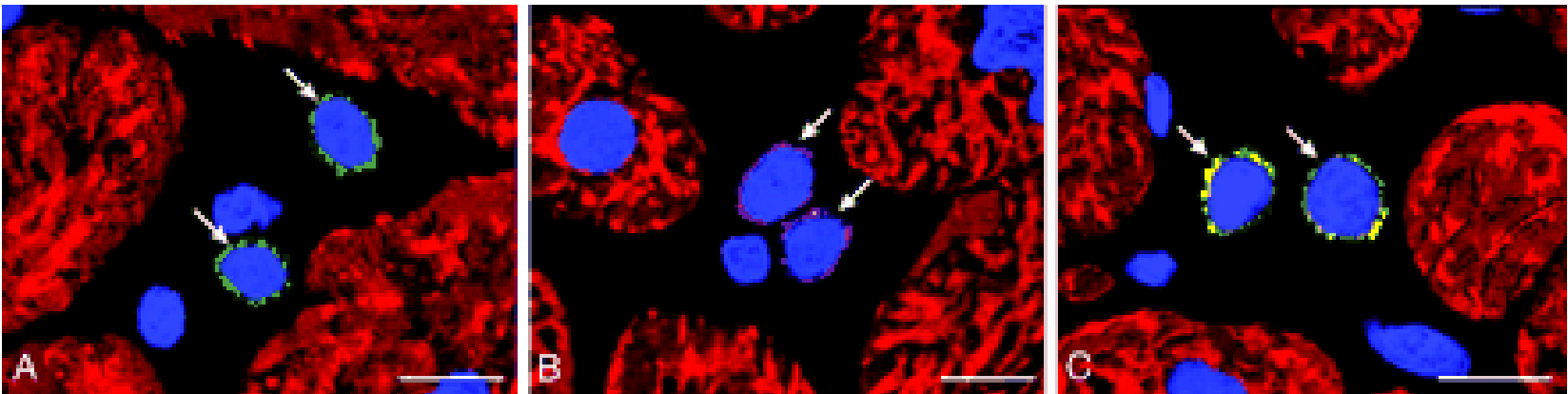


# Experimental Application of Purine and Pyrimidine Metabolism

- Monoclonal antibody technology
- Cell division assays



# Monoclonal antibody production

**Monoclonal antibody:**

-result of one B cell clone

-one antibody to one antigen

**Polyclonal antibody:**

-results from more than one B cell clone

-many antibodies to many antigens

- To use antibodies for a diagnostic test
  - e.g. to detect HIV, pregnancy
  - can detect a specific HIV protein/ pregnancy hormone (eg. hCG)
- You require a source of antibodies made against the HIV/hCG protein.

# B cell immune response

- You have an array of B-cells with capability to produce antibodies against a huge array of foreign proteins (eg HIV, malaria).
- When encountering an antigen the B-cell
  - a) secretes the antibody recognising the antigen AND
  - b) B-cell divides so that many more B-cells can secrete the antibody.

