

## Indirect Immunofluorescence: HIV-1&2 Dual Infections

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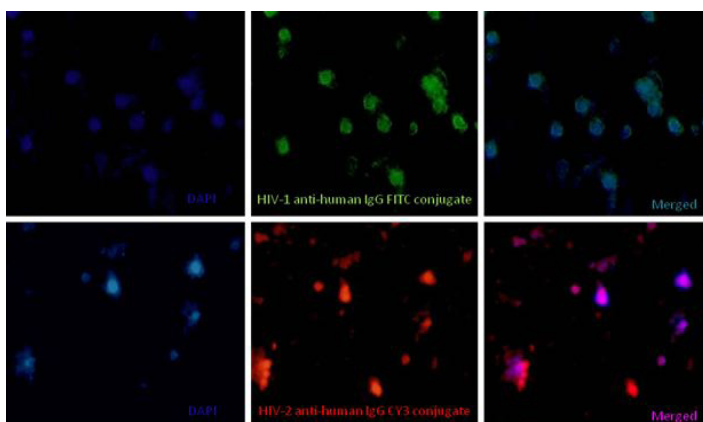
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### Abstract

HIV-1 is extensively studied; however scarce data exists on HIV-2 and HIV-D (HIV-1 and HIV-2 dual infections) from the endemic regions [1]. In this context, while studying HIV-D we established an improved indirect immunofluorescence Assay (IFA) for virus identification, that can be used both in experimental and diagnostic virology [2].

Indirect Immunofluorescence is tricky when simultaneous detection of two viruses is attempted [3]. The challenge is to reduce non-specific interactions without impairing antibody-epitope binding with improved techniques to reduce background and other problems in visualizing the antigen of interest [4]. We optimized an IFA for HIV-1 and HIV-2 detection in dual infection, where independent unlabeled primary antibodies for HIV-1 and HIV-2 were detected with a fluorophore-labelled secondary antibody to confirm dual infection; simultaneously yet independently for HIV-1 and HIV-2 on the dually infected PBMCs [2].

Previous studies have used IFA for studying HIV-1 and HIV-2 antibodies and their differences [4]. To our knowledge this is the first report of an Indirect Immunofluorescence assay performed for assessing and confirming dual HIV-1 and HIV-2 infection during the virus isolation [2]. However, simultaneous detection the two viruses in HIV-D, obviating independent processing for the two viruses remains a goal to achieve.



**Figure 1:** Indirect Immunofluorescence images of HIV-1 and HIV-2 infected patient's PBMC's. Nucleus of the PBMC's is Stained with DAPI [2-(4-amidinophenyl)-1H-indole-6-carboxamide] (Blue); HIV-1 infected PBMC's Stained with HIV-1 Positive Serum Followed by FITC Conju-

gated to Anti-Human IgG (Green); HIV-2 Infected PBMC's Stained Using HIV-2 Positive Serum Followed by CY3 Conjugated to Anti-Human IgG (Red). The images were taken at 100 X using Olympus IX51 Microscope with pE excitation system and were merged using Cell F software.

**Keywords:** Antibody; HIV-1; HIV-2; HIV-1 and HIV-2 Dual Infections (HIV-D) Indirect Immunofluorescence Assay (IFA); Virus Isolation

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