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Research paper

Detection of anti-dsDNA antibodies by computer-aided automated immunofluorescence analysis

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ABSTRACT

Introduction: NOVA View is a computer aided fluorescence microscope that is used for the automated reading and interpretation of indirect immunofluorescent tests in diagnostic immunology. The objective of the present study was to evaluate the performance of the NOVA View® system for the measurement of anti-dsDNA antibodies using the *Crithidia luciliae* indirect immunofluorescence test (CLIFT) technology.
Methods: Analytical performance of NOVA View CLIFT was assessed in repeatability (within run) and reproducibility (between runs and instruments) studies. Two hundred-fifty patient samples (N = 200 consecutive samples and N = 50 samples from systemic lupus erythematosus patients) were tested to evaluate the agreement between results generated with NOVA View CLIFT, and those obtained with manual microscopic reading of the same slides. Positivity rate in SLE was assessed on the 50 SLE samples.
Results: The NOVA View system showed high level of repeatability and reproducibility within runs, between runs, and between instruments. Agreement of NOVA View software interpretation and digital image reading results with manual microscopic reading results was 96.0%, and the same positivity rate was obtained on SLE samples by NOVA View digital image reading as that of manual microscopic reading (36.0% vs. 38.0%, respectively).
Conclusion: Results generated by NOVA View CLIFT were equivalent to those obtained by manual microscopic reading on a large routine sample set. NOVA View demonstrated consistency within and between runs, and between instruments. Automation of CLIFT provides reliability and is a suitable alternative for routine clinical laboratories.

1. Introduction

A variety of methods has been developed over the years to measure antibodies to double stranded (ds)DNA, a key diagnostic marker of systemic lupus erythematosus (SLE). These methods include the Farr assay (Mahler and Fritzler, 2007), ELISA (Mahler and Fritzler, 2007; Tan et al., 1982; Aarden et al., 1975a; Fish and Ziff, 1981; Pincus et al., 1969), other solid phase immunoassays (Infantino et al., 2015; Venner et al., 2013; Hillebrand et al., 2013; Lemarie et al., 2011), and the *Crithidia luciliae* indirect immunofluorescence test (CLIFT) (Somerfield et al., 1981; Sontheimer and Gilliam, 1978; Stingl et al., 1976). As current solid phase immunoassays have variable performance due to lack of standardization (Chiaro et al., 2011), CLIFT is often regarded as a reference method, because of its high clinical specificity (Haugbro et al., 2004). CLIFT uses the hemoflagellate, *C. luciliae*, as the substrate. This protozoon, a single-cell organism, possesses a large modified mitochondrion, called kinetoplast, containing a network of circular dsDNA (Aarden et al., 1975a). This network of dsDNA appears to be free of histones or other mammalian nuclear antigens (Aarden et al., 1975b; Crowe and Kushner, 1977). Therefore, reactivity against the kinetoplast is specific for anti-dsDNA antibodies (Fig. 1).

The indirect immunofluorescence (IIF) procedure, however, is time consuming and manual labor intensive, and the microscopic interpretation has high intra- and inter-laboratory variability (Copple et al., 2012, 2014; Van et al., 2009; Sack et al., 2009). There is a strong need for automation of not only CLIFT, but also other IIF assays, to increase efficiency and to improve the consistency of the results. Recently, computer-aided automated systems have become available for the interpretation of IIF assays, such as NOVA View (Inova Diagnostics, Inc.), Aklides (Medipan GmbH), EUROPattern (Euroimmun AG), Zenit G-Sight (Menarini Diagnostics), Helios (Aesku) and Image Navigator (ImmunoConcepts), and

Abbreviations: CAP, College of American Pathologists; CLIA, clinical laboratory improvement amendments; CLIFT, *Crithidia luciliae* indirect immunofluorescence test; IIF, indirect immunofluorescence; SLE, systemic lupus erythematosus; FITC, fluorescein 5-isothiocyanate; DAPI, 4′, 6-diamidino-2-phenylindole, dihyrochloride.

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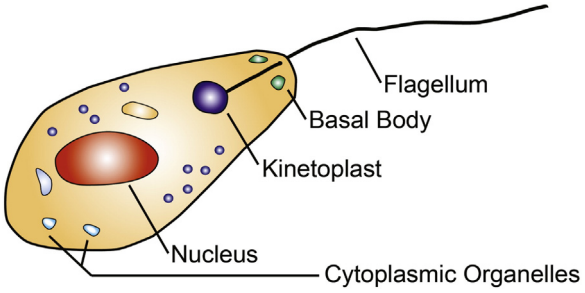

Fig. 1. The structure of the *Crithidia luciliae* organism.

are making their way to the clinical laboratory (Copple et al., 2014; Bossuyt et al., 2013; Bizzaro et al., 2014; Knutter et al., 2012; Mahler et al., 2014). These systems not only produce results that show good agreement with manual reading, but they are also able to leverage the technology to add additional value to the traditional IIF assay results. As an example, one study has found that the system-generated light intensity measurements taken during antinuclear antibodies (ANA) test correlated with likelihood for disease, and has raised the possibility of using this information for patient stratification (Schouwers et al., 2014). Automated applications are now available on multiple devices for detecting ANA and anti-neutrophil cytoplasmic antibodies (ANCA), but publications on the CLIFT assay are scarce (Buzzulini et al., 2014; Melegari et al., 2012; Soda et al., 2011; Gerlach et al., 2015). Therefore our goal was to evaluate the performance of the NOVA View system for the detection of anti-dsDNA antibodies with the CLIFT method.

