Non organ specific autoantibodies (AABs), are directed against highly conserved antigens of the body’s own cells (Holborow et al., 1957). Diagnostically relevant target structures are predominantly located in the cell nucleus (antinuclear antibodies, ANA), but also in the cytoplasm. Screening for these AABs constitutes a major part of the diagnostic procedure for connective tissue and autoimmune liver diseases. Evidence is usually collected in a multistage diagnostic process in which initial screening is carried out using indirect immunofluorescence testing (IIF) on HEp-2 cells, an epithelial cell line derived from a human laryngeal carcinoma (Hahon et al., 1975). HEp-2 cells have replaced the frozen sections of organs, which were originally used as a substrate. Used in primary screening, the main advantage of IIF on HEp-2 cells is that it provides a good overview of most of the diagnostically relevant non-organ-specific AABs and their concentrations. Originally, immunological laboratories prepared their own HEp-2 cells, and diagnostic use of these cells was subject to great variability because of individual culture and fixation conditions. Nowadays preparations of acceptable quality are available from manufacturers of diagnostic equipment on standardized microscope slides as in vitro diagnostic material (EU Directive, 1998). The use of cells in IIF cannot be replaced by the use of lysed HEp-2 cells in enzyme immunoassays because only immunofluorescence can deliver information about all diagnostically relevant AABs. In spite of the now-acceptable quality of the available HEp-2 preparations for immunofluorescence, the results from different laboratories can differ considerably. This paper therefore aims to summarize current recommendations concerning IIF methodology and the interpretation of immunofluorescence patterns on HEp-2 cells. These recommendations will contribute to improve the comparability of diagnostic procedures and are intended to aid the interpretation of findings, independent of the laboratory in which the tests are performed. The recommendations were developed in close collaboration with clinical and laboratory scientists with the focus to improve diagnostic procedures for autoimmune diseases (Sack et al., 2007).
Reagents and Test Preparation

HEp-2 Cells

HEp-2 cells are available from numerous suppliers as a CE-certified and/or FDA approved diagnostic aid. They can also be obtained from cell banks (such as American Tissue and Cell Collection CCL-23; http://www.atcc.org) as a cell line for scientific investigations. The cells have been found to be heterogeneous in their morphology, antigen expression and cell division behavior. Modified HEp-2 cells also exist in which the expression of particular antigens, such as Ro60 has been increased by transfection. Certain criteria are important for the judgment of the cells’ quality and can be heavily influenced in the production process by cell culture conditions, cell preparation, microscope slide preparation, fixation and the processing instructions given.

These criteria are:
1. cell density and distribution on the microscope slide,
2. number of mitoses (at least 3 to 5 mitoses per visual field at 200 × magnification),
3. expression of target antigens for relevant autoantibodies,
4. maintenance of morphology,
5. background fluorescence

Before it is used in the laboratory, the quality of each batch should be tested using defined monitoring procedures. There is so far no standardization of the preparation of HEp-2 cells or the composition of the test kits among manufacturers, although this would make it possible to ensure that differences in titer levels and fluorescence patterns detected on evaluation do not arise as a result of preparation procedures. Furthermore, only standardized procedures make possible longitudinal measurements and comparison of results from different laboratories. Batch monitoring requires the use of one negative serum and at least three positive serum samples with different fluorescence patterns resulting from defined antibody reactivity (e.g. centromeres, dsDNA, Ro/SS-A). These must be used in alternation each time the test is run.

Workplace

The preparation of IIF tests and subsequent evaluation by fluorescence microscopy are typically carried out in separate areas. In immunofluorescence laboratories it is especially important to ensure low levels of dust. The microscopy room must be large enough to allow two people to work simultaneously and must be adequately ventilated. Considerable amounts of heat can be produced, especially when microscopes with high-pressure mercury lamps are used. A network connection for laboratory computers simplifies working procedures and documentation.