# Mouse monoclonal antibody production

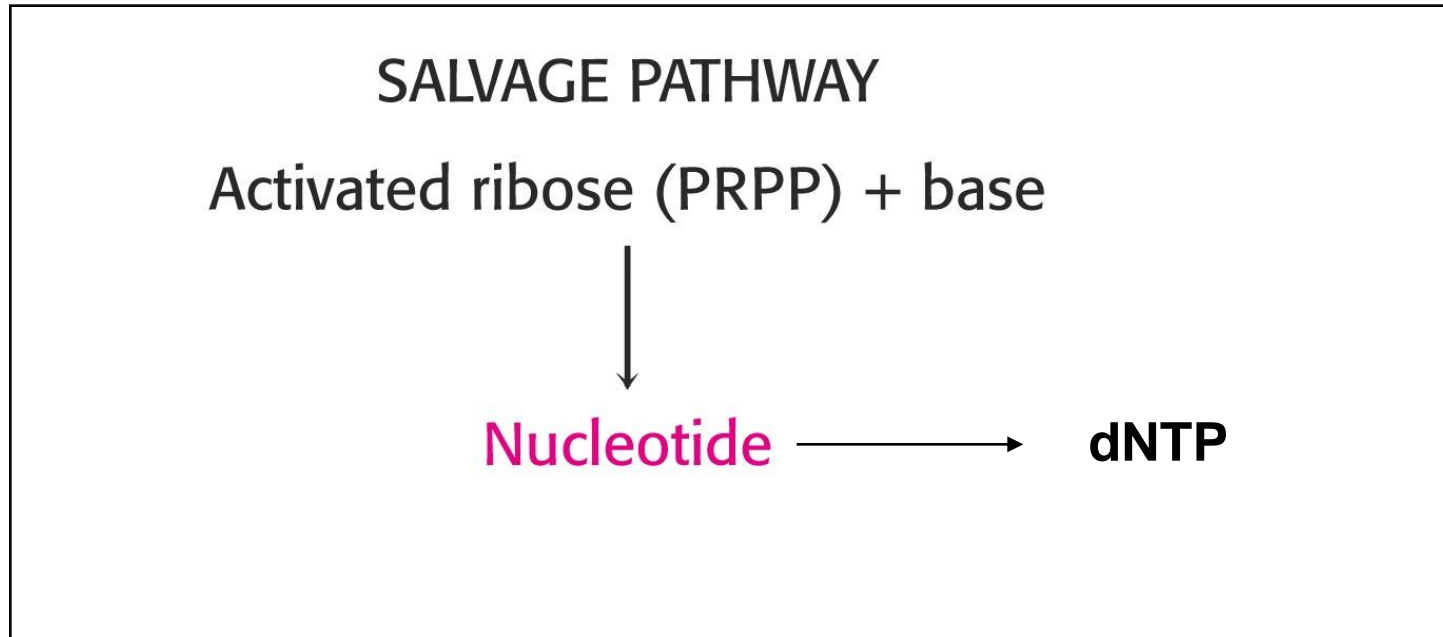
- Inject the antigen (HIV protein 3 times - over 6 week period)
- Bleed
  - red blood cells                      white blood cells
  - serum proteins                      antibodies
  - Anti-HIV antibody
- To obtain enough antibodies for a HIV diagnostic test
  - need to inject many many many many animals
  - take their blood
  - purify the antibodies from the blood
  - then purify the specific antibodies required from all the other antibodies

# OR

- Obtain the B-cell that makes the anti-HIV antibody and grow this B-cell (remember : B-cells secrete antibodies)
- Hibridoma's
  - B-cells die in culture after a few days
  - Myeloma cells (cancer cells that grow well in culture)
  - B-cell/myeloma fused cells
    - grow well in culture (characteristics of the myeloma cell) AND
    - secrete antibodies (characteristic of the B-cell)
  - It is necessary to STOP the myeloma cells growing so that ONLY the fused cells grow

# HAT

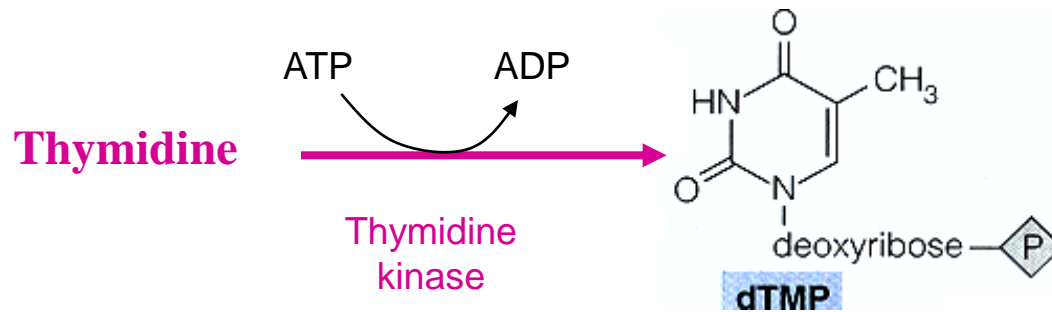
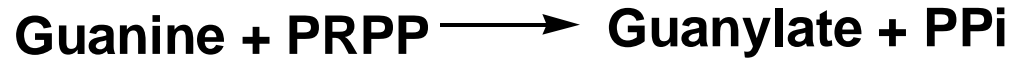
(Hypoxanthine + Aminopterin + Thymidine)



