Immunology – II
BC402

Immunological Methods and Applications

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Suggested Reading:

Immunology by Barbara Anne Osborne and Janis Kuby

Terminologies

- *In vivo*
  - Involve whole animal
- *In vitro*
  - Defined populations of immune cells are studied under controlled lab conditions

- Study of immune system requires suitable animal models
  - For vaccine development – is the animal model susceptible to the disease?
  - Mouse most often used
  - Inbred strains reduce variation caused by differences in genetic backgrounds
    - 20 or more generations of brother-sister mating
  - Have to abide by IACUC guidelines
- Adoptive Transfer
  - Immune system of model animal can be eliminated
  - Replaced with immune cells of animal to be studied

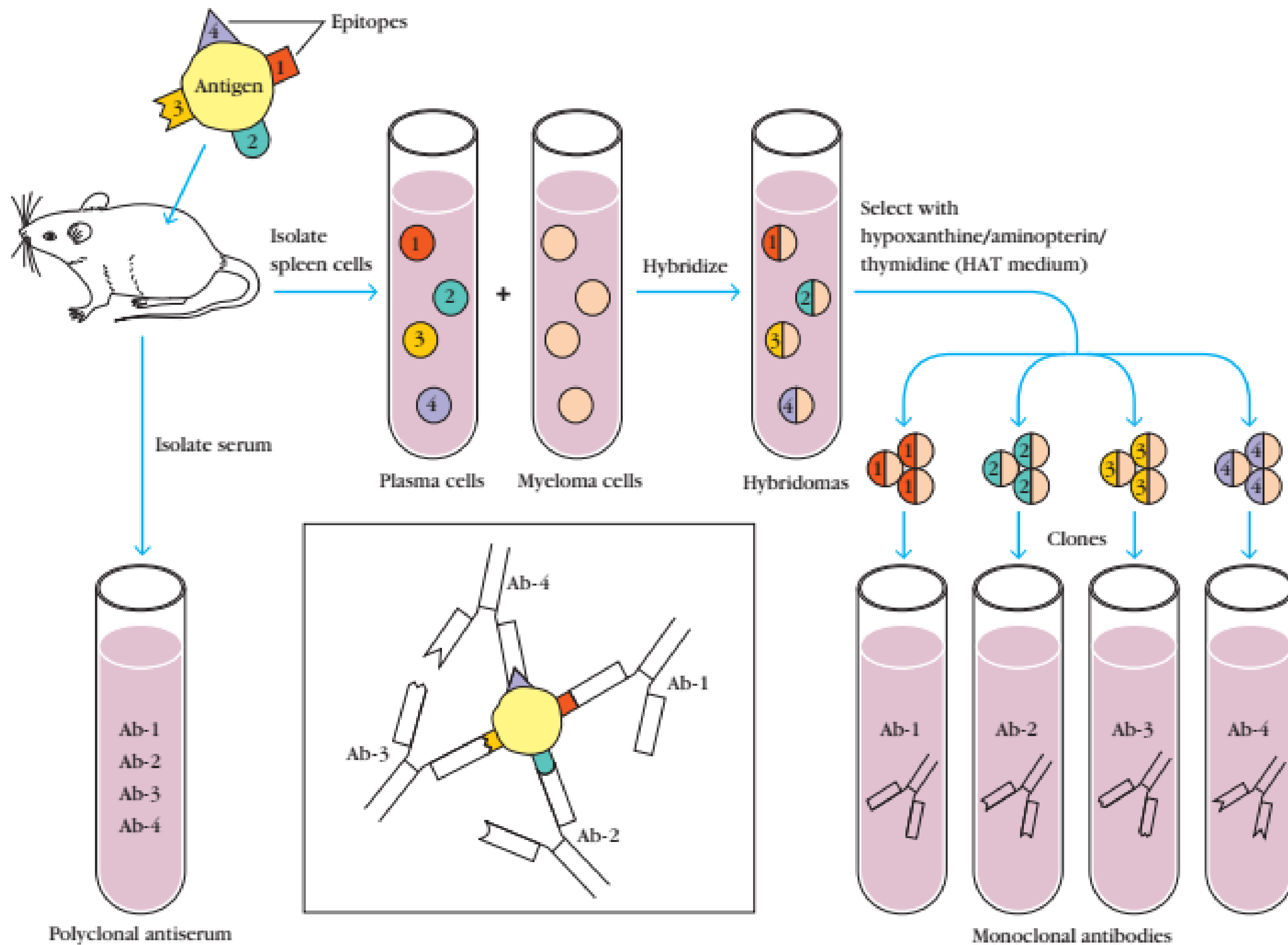
Polyclonal Antibodies Are Secreted by Multiple Clones of Antigen-Specific B Cells

- Use of polyclonal antibodies
  - Immunizing animal (mouse, rabbit) or human with antigen one or more times
  - Taking blood samples, purifying the antibodies from the serum
  - Results in a mixture of antibodies directed towards variety of different epitopes
  - Disadvantages:
    - Ill-defined cross-reactivities with related antigens
    - Range of cross-reactivity to desired antigen might vary from bleed to bleed
    - Animal might die, causing you to start over

A Monoclonal Antibody Is the Product of a Single Stimulated B Cell

- Use of monoclonal antibodies
  - Product of single, stimulated B cell
    - Supply of antibody specific for one epitope
  - Uses:
    - Can be specific for specific targe cells and conjugated to toxins
    - More sensitive/specific ELISAs

- Cell Culture Systems
    - Cells are cultured and studied
    - Specialized media
    - Can be used for:
        - Testing effects of contaminants on immune cells
        - Testing drugs
        - Producing monoclonal antibodies
    - Cell line
        - Cells that have been transformed – propagate indefinitely (cancerous cells)



- Köhler and Milstein: used myeloma cells lacking the enzyme *h*ypoxanthine *g*uanine *p*hosphoribosyl *t*ransferase (HGPRT).
- The mutant tumor cells and tumor-tumor hybrids would be unable to synthesize new DNA by either the salvage or the de novo pathways and would eventually die.
- Hybridomas formed by fusion between B cells and tumor cells, the B-cell parent would provide the HGPRT, and so these hybrids would survive in the selection medium.

The conventional polyclonal antiserum produced in response to a complex antigen contains a mixture of monoclonal antibodies, each specifi c for one of the four epitopes shown on the antigen (inset). In contrast, a monoclonal antibody, which is derived from a single plasma cell, is specific for one epitope on a complex antigen. The outline of the basic method for obtaining a monoclonal antibody is illustrated here.

- Fusion producing hybridoma

A **hybridoma** possesses the immortal growth properties of the myelomacell parent and secreted the unique antibody produced by the B-cell parent.

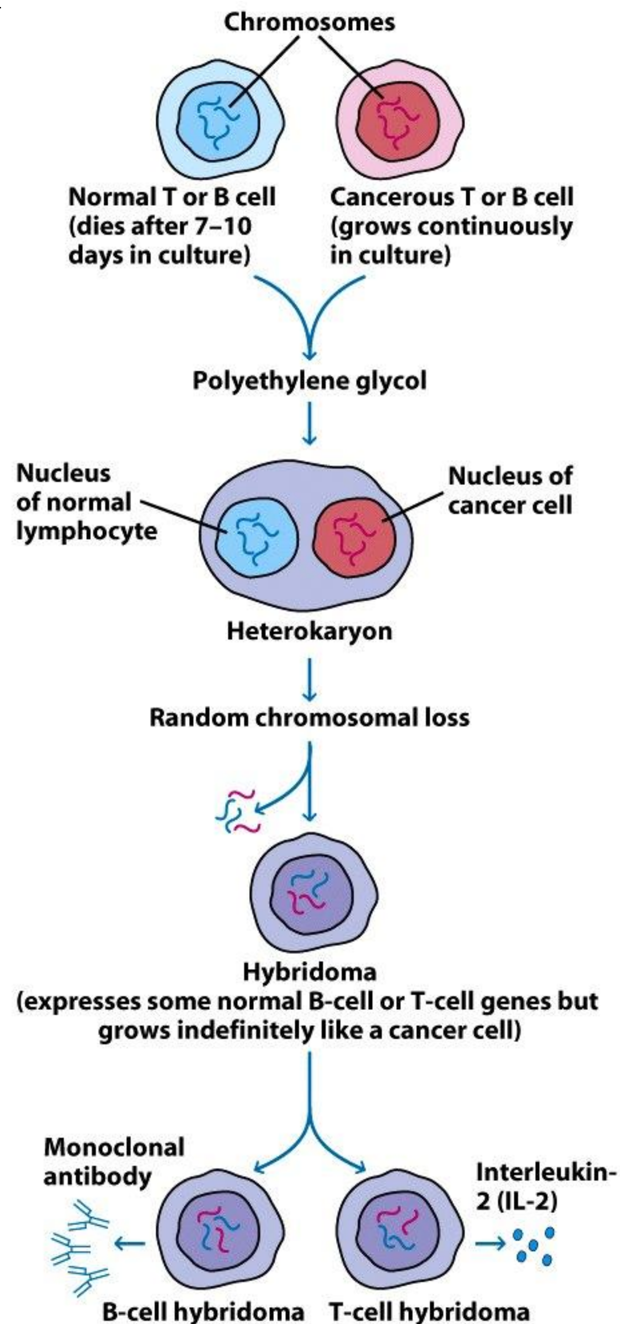


Figure 22-1
Kuby IMMUNOLOGY, Sixth Edition
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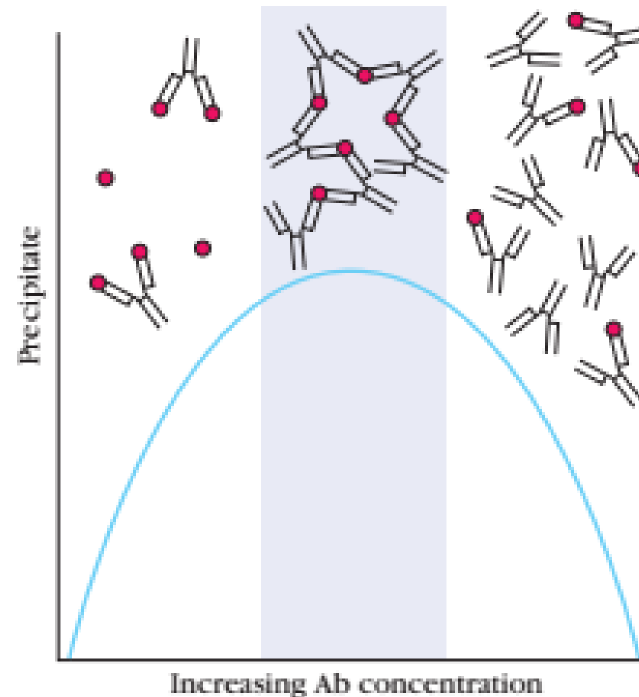
ABZYMES

mAbs can specifically bind and stabilize the transition state of a chemical reaction, thus directly mimicking the activity of enzymes.
Such antibodies with enzyme-like activities are referred to as **abzymes**.