NOVA View is a computer controlled automated fluorescence microscope, utilizing a light-emitting diode (LED) light source, a dual band DAPI FITC/HC filter, and a charge coupled device (CCD) camera to acquire digital images of predetermined areas of IIF slides. The system uses a dedicated CLIFT reagent kit, containing a DNA-binding dye, 4′,6-diamidino-2-phenylindole (DAPI), for counterstaining cell nuclei and other DNA-containing organelles. Slides are loaded to the instrument in carriers that can accommodate up to five slides. Images are taken using a 40× microscope objective. Kinetoplasts and nuclei are recognized in the DAPI channel by the use of an adaptive threshold algorithm, and are differentiated from each other based on size distribution (Fig. 2). To ensure accurate cell recognition, overlapping regions and artifacts are excluded based on predefined criteria, like cell size, shape and relation to neighboring objects. A brightness value (measured as Light Intensity Units, LIU) is determined for each kinetoplast in the FITC fluorescence channel, and the median value is used for sample classification. An adaptive capturing strategy (adjusting focus and exposure time between wells) ensures rapid imaging of the wells. A minimum of three digital images of three different fields of view are generated. The software categorizes results as positive or negative based on a pre-determined cut-off LIU value, and presents the digital images for operator confirmation. All digital images are archived for future review (Fig. 3).

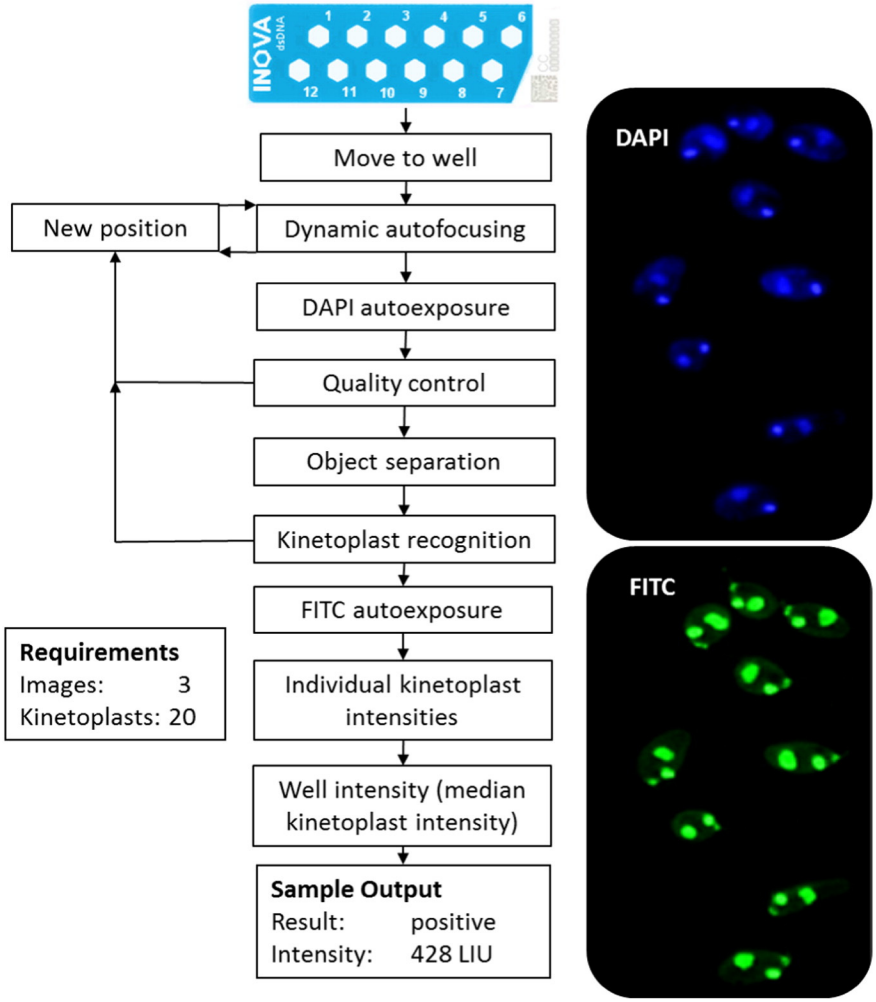

Fig. 2. Schematic representation of the NOVA View software algorithm for the imaging and interpretation of the *Crithidia luciliae* immunofluorescent test.

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