- Hypoxanthine: substrate for the salvage pathway
- Thymidine: substrate for the salvage pathway
- Aminopterin: inhibits denovo dTMP synthesis (via inhibition of dihydrofolate reductase)

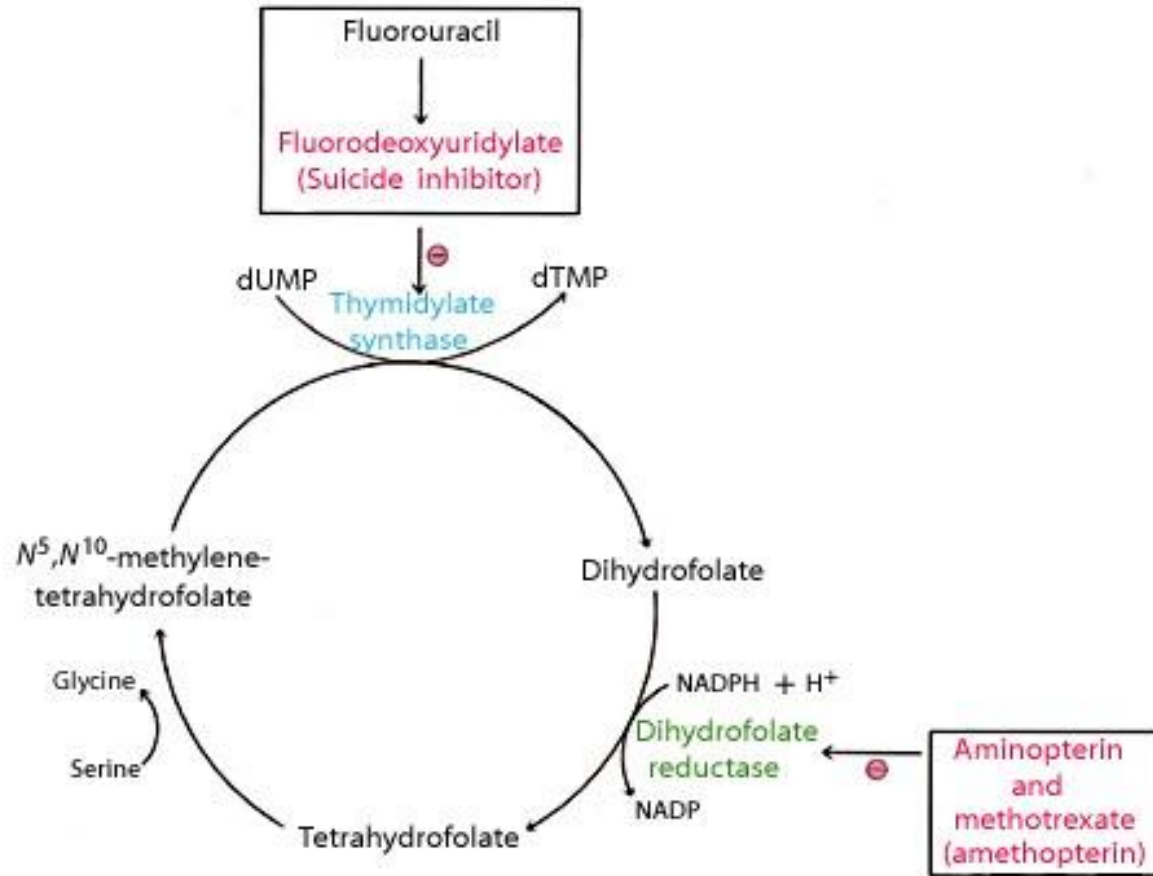
## Salvage pathway

hypoxanthine-guanine  
phosphoribosyl transferase (HGPRT)