Immunoprecipitation- Based Techniques

Immunoprecipitation Can Be Performed in Solution



- When bi or multivalent antibodies are mixed in solution with antigen, the antibodies can form cross-linkages with two or more antigen molecules, leading to the formation of a cross-linked precipitate (middle panel of graph).
- Precipitate formation requires that neither antigen (left panel in graph) nor antibody (right panel in graph) molecules are in excess.
- In either of these two cases, primarily monovalent binding takes place.

Immunoprecipitation of Soluble Antigens Can Be Performed in Gel Matrices

FIGURE 20-3 Immunodiffusion in agar gels can be used to assay for the presence of antibodies and determine cross-reactivity patterns between complex antigens and antibody samples. In this example, viral antigen was placed in the center well, and serum samples from different individuals were introduced into each of the surrounding wells. Note that the serum samples of individuals B and D are negative for antiviral antibodies, in that there is no line of precipitate between the serum sample on the outside and the viral sample in the center; all other serum samples are positive for antiviral antibodies. *[ASM MicrobeLibrary.org © Thomas*

Ouchterlony double immunodiffusion

Whether 2 Ag share common epitopes?

Diffusion patterns

- Fusion of lines at their junction to form an arc
  - Serologic identity / presence of common epitope
- Crossed lines
  - Demonstrates 2 separate reactions
  - Compared antigens shared no common epitopes
- Fusion of 2 lines with spur
  - Partial identity

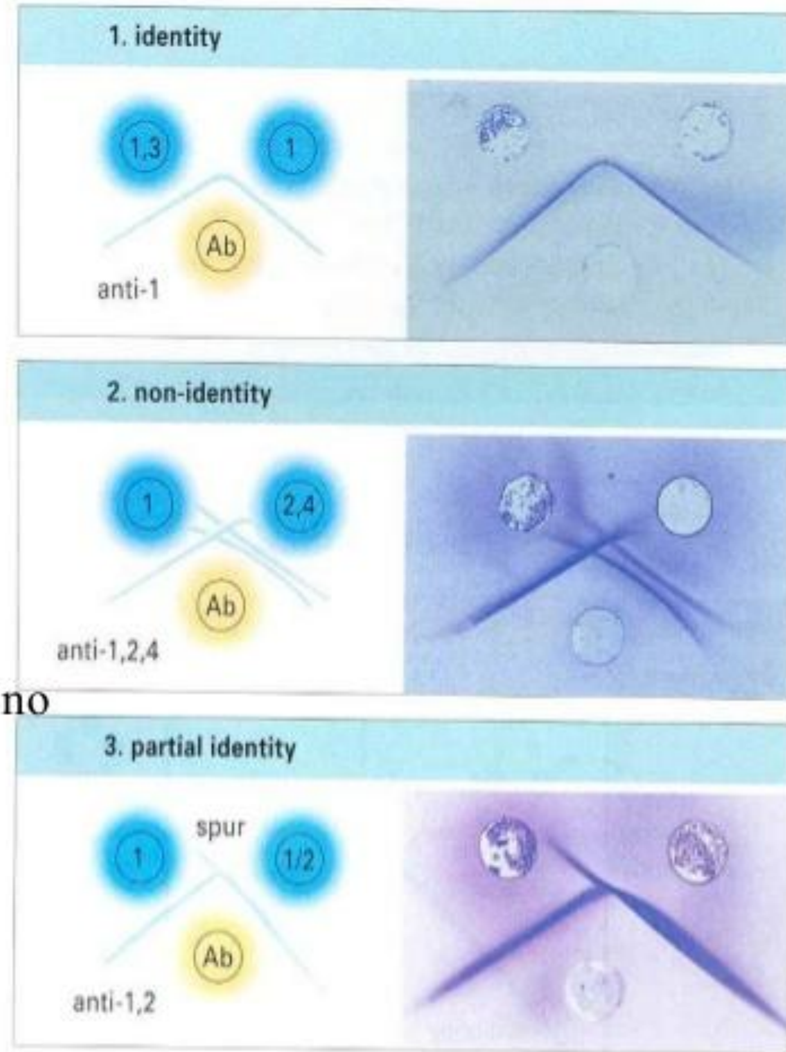


TABLE 20-1 Sensitivity of various immunoassays

Assay	Sensitivity* (µg antibody/ml)
Precipitation reaction in fluids	20–200
Precipitation reaction in gels	
Ouchterlony double immunodiffusion	20–200
Agglutination reactions	
Direct	0.3
Agglutination inhibition	0.006–0.06
Radioimmunoassay (RIA)	0.0006–0.006
Enzyme-linked immunosorbent assay (ELISA)	~0.0001–0.01
ELISA using chemiluminescence	~0.00001–0.01†
Immunofluorescence	1.0
Flow cytometry	0.006–0.06

*The sensitivity depends on the affinity of the antibody used for the assay as well as the epitope density and distribution on the antigen.

† Note that the sensitivity of chemiluminescence-based ELISA assays can be made to match that of RIA.

Source: Updated and adapted from N. R. Rose et al., eds., 1997, *Manual of Clinical Laboratory Immunology,* 5th ed., Washington, DC: American Society for Microbiology.

Agglutination Reactions

Hemagglutination Reactions Can Be Used to Detect Any Antigen Conjugated to the Surface of Red Blood Cells

Hemagglutination Inhibition Reactions Are Used to Detect the Presence of Viruses and of Antiviral Antibodies

Bacterial Agglutination Can Be Used to Detect Antibodies to Bacteria

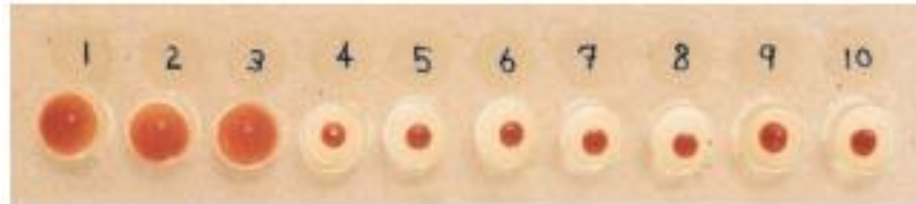
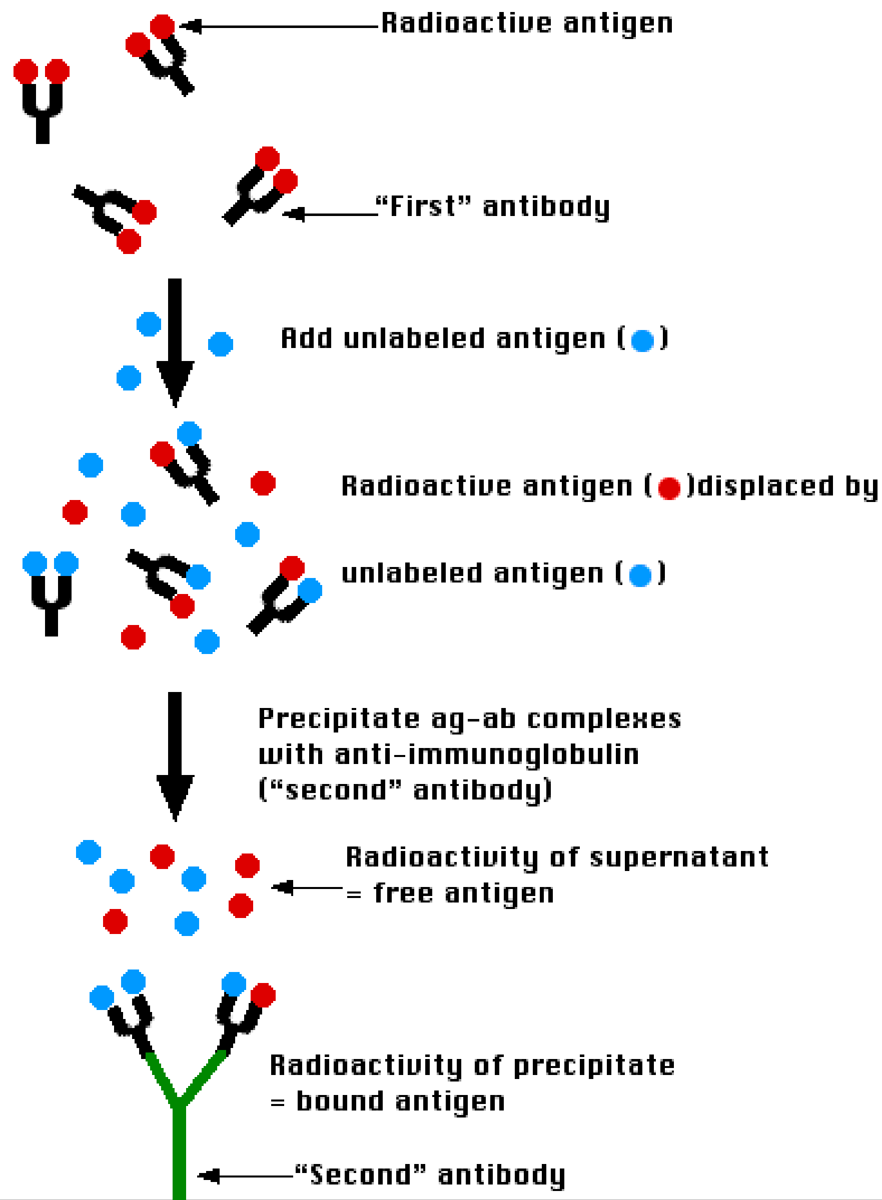


FIGURE 20-4 Demonstration of hemagglutination using antibodies against sheep red blood cells (SRBCs). The control tube (10) contains only SRBCs, which settle into a solid "button." The experimental tubes 1 to 9 contain a constant number of SRBCs plus serial twofold dilutions of anti-SRBC serum. The spread pattern in the experimental series indicates positive hemagglutination through tube 3. *[Louisiana State University Medical Center/MIP. Courtesy of Harriet C. W. Thompson.]*

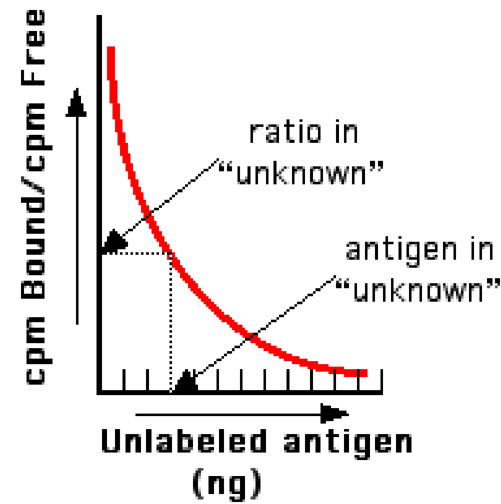
Radioimmunoassays (RIA)

Are Used to Measure the Concentrations of Biologically Relevant Proteins (Antigens) and Hormones in Body Fluids



Gamma Counter

- From these data, a standard binding curve, like the one shown in red, can be drawn.
- The samples to be assayed (the unknowns) are run in parallel.
- After determining the ratio of bound to free antigen in each unknown, the antigen concentrations can be read directly from the standard curve.



