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# Indirect Fluorescent Antibody (IFA) Assay

## Application

IFA is an assay which uses fluorescent microscopy to detect antibodies to specific antigenic material. This test is often used to confirm positive results obtained by ELISA (Enzyme Linked Immunosorbent Assay) or MFIA® (Multiplexed Fluorometric ImmunoAssay®). It is typically used as a confirmation test as the location of antibody-antigen reactions can be visualized within an infected cell.

## Assay

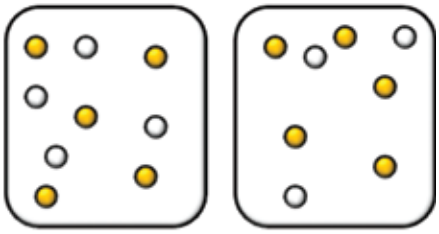
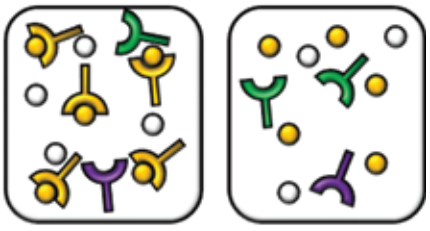
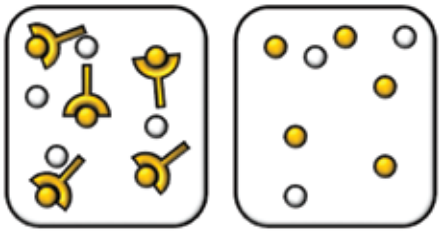


**Figure 1:** Infected cells ( ) and uninfected cells ( ) are fixed to wells on a glass microscope slide; the fixative is typically cold acetone. This fixative permeabilizes the cell membrane, making intracellular infectious agent antigens more accessible to antibodies.



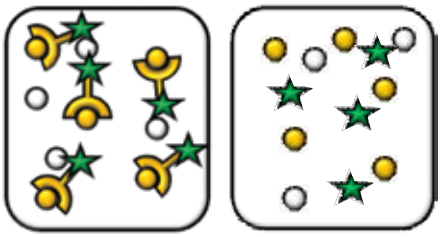
**Positive Sample** **Negative Sample**

**Figure 2:** Diluted serum samples are added to individual slide wells and incubated. Antibodies specific to the antigen presented by the infected cells are then bound ( ). The other antibodies ( and ) remain free in the serum.



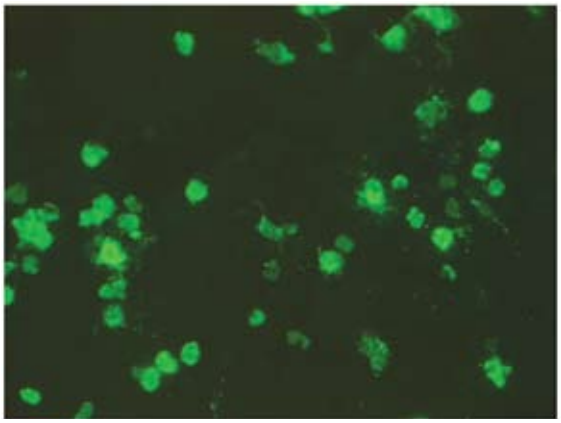
**Positive Sample** **Negative Sample**

**Figure 3:** Unbound antibodies are removed by washing.



**Positive Sample** **Negative Sample**

**Figure 4:** A fluorescent dye-conjugated anti-immunoglobulin ( ) is used to reveal the binding of primary antibodies to the virus-infected cells. After the unbound conjugate is removed via washing *(not shown)*, the slides are covered with buffered glycerol and examined with a fluorescence microscope. Bright, granular fluorescence is typical of a specific antibody viral antigen reaction.



**Positive Sample**

**Negative Sample**

**Figure 5:** An example of positive and negative IFA; Mouse Parvovirus. Positive assay shows bright granular fluorescence in the nucleus of the cell, indicating viral infection in the correct location for mouse parvovirus. Negative assay shows dark cells, indicating that no mouse parvovirus antibodies are present in the serum.

## Advantages

Inexpensive to perform. The morphology and location of fluorescence can be evaluated to differentiate specific from non-specific reactions.

## Disadvantages

Fluorescent microscope is required. Due to efficiency limitations, IFA is not a primary serologic screening tool. Interpretation is subjective. Results are not quantitative. Limited to one antigen per slide. Nonspecific fluorescence is common and intensity of fluorescence is variable. As with all serologic tests, IFA does not detect the infectious organism; only provides a historical indication of infection (antibodies). As they do not produce antibodies, IFA is not suitable for use with immunodeficient animals.