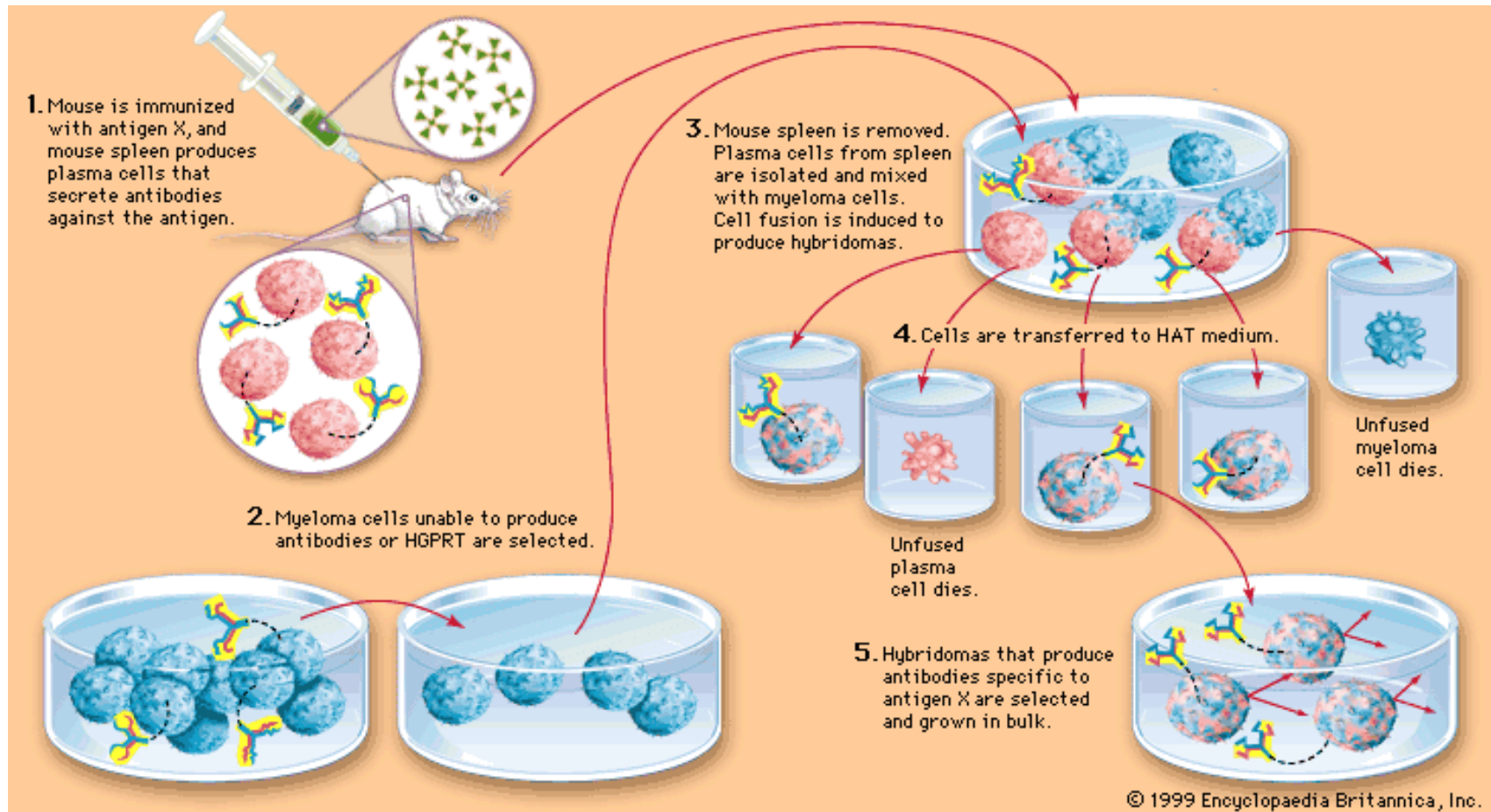




## Aminopterin



- B-cells
  - CAN grow in HAT as they have salvage pathway enzymes ... but die after a few days in culture
- Myeloma cells
  - B cell cancer (specifically, plasma cell cancer)
  - CANNOT grow in HAT as they do not have the salvage pathway enzymes (select cells that are missing hypoxanthine guanine phosphoribosyl transferase: HGPRT) & de novo dTMP is inhibited
- Fused cells
  - Grow well in culture (myeloma cells)
  - Can grown in HAT



- Hybridoma cells

- Myeloma cells fuse with B cells
- Grow and secrete antibody.
- It is now necessary to select for the B/M fused cell that secretes the antibody of interest e.g. anti-HIV protein antibody.