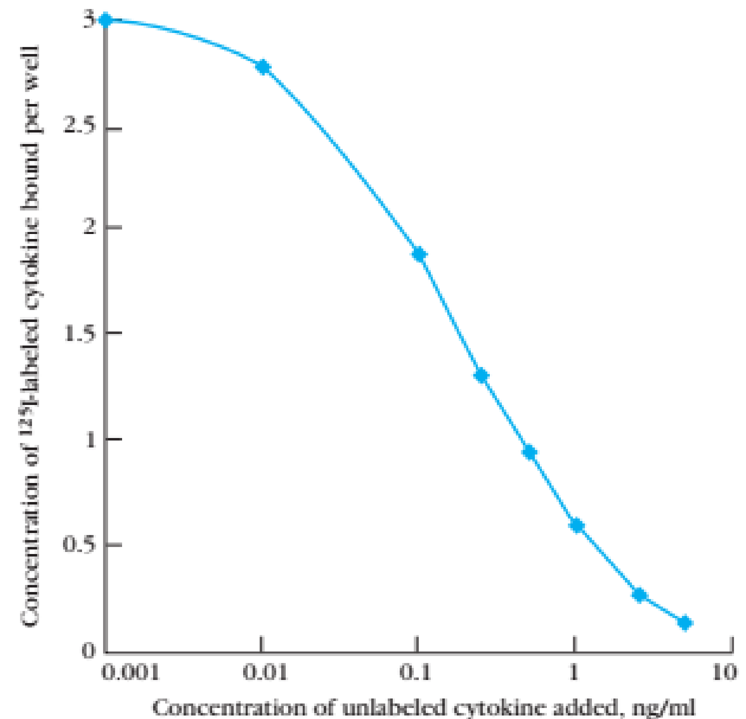
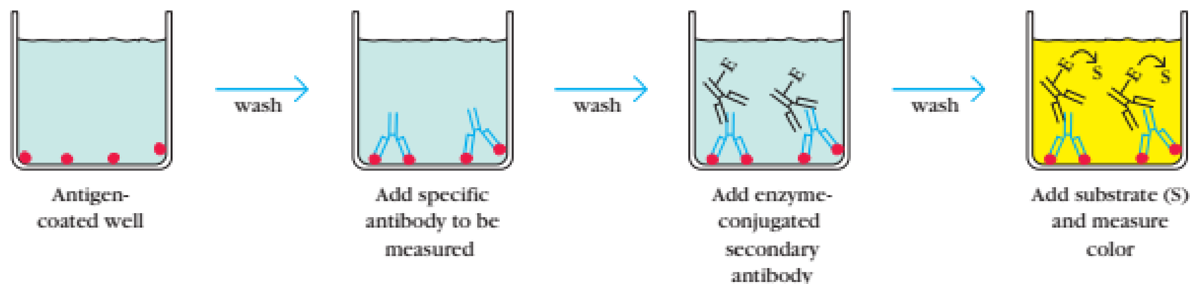


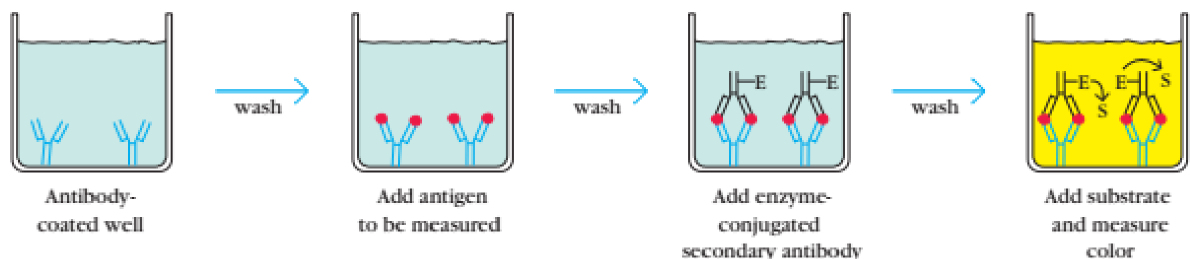
FIGURE 20-6 A competitive, solid-phase radioimmunoassay (RIA) to measure cytokine concentrations in serum. Anti-

- The main drawbacks to radioimmunoassay are the expense and hazards if preparing and handling the radioactive antigen.
- Both ^{125}I or ^{131}I emit gamma radiation that requires special counting equipment;
- The body concentrates iodine atoms — radioactive or not — in the thyroid gland where they are incorporated in thyroxine (T4).

- Despite these drawbacks, RIA has become a major tool in the clinical laboratory where it is used to assay
- plasma levels of:
  - most of our hormones;
  - digitoxin or digoxin in patients receiving these drugs;
  - certain abused drugs
- for the presence of hepatitis B surface antigen (HBsAg) in donated blood;
- anti-DNA antibodies in systemic lupus erythematosus (SLE).

ELISA Assays Use Antibodies or Antigens Covalently Bound to Enzymes





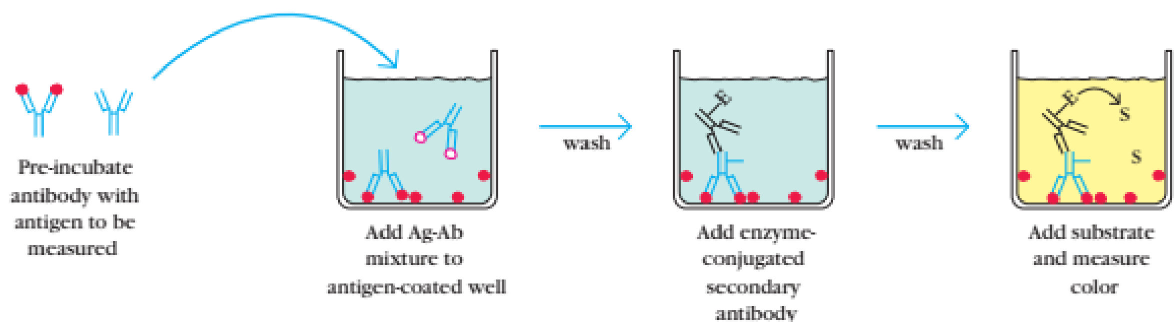


FIGURE 20-7 Variations in enzyme-linked immunosorbent assay (ELISA) technique allow determination of antibody or antigen. Each assay can be used qualitatively or quantitatively by comparison with standard curves prepared with known concentrations of antibody or antigen. Antibody can be determined with an indirect ELISA (a), whereas antigen can be determined with a sandwich ELISA (b) or competitive ELISA (c). In the competitive ELISA, which is an inhibition-type assay that is identical in principle to the competition RIA described above, the concentration of antigen is inversely proportional to the color produced.

- Protein Biochemistry
  - Biotin labels
    - Biotin – small molecule that can be bound to antibody
    - Used in ELISA
    - Reacts with avidin to produce color change

