▶ Fully automated calibration
- ▶ Saving of time and material
- ▶ Objective pattern recognition
- ▶ Online global data sharing
- ▶ Archiving and data base
- ▶ LIMS connectable



 **KLIDES<sup>®</sup>**



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