# Enzyme Linked Immunosorbent Assay

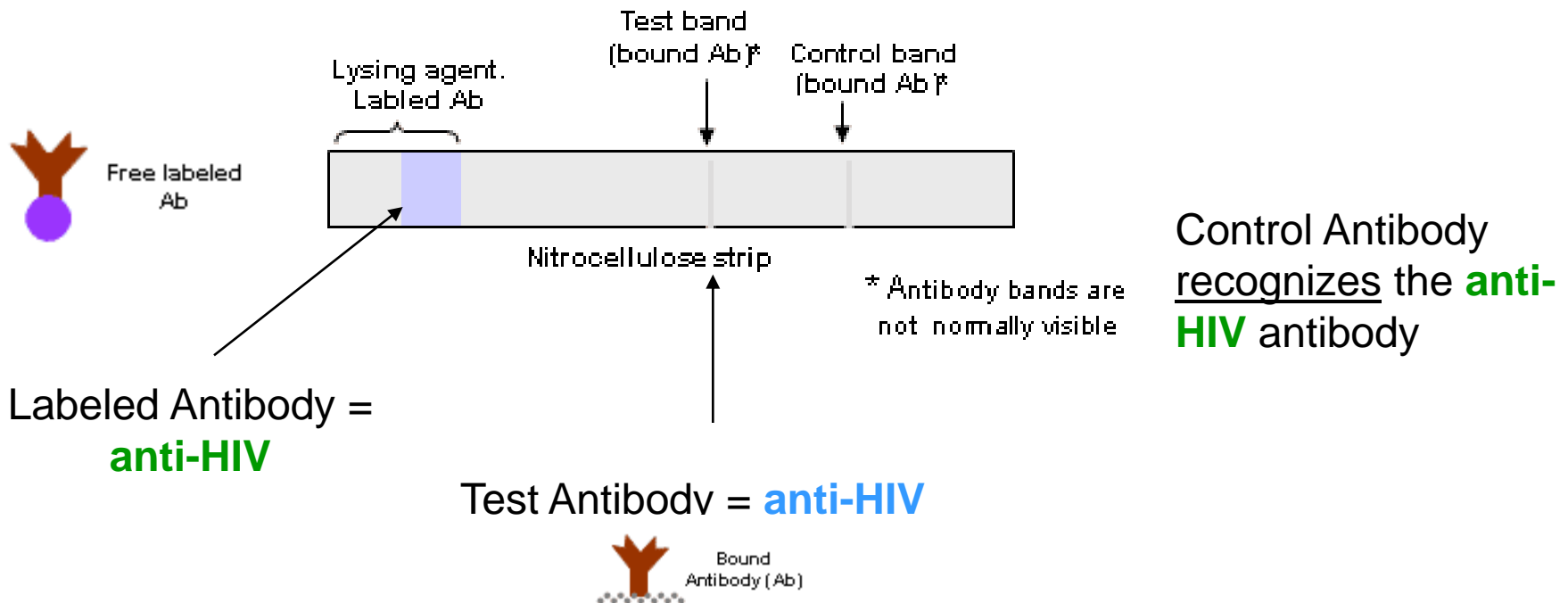
- ELISA consists of
  - partially purified HIV antigen is bound to plastic
  - hybridoma-secreted antibody is used as the probe
  - enzyme-linked anti-mouse antibody is then used to probe for the presence of bound antibody
  - the enzyme-linked antibody remains bound in a well only if the hybridoma has produced antibodies that bind to HIV proteins
    - Catalyses a reaction to produce eg a colored product
    - The intensity of the color produced gives indication of the concentration of the antibodies being assayed
    - If B/M fused cell secretes anti-HIV antibody, it will cause a colour change in the ELISA