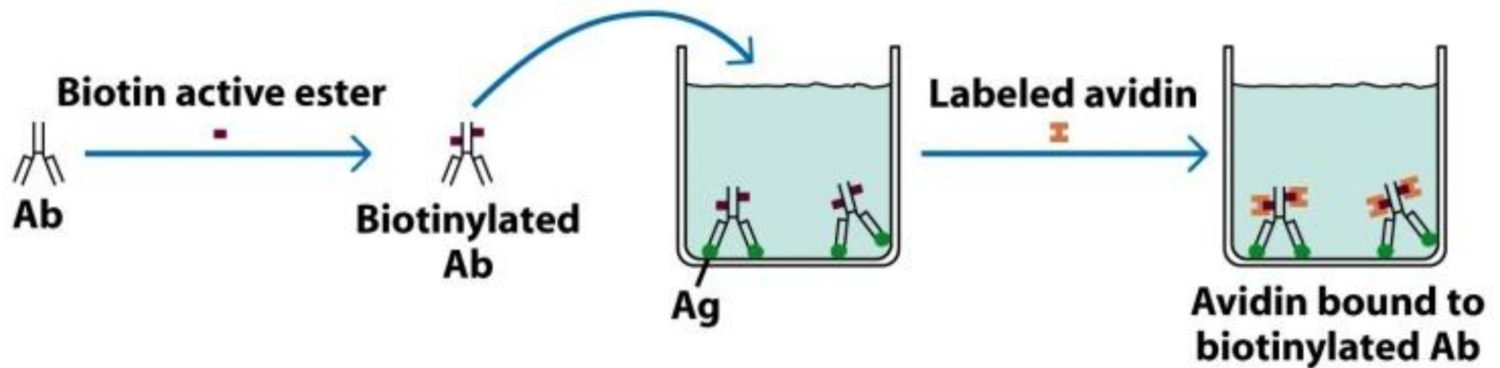


Figure 22-2
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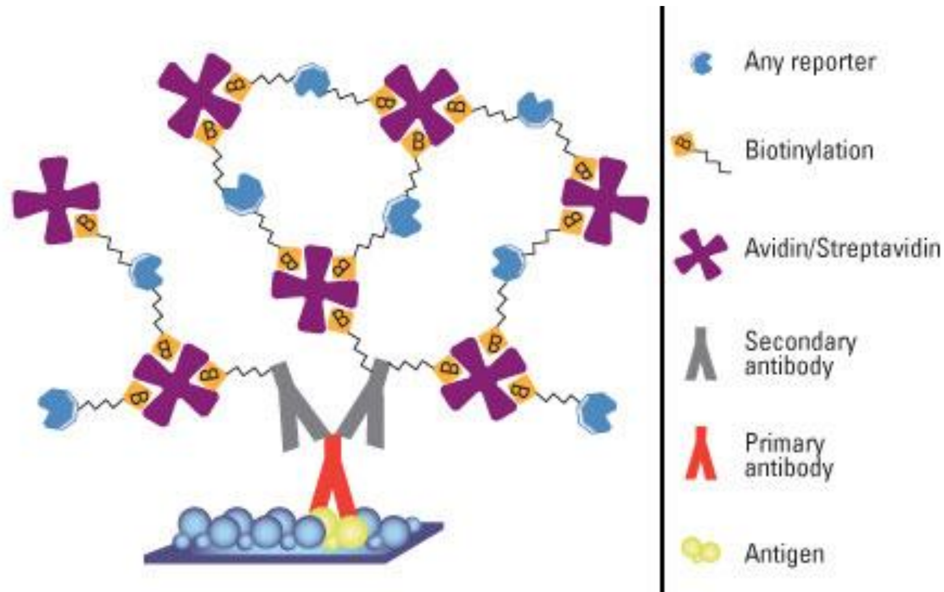
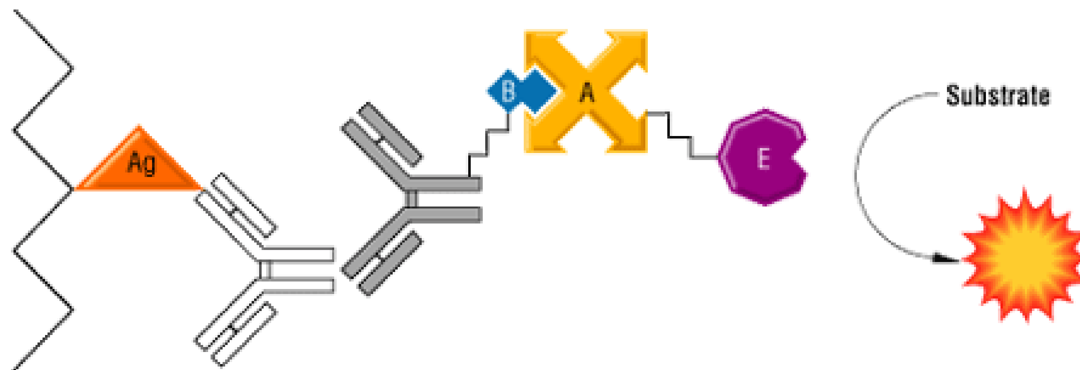
Schematic representation of the avidin-biotin complex (ABC) staining method.



The Labeled Streptavidin Biotin (LSAB) Staining Method



- Let’s say I’m trying to develop an ELISA to detect HS (harbor seal) IgG antibody levels in serum
  - Need a monoclonal antibody specific for HS IgG
  - So, I isolate HS IgG using column chromatography, inject mouse, mouse produces anti-IgG (remember there are idiotypic differences between IgG of mouse and another species)
  - Extract spleen (there are some B cells producing antibodies specific to the HS IgG I innoculated with); perform fusion to create hybridomas
  - After a few weeks, I have some living hybridomas – perform ELISA to see if they are producing antibody
  - Isolate the hybridomas (want to make sure I only have clones from 1 B cell)
  - My ELISA tells me they are producing anti-HS IgG but I want to see if the epitope is on the light or heavy chain
    - Coat plate with isolated HS IgG, then add media from monoclonals containing anti-HS IgG, followed by biotinylated anti-mouse
  - Therefore, I can use a Western blot to see this
    - Next 2 slides

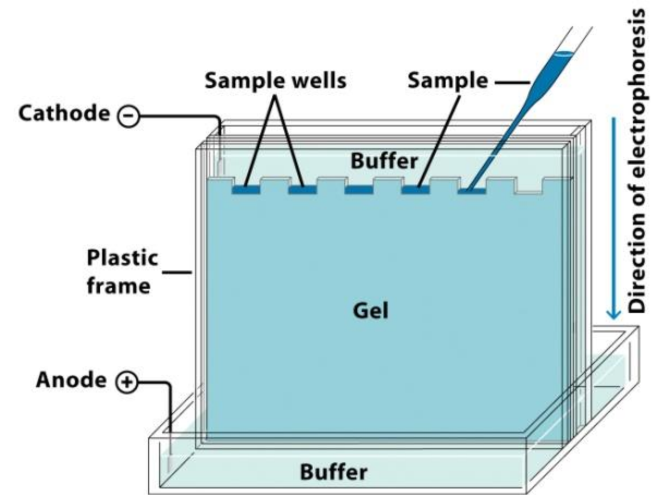


Figure 22-3a
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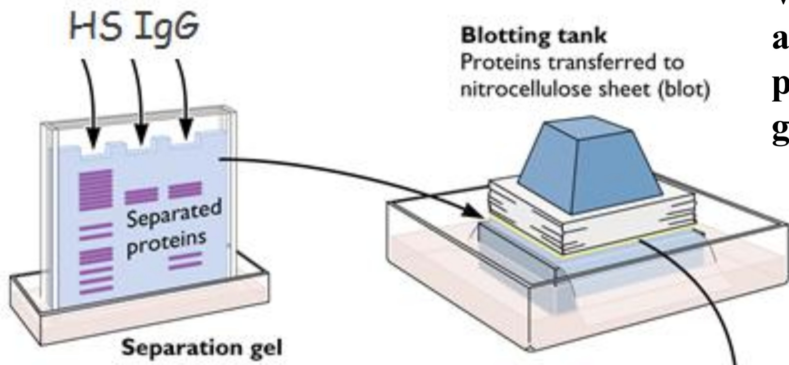
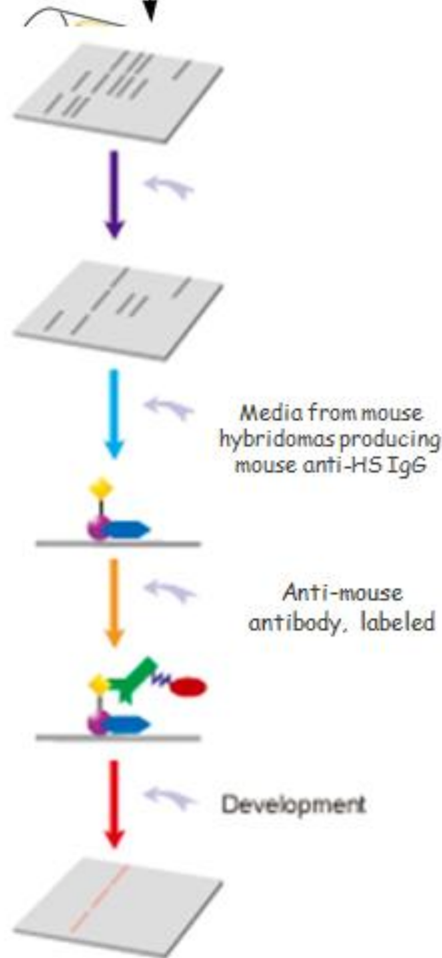
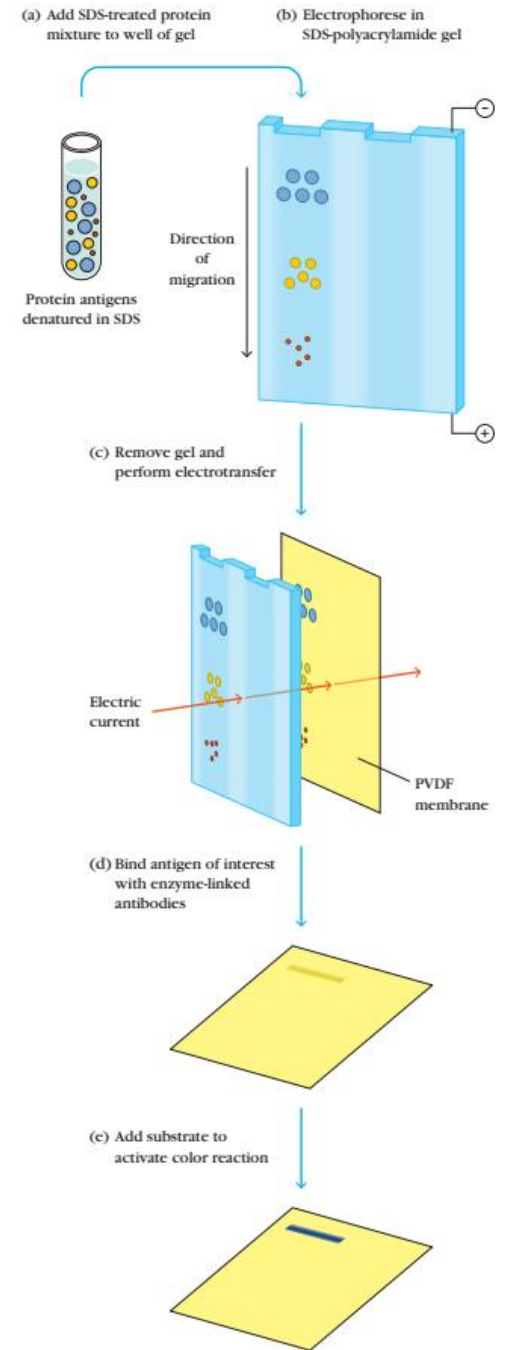
- Protein Biochemistry
  - Gel Electrophoresis
    - SDS-PAGE
      - SDS is a detergent, binds to proteins and destroys tertiary and secondary structure
      - Proteins can be separated according to molecular weight
        - Separation of antibody classes (different heavy chains, separation of light and heavy chains)
        - Run IgG I’m interested in looking at (HS-IgG I injected into mouse)
        - This can then be used in Western Blot (next slide)

Western blotting uses antibodies to identify protein bands following gel electrophoresis.

