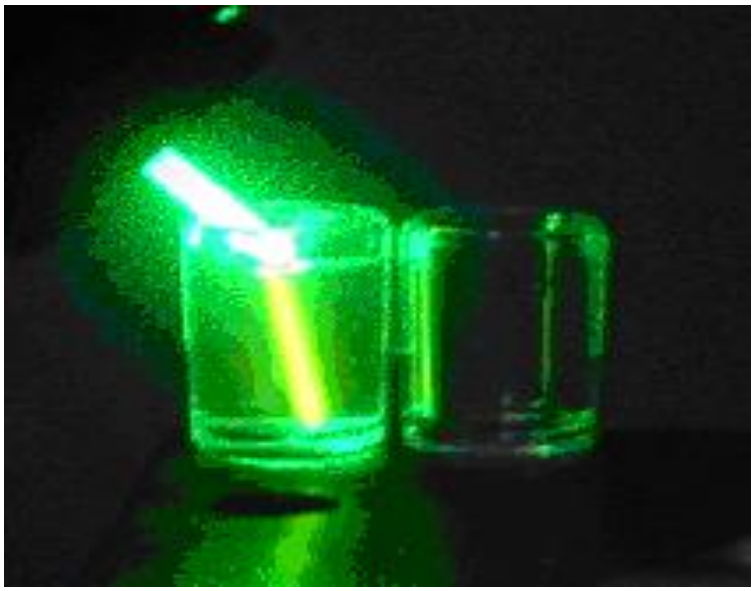
Fluorescent Immunoassay

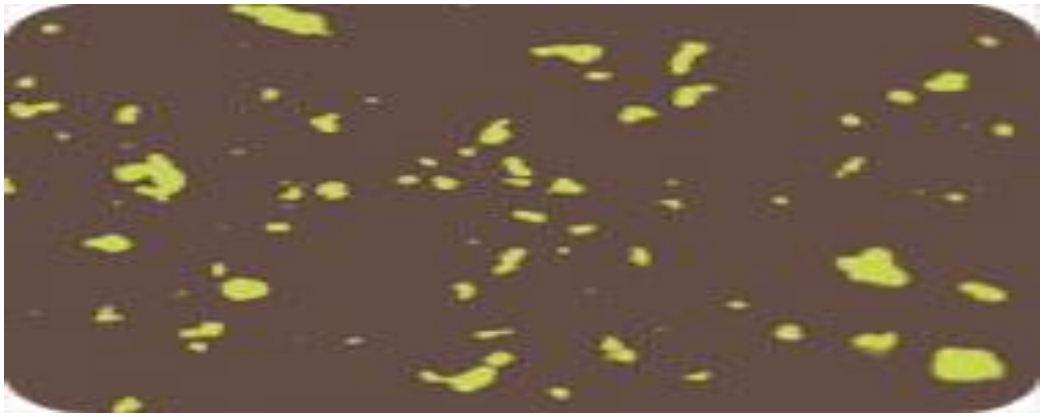


Fluorescent Immunoassay Markers

- Fluorophores or fluorochromes
- Ability to absorb energy and emit light
- Two most commonly used:
  - Fluorescein – green
  - Tetramethylrhodamine – red
- Tests may be qualitative or quantitative
- Complex must form for fluorescence to occur.

Fluorescent Immunoassay

- Antibodies and bacteria are fixed on a glass-plate.
- non-bounded antibodies are washed out, antibody-bacteria-complexes ("sandwiches") remain.
- The "sandwich" becomes visible by adding fluorescent anti bovine immunoglobulin which can be seen as green light in the fluorescence microscope.



- **Fluorescent Immunoassay**
  1- Direct immunofluorescence

  – Tagged antibody added to unknown antigen fixed to slide

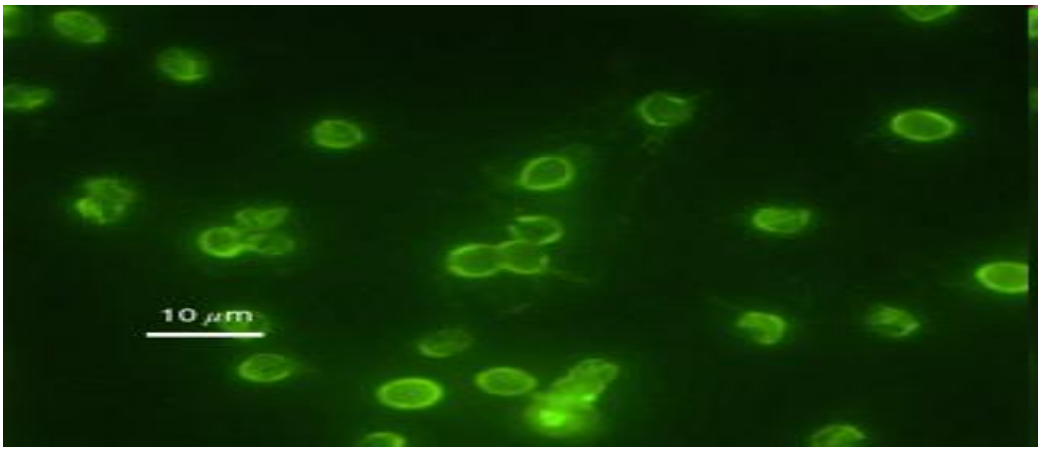
  – If patient antigen present = fluorescence

  2- Indirect immunofluorescence – sandwich assay
  - Patient plus known fixed antigen
  - Allow to react and wash off unbound reactants
  - Add tagged anti-antibody

Fluorescence

Positive Immunofluorescence

- *Cryptosporidium parvum* oocysts



Advantage/disadvantage

Advantage:

Detect more than one target (more popular dual stain)

Sensitive

Disadvantage:

Need fluorescent microscope (UV Lamp- worming up , expiration date)

Sample on slide- single washing- less specific

Results-subjective should be read immediately-fading especially when exposed to light

Applications

1. Detection of Autoimmune diseases.

2. Detection of infectious diseases- *Toxoplasma, Leishmania*

3. Phenotyping of lymphocytes