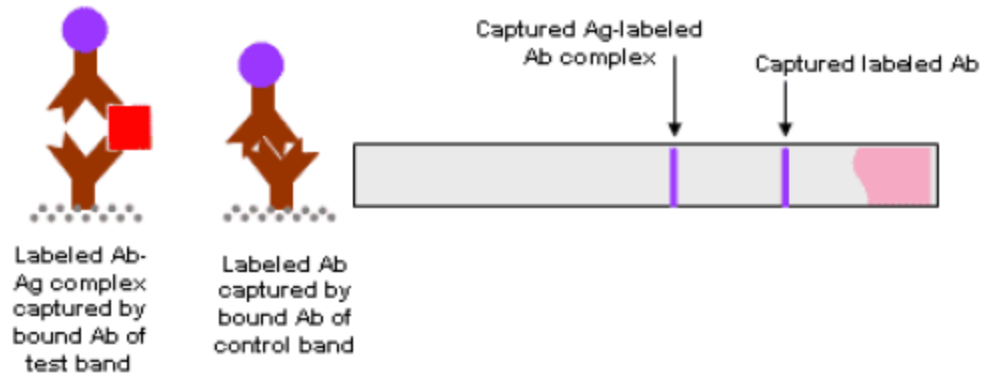
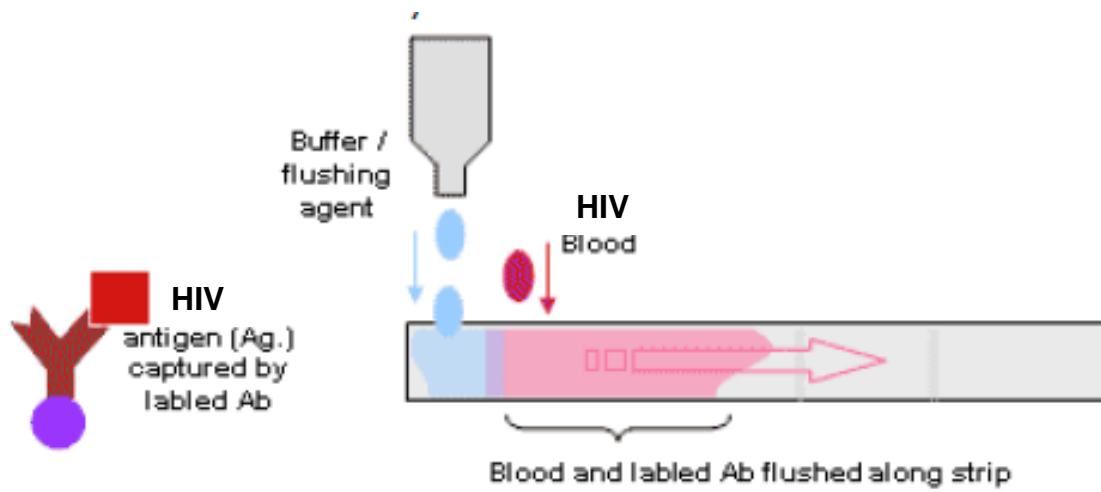
# Can now use anti-HIV antibody to make a rapid diagnostic test

Add blood

If blood contains **HIV**, it will bind to the **anti-HIV** on the plate

Test **anti-HIV** will recognize the complex of **HIV** antigen bound to the labelled **anti-HIV** antibody





# Cell division assays

## *Cell proliferation assays*

- Cells divide in response to stimuli
- Cell proliferation can be measured

# Methods

1. Counting the cells under the microscope  
– Hemocytometer (tissue culture)
2. Counting the cells with flow cytometry
3. Using a radioactive precursor  
(metabolite) to follow changes and  
measure DNA



# Measuring DNA

- Cells placed with stimulant and **tritiated thymidine** added
- The cells divide
- [DNA] increases
  - Tritiated thymidine in DNA increases
  - Cells are lysed and cell contents passed through a filter which traps the DNA.
  - Measure how much tritium is present. The more tritium and the more cells present.
  - For RNA - use tritiated uracil

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