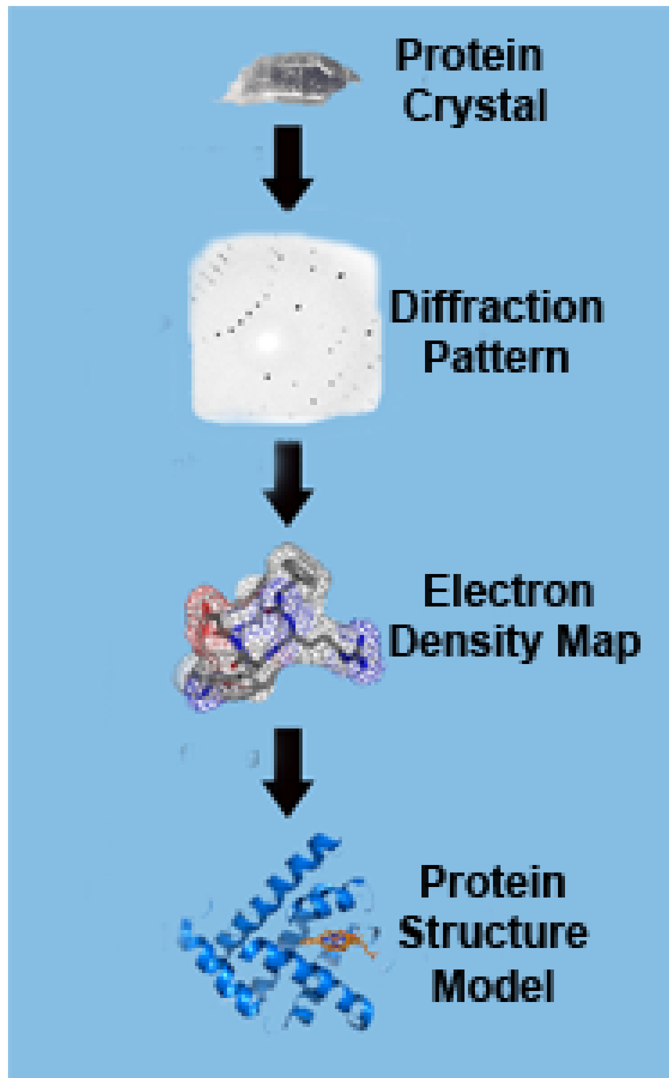






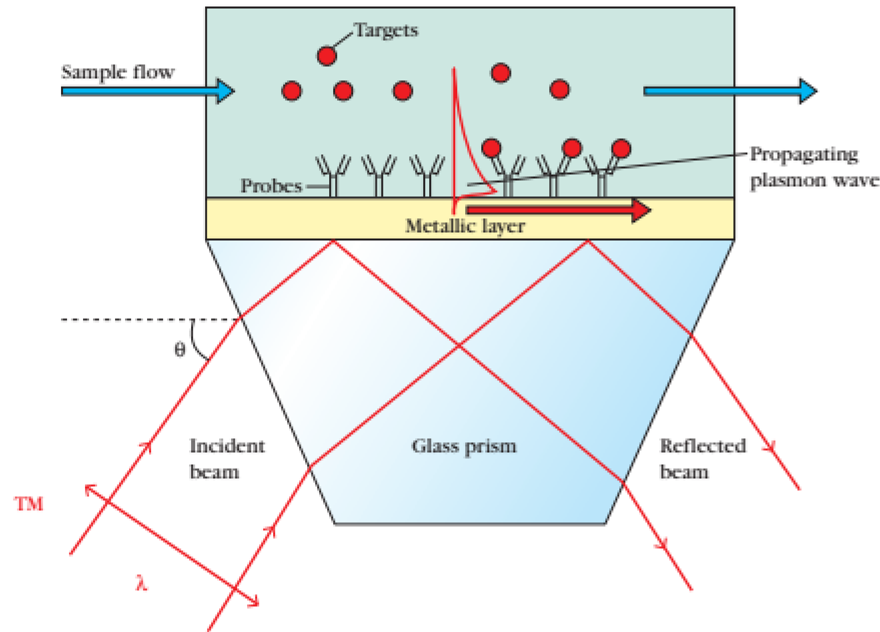
ELISPOT Assays Measure Molecules Secreted by Individual Cells

FIGURE 20-9 ELISPOT measurements of interferon γ secretion by NKT cells. A capture antibody to interferon γ was bound to a polyvinylidene difluoride–coated well of a microplate, and NKT cells were added at the appropriate concentration. After a 17-hour stimulation with phorbol myristoyl acetate and ionomycin, the cells were washed off and a biotin-conjugated, interferon-γ specific detection antibody was added and allowed to bind. Following removal of excess detecting antibody, streptavidin-conjugated alkaline phosphatase was added, followed (after washing out excess enzyme) by the substrate BCIP/NBT. BCIP/NBT is converted from a colorless solution to the brown-black substrate seen in the figure. The photograph shows the results of PMA/ionomycin stimulation of the NKT cells. Each brown-black spot represents the site of a cell that secreted interferon γ. These spots can be counted under a dissecting microscope at low power. *[Nicole Cunningham and Jenni Punt, Haverford College.]*



- Protein Biochemistry
  - X-ray crystallography
    - Limit of light microscopy is resolution
    - X-rays are transmitted through crystallized protein
      - Different atoms will scatter the x-rays differently
      - Pattern contains information of position of atoms within the molecule
      - Detector records pattern of spots
      - Mathematical deduction leads to calculation of structure

Surface Plasmon Resonance Is Now Commonly Used for Measurements of Antibody Affinity



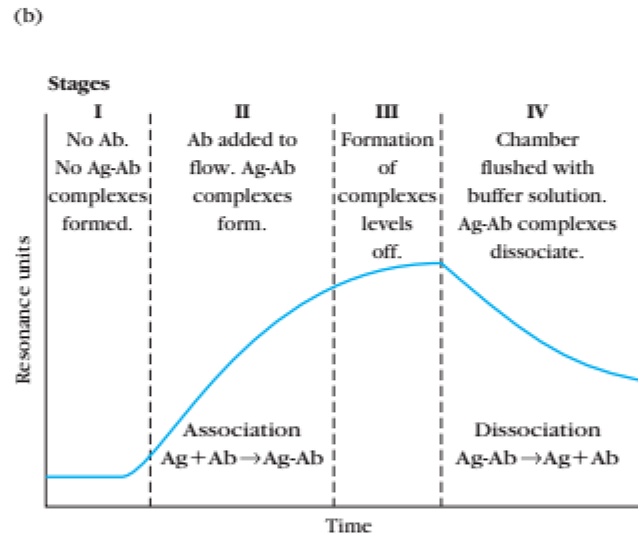
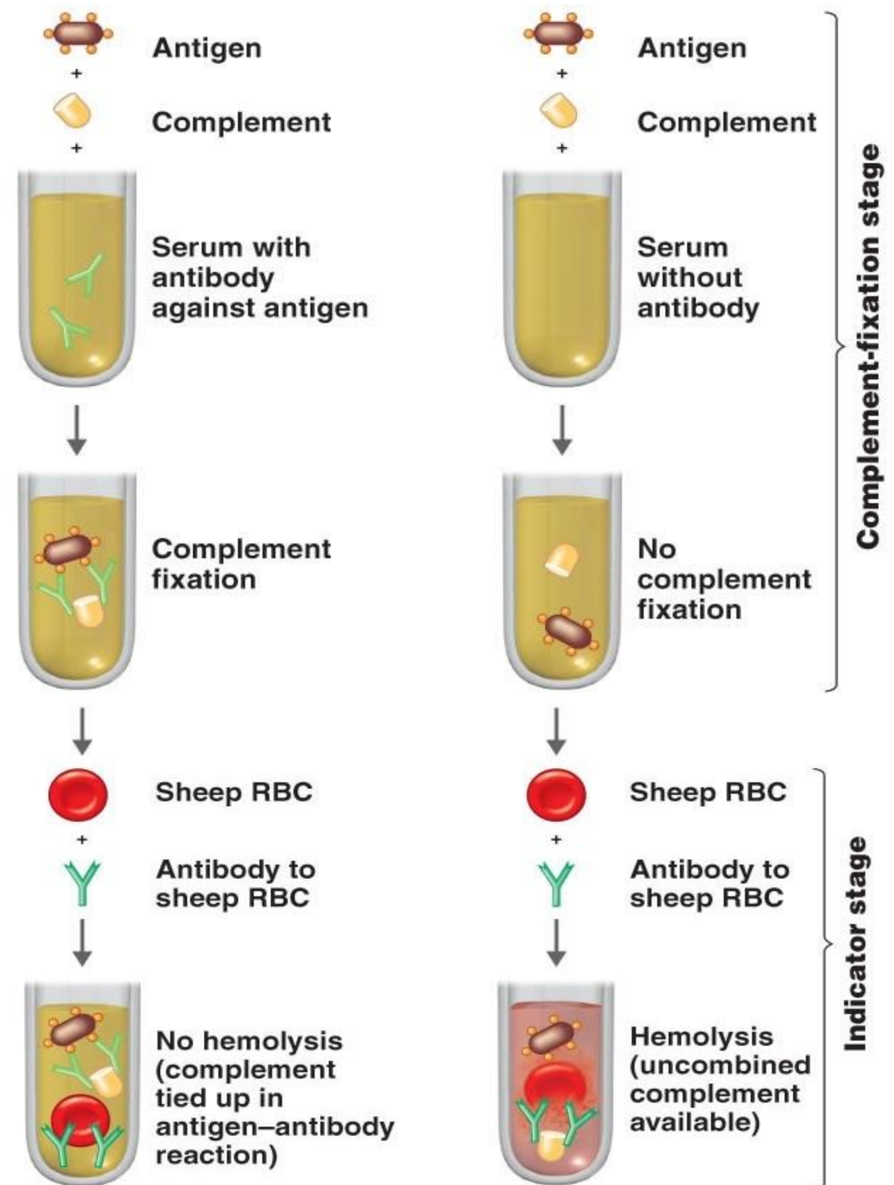


FIGURE 20-13 Surface plasmon resonance (SPR). (a) A buffer solution containing antibody is passed through a flow chamber, one wall of which contains a layer of immobilized antigen. As explained in the text, formation of antigen-antibody complexes on this layer causes a change in the resonant angle of a beam of polarized light against the back face of the layer. A sensitive detector records changes in the resonant angle as antigen-antibody complexes form. (b) Interpretation of a sensorgram. There are four stages in the plot of the detector response (expressed as resonance units, which represent a change of 0.0001 degree in the resonance angle) versus time. Stage I: Buffer is passed through the flow chamber. No Ag-Ab complexes are present, establishing a baseline. Stage II: Antibody is introduced into the flow and Ag-Ab complexes form. The ascending slope of this curve is proportional to the forward rate of the reaction. Stage III: The curve plateaus when all sites that can be bound at the prevailing antibody concentration are filled. The height of the plateau is directly proportional to the antibody concentration. Stage IV: The flow cell is flushed with buffer containing no antibody and the Ag-Ab complexes dissociate. The rate of dissociation is proportional to the slope of the dissociation curve. The ratio of the slopes, ascending over descending, equals $k_1/k_2 = k_a$.

Complement Fixation Test

- **In the positive test** : The available complement is fixed by Ag-Ab complex and no hemolysis of sheep RBCs occurs. So the test is positive for presence of antibodies.
- **In the negative test** : No Ag-Ab reaction occurs and the complement is free. This free complement binds to the complex of sheep RBC and it's antibody to cause hemolysis, causing the development of pink color.
- **Controls should be used along with the test to ensure that**
  - Antigen and serum are not anti complimentary
  - The appropriate amount of complement is used and
  - The sheep red blood cells do not undergo autolysis



(a) Positive test. All available complement is fixed by the antigen–antibody reaction; no hemolysis occurs, so the test is positive for the presence of antibodies.

(b) Negative test. No antigen–antibody reaction occurs. The complement remains, and the red blood cells are lysed in the indicator stage, so the test is negative.

MICROSCOPY: Staining

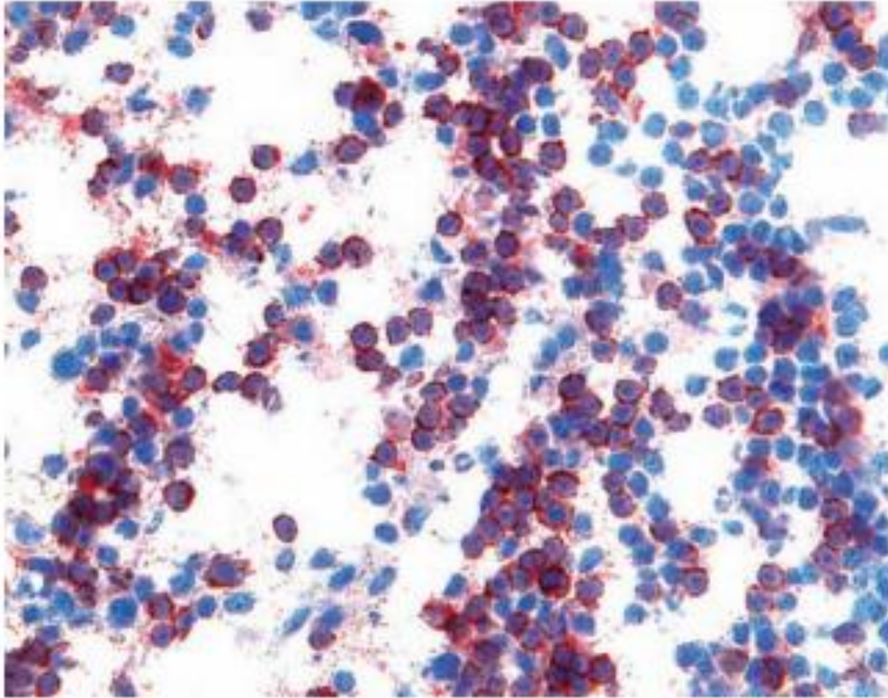


FIGURE 20-14 Immunohistochemical staining of lymph node. Human lymph node material was embedded in paraffin, fixed, and stained with antibody to CD4 (red stained cells). The lymph node was then counter-stained with hematoxylin (blue). *[Courtesy R&D Systems, Inc., Minneapolis, MN, USA.]*

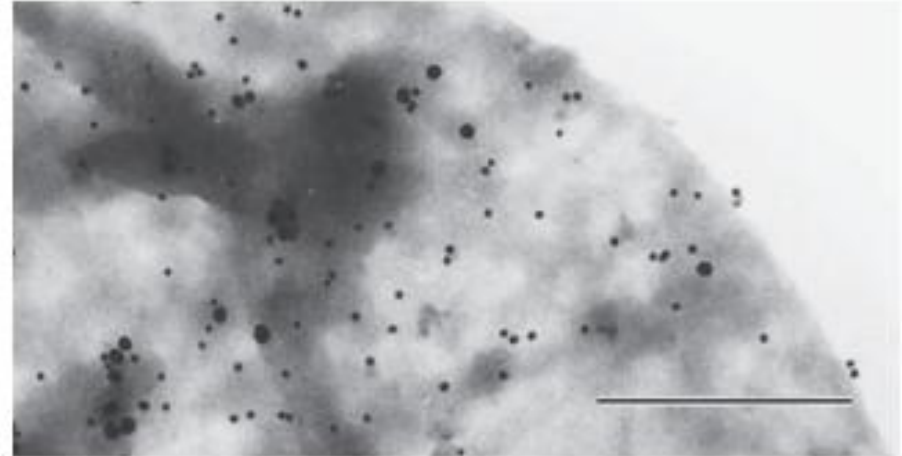
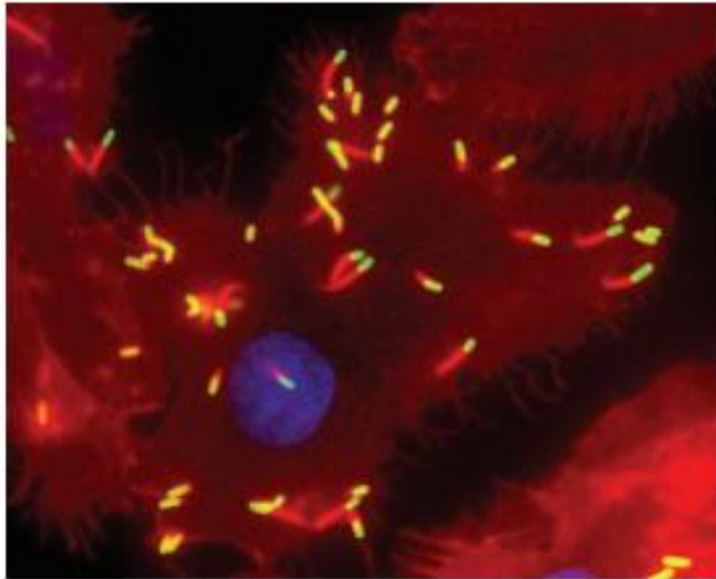


FIGURE 20-15 An immunoelectronmicrograph of the surface of a B-cell lymphoma was stained with two antibodies: one against class II MHC molecules labeled with 30-nm gold particles and another against MHC class I molecules labeled with 15-nm gold particles. The density of class I molecules exceeds that of class II on this cell. Bar = 500 nm. *[From A. Jenei et al., 1997, PNAS 94:7269–7274; courtesy of A. Jenei and S. Damjanovich, University Medical School of Debrecen, Hungary.]*

MICROSCOPY: Immunofluorescence-Based Imaging Techniques

(a)



(b)

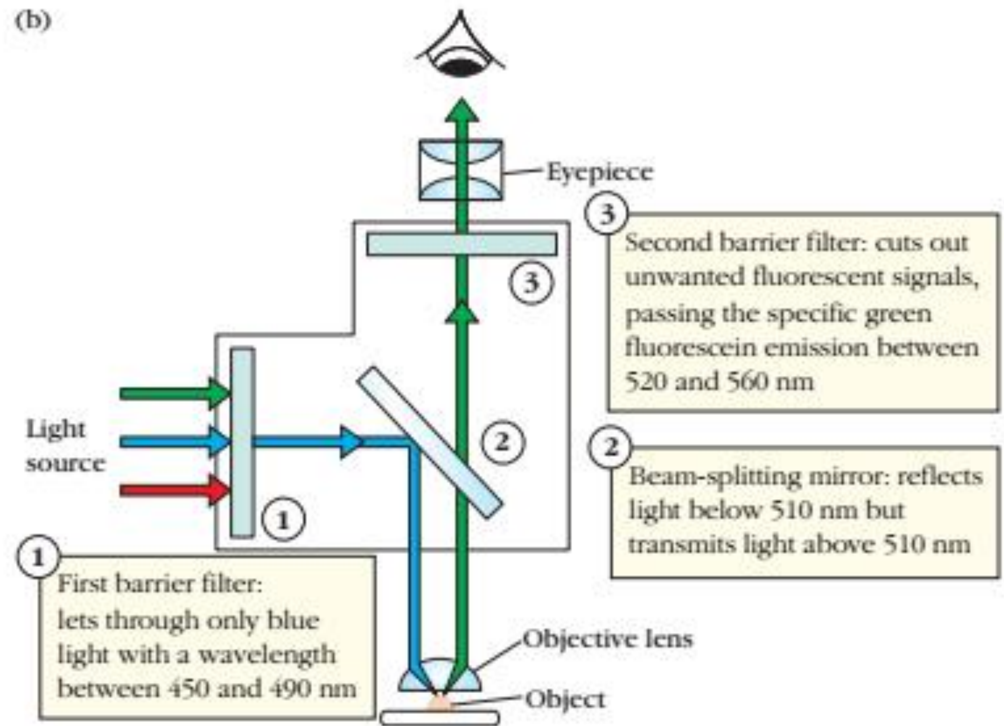


FIGURE 20-16 Fluorescently-labeled cells and the passage of light through a fluorescence microscope. (a) Fluorescence microscopic image of a human dendritic cell infected with engineered *Listeria monocytogenes*. The green fluorescence is generated by Alexa fluor 488-conjugated phalloidin (which binds actin filaments). Red fluorescence derives from red fluorescent protein expressed by the bacterium. DNA is stained with DAPI (blue). (b) Light from a source passes through a barrier filter that only allows passage of blue light of particular wavelengths. The light is then directed onto the sample by a dichroic mirror that reflects light of short wavelengths (below approximately 510 nm) but allows passage of higher wavelengths. When the blue light interacts with the sample, any fluorescent molecules excited by it emit fluorescence that then passes through the dichroic mirror, through a second barrier filter, and then is transmitted to the eyepiece. *[(a) Image courtesy of Dr. Keith Bahjat, Earle A. Chiles Research Institute.]*

MICROSCOPY: Confocal Microscopy

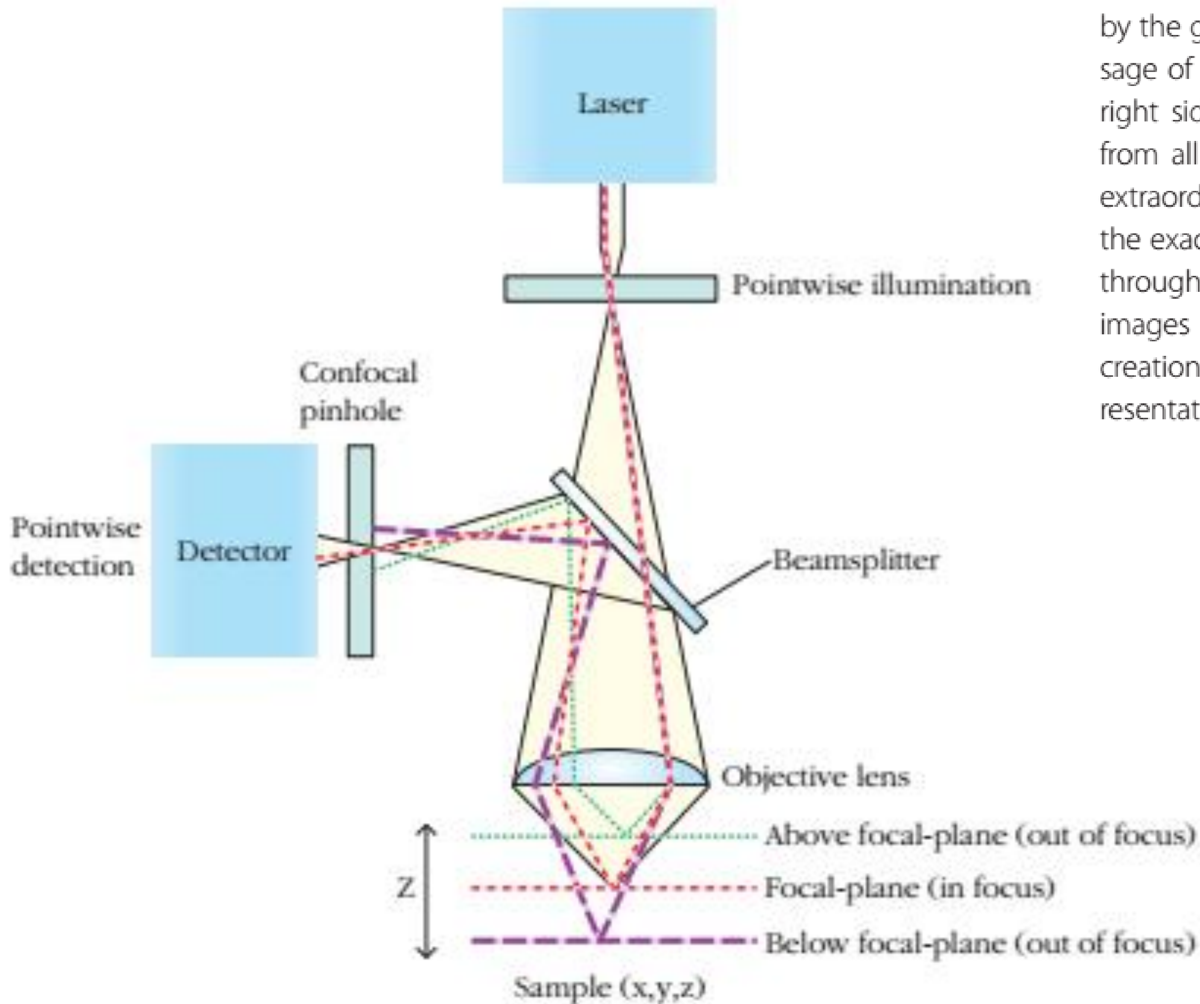
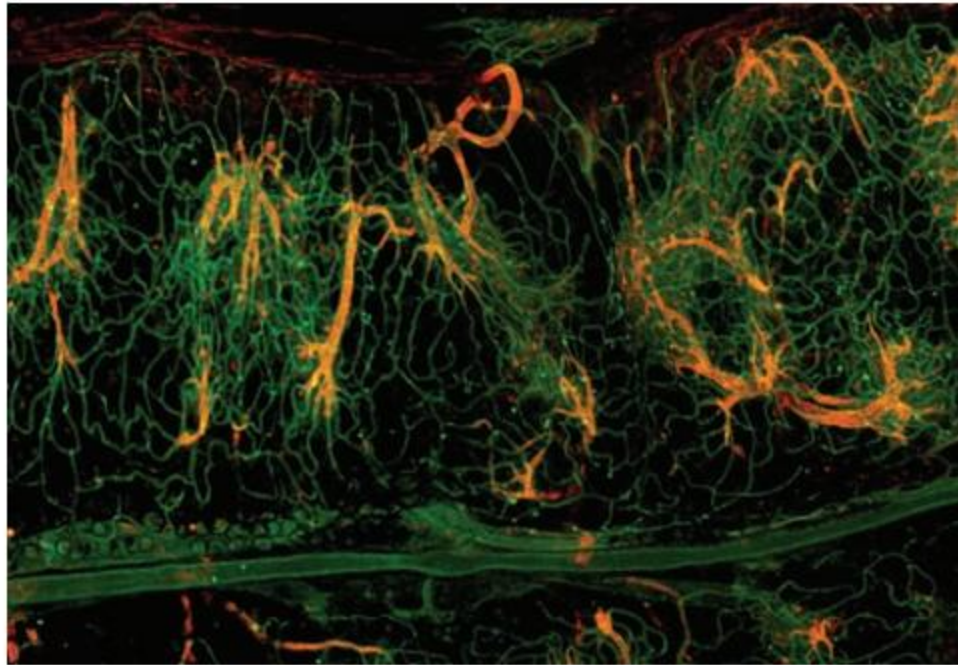


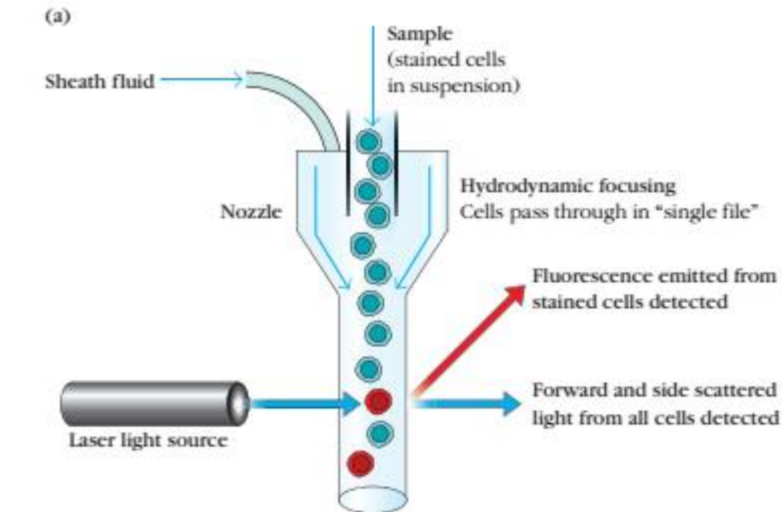
FIGURE 20-17 The principles of confocal microscopy. (a) The sample is illuminated by a laser beam that excites fluorescence from dyes in several different focal planes, represented here by the green, red, and purple lines. However, passage of light through the pinhole (shown on the right side of the image) filters out light emitted from all but a single focal plane, resulting in an extraordinarily clear image. Computer control of the exact plane from which light can be received through the pinhole allows the development of images from a number of focal planes and the creation of a composite three-dimensional representation such as that shown in Figure 20-1.

(b)



(b) Confocal image of a mouse omentum. The omentum was stained with green dye-conjugated antibodies specific for CD31, which stains all endothelial vessels in the omentum. A second, red dye-conjugated antibody was then added specific for the molecule DARC, that is present only on the endothelial cells of the post-capillary end venules (the vessels from which extravasation of immune cells can occur). The red and green images were acquired separately and merged. Vessels staining both red and green appear yellow in this image. *[(b) Courtesy Aude Thiriot and Ulrich von Andrian, Harvard Medical School.]*

Flow Cytometry: Fluorescence Activated Cell Sorter (FACS)



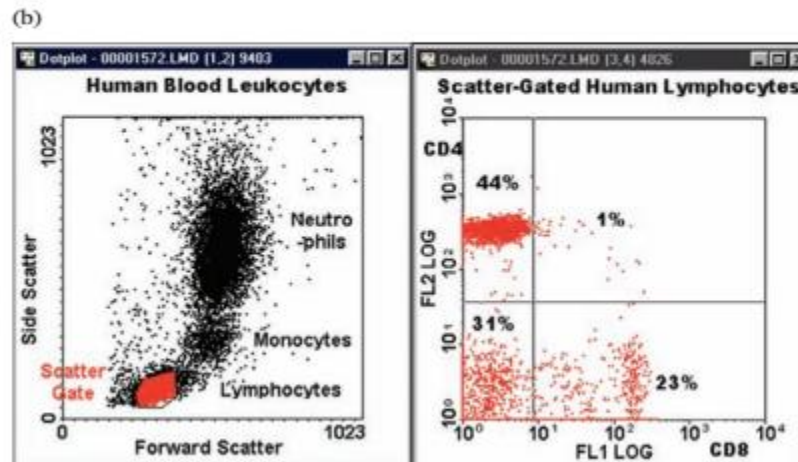


FIGURE 20-19 Principles of flow cytometry. (a) Cells introduced into the sample injection port are focused within a stream of sheath fluid and pass one by one in front of the laser beam. Forward-scattered light is detected by a photodiode. Side-scattered light and emitted fluorescence of various wavelengths is detected by photomultiplier tubes, after passage through a series of dichroic mirrors and light filters. All of the information obtained from individual cells is integrated by the software and can be expressed in a number of formats, such as that shown in (b). (b) On the left is a scatter plot of forward scatter (abscissa) versus side scatter (ordinate) of a sample of human white blood cells. Lymphocytes are gated and displayed in red. On the right is a plot of lymphocytes stained with anti-CD4 (ordinate) or anti-CD8 (abscissa) antibodies. *[(a), www.sonyinsider.com/wp-content/uploads/2010/02/Flow-Cytometry-Diagram2.jpg; (b), Courtesy University of Massachusetts, Amherst, Department of Microbiology.]*

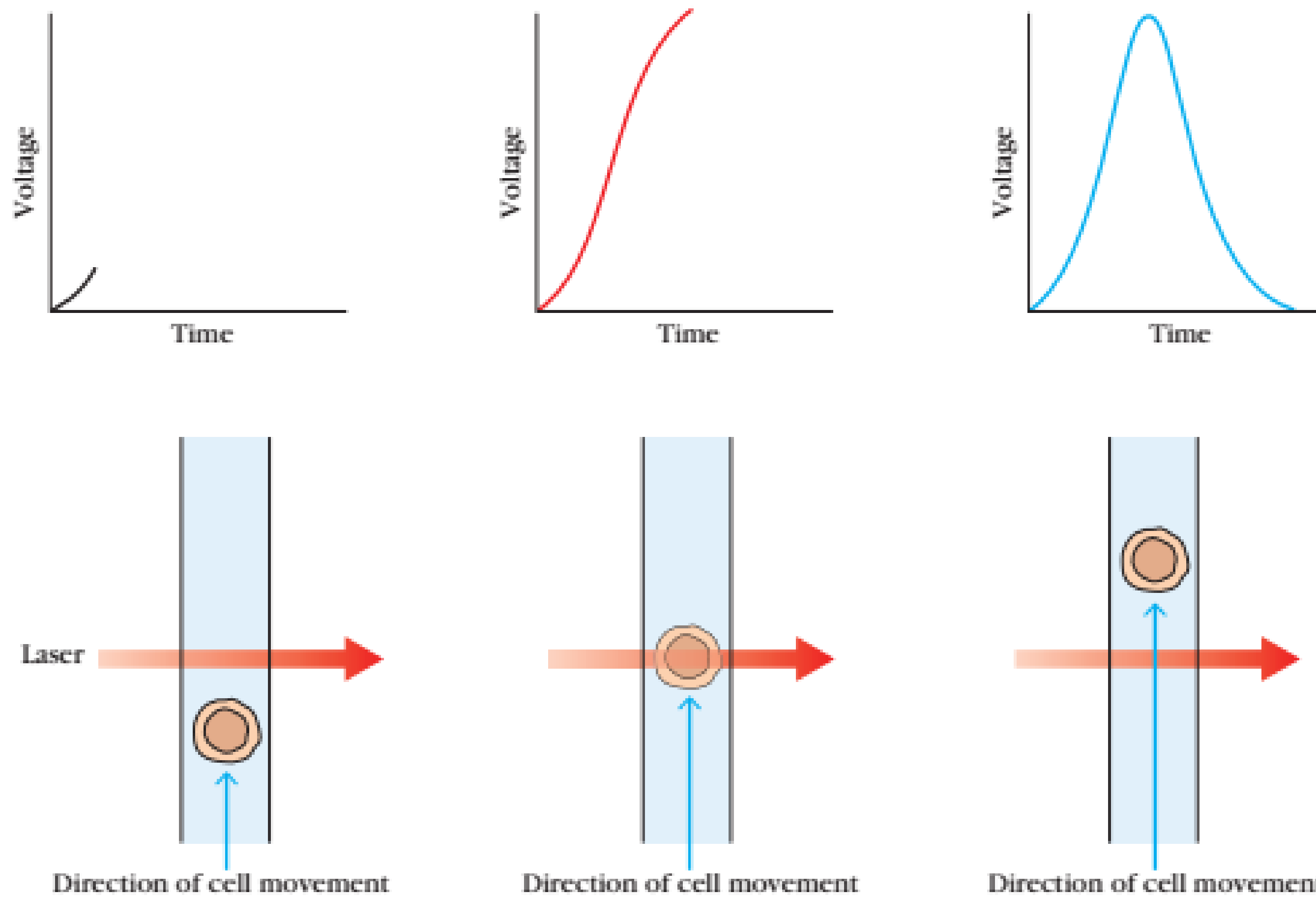


FIGURE 2

The voltage pulse generated by a cell passing in front of the laser beam. As cells pass in front of the laser beam, they emit or scatter light that is detected by a series of photomultiplier tubes (PMTs). The PMT receives the photons and converts them into a voltage pulse whose height, width and area is digitized and represented by the cytometer software. *[J. Punt]*

Cell Complexity

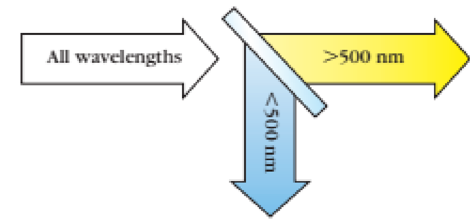


FIGURE 1

Flow cytometers contain dichroic mirrors. Dichroic mirrors allow passage of light of some wavelengths and reflect light of others. In this example, the dichroic mirror is enabling passage of light of wavelengths longer than 500 nm, but is reflecting light of shorter wavelengths in a direction perpendicular to the incident light. *[J. Punt]*

FL1
530/30
SSC
488/10
FL2
585/42
90/10 beam splitter
DM 560SP
DM 640LP
670LP
FL3
Fluorescence collection lens
488 nm blue laser
Focusing lens
Flow cell
488/10
FSC diode

Cell Size

Key:

FSC – forward scatter detector	DM – Dichroic mirror
SSC – side scatter detector	SP – shortpass filter
FL1, FL2, FL3 – fluorescence detectors	LP – longpass filter

FIGURE 3

A simple flow cytometry setup. Cells passing through the flow cell are interrogated by the laser and scattered and fluorescent light is directed through the series of mirrors and filters to the appropriate PMTs. There, the induced voltages are digitized and represented by the software in graphical form. Since each parameter of light scatter or fluorescence is recorded for each cell detected, results can be displayed that include any combination of parameters for the cell population being studied. A variety of display styles are available depending on the graphing software used by the investigator. (See text for further details.) *[http://ars.els-cdn.com/content/image/1-s2.0-S0167779911001958-gr1.jpg]*

- Fluorescent Technology
  - Green fluorescent protein
    - Isolated from bioluminescent jellyfish, naturally occurring
    - Can be used to visualize live cells

Cell Cycle Analysis

Tritiated (^{3}H) Thymidine Uptake

Was One of the First Methods Used to Assess Cell Division. ^{3}H thymidine uptake assays were the first to be used routinely to measure cell division in lymphocyte cultures.

They rely on the fact that dividing cells synthesize DNA at a rapid pace, and radioactive thymidine in the culture fluid will therefore be quickly incorporated into high molecular weight DNA.

Colorimetric Assays

The tetrazolium compound MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide, a yellow tetrazole) is reduced by metabolically active cells to form insoluble, purple formazan dye crystals (Abs at 570nm).

Bromodeoxyuridine-Based Assays

Use Antibodies to Detect Newly Synthesized DNA.

When introduced into cells, *br*omo*d*eoxy*u*ridine (BrdU) is rapidly phosphorylated to bromodeoxyuridyl triphosphate (an analogue for deoxythymidine triphosphate) and is incorporated in its place into newly synthesized DNA.

Cells that divide following BrdU incorporation can then be identified using antibodies to BrdU.

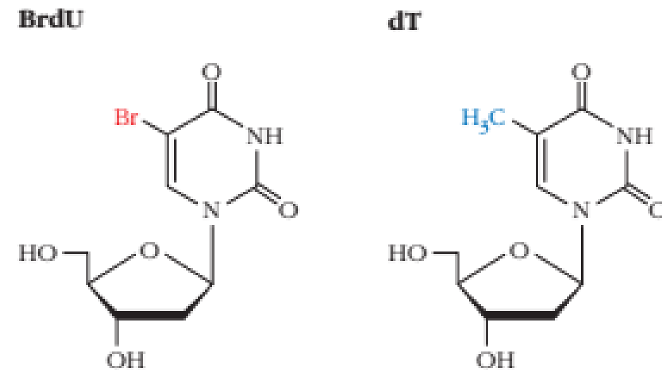


FIGURE 20-20 Bromodeoxyuridine incorporates into DNA in place of deoxythymidine during DNA synthesis. Bromodeoxyuridine (BrdU) is a thymidine analog, as the large bromine group serves to mimic the size and shape of the methyl group of thymidine. It is incorporated into DNA instead of thymidine and can be detected by anti-BrdU antibodies. *[http://openwetware.org/images/thumb/c/cc/BrdU_vs_dT.svg/250px-BrdU_vs_dT.svg.png.]*

Propidium Iodide Enables Analysis of the Cell Cycle Status of Cell Populations

G1 cells will have half the DNA of G2 cells, or cells about to undergo mitosis, and cells that are currently replicating DNA and are therefore in S phase will have an intermediate value. Apoptotic cells and fragments that have begun to break down their DNA will appear as events with less than G1 amounts of DNA.

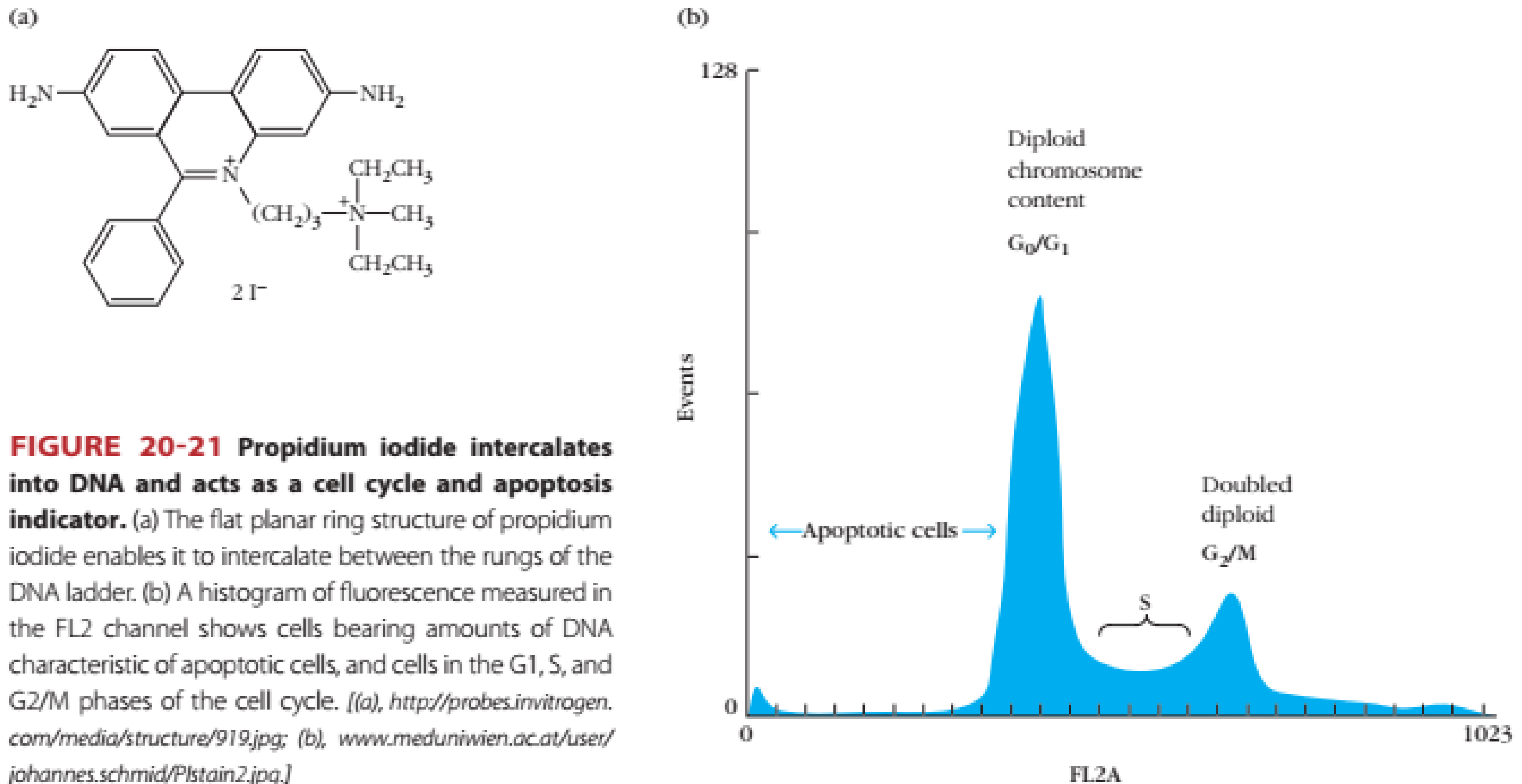


FIGURE 20-21 Propidium iodide intercalates into DNA and acts as a cell cycle and apoptosis indicator. (a) The flat planar ring structure of propidium iodide enables it to intercalate between the rungs of the DNA ladder. (b) A histogram of fluorescence measured in the FL2 channel shows cells bearing amounts of DNA characteristic of apoptotic cells, and cells in the G1, S, and G2/M phases of the cell cycle. *[(a), http://probes.invitrogen.com/media/structure/919.jpg; (b), www.meduniwien.ac.at/user/johannes.schmid/PIstain2.jpg.]*

Carboxyfluorescein Succinimidyl Ester Can Be Used to Follow Cell Division

The diacetyl groups enable the CFDASE to enter the cell, and are then cleaved by intracellular esterases.

CFSE remains trapped within the cytoplasm.
In the cytoplasm, molecules of CFSE are efficiently and covalently attached to intracytoplasmic proteins, with the succinimidyl ester acting as a leaving group.

The amount of fluorescence emitted