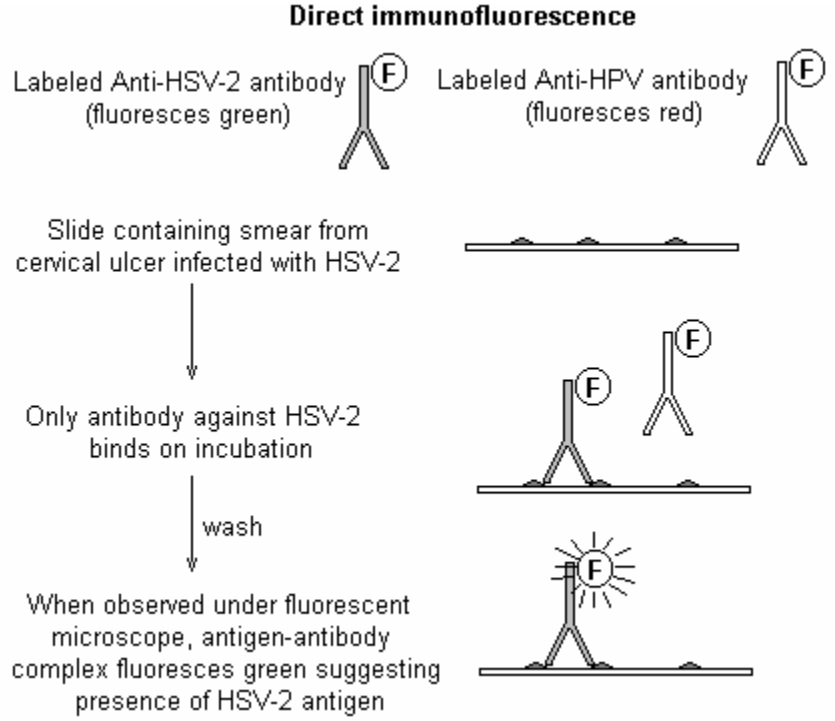
IMMUNOFLUORESCENCE

Immunofluorescence is an antigen-antibody reaction where the antibodies are tagged (labelled) with a fluorescent dye and the antigen-antibody complex is visualized using ultra-violet (fluorescent) microscope. Fluorochromes are dyes that absorb ultra-violet rays and emit visible light. This process is called fluorescence. Commonly used fluorochromes are Acridine Orange, Rhodamine, Lissamine and Calcofluor white. However, these fluorochromes are used for general fluorescence. When fluorescein (FITC) is excited by a blue (wavelength 488nm) light, it will emit a green (520nm) colour. Phycoerythrin (PE) emits an orange (570nm) colour. The fluorochromes commonly used in immunofluorescence are fluorescein isothiocyanate (green) and and tetramethyl rhodamine isothiocyanate (red).

Types of immunofluorescence:

- Direct immunofluorescence
- Indirect immunofluorescence
- Microimmunofluorescence

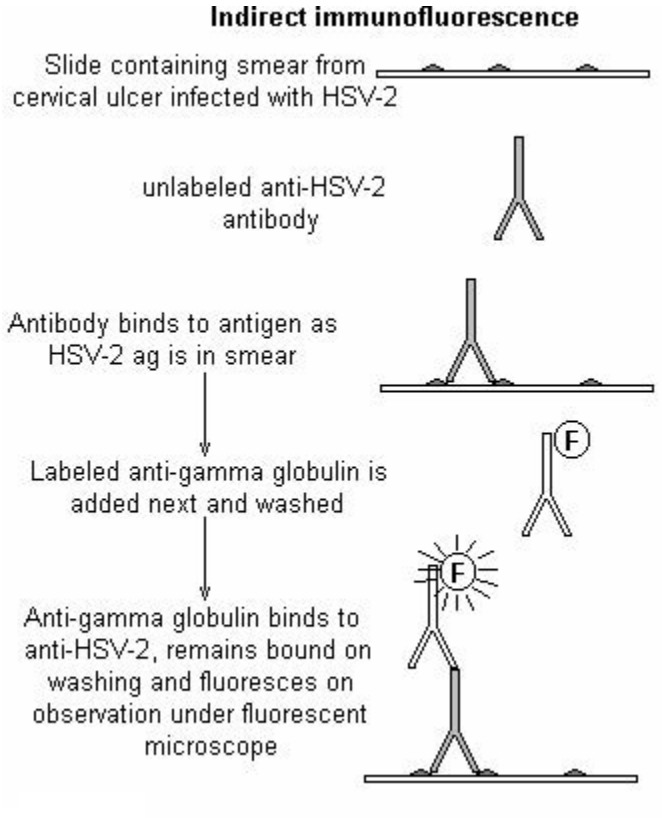
Direct immunofluorescence:
This technique is used to detect antigen in clinical specimens using specific fluorochrome labeled antibody. The steps involved are: Fixation of smear on the slide, treating with labeled antibody, incubation, washing to remove unbound excess labeled antibody and visualization under fluorescent microscope. When viewed under fluorescent microscope, the field is dark and areas with bound antibody fluoresce green.



This technique can be used to detect viral, parasitic, tumor antigens from patient specimens or monolayer of cells. Another application is identification of anatomic distribution of an antigen within a tissue or within compartments of a cell.

Indirect immunofluorescence:

Indirect immunofluorescence is employed to detect antibodies in patient serum. The antigen on smear are made to react with specific unlabeled antibody (raised in mouse) and washed. The unbound antibody gets washed off. The presence of specific mouse antibody bound to the antigen on smear is detected by adding another antibody. The second antibody is labeled anti-gamma globulin (rabbit antibody against mouse antibody) antibodies. This antibody binds to Fc portion of first antibody and persists despite washing. The presence of the second antibody is detecting by observing under fluorescent microscope. It is often used to detect autoantibodies. Commonly used in the detection of anti-nuclear antibodies (ANA) found in the serum of patients with SLE.



Microimmunofluorescence:
This is a serological technique employed to detect antibodies in patient serum. It works on the same principle as that of indirect immunofluorescence but is performed on Teflon slides with many wells dotted with antigens. This technique is used in the serodiagnosis of Q fever, Mediterranean spotted fever, Detection of IgG, IgA and IgM Antibodies to Chlamydia, toxoplasmosis, epidemic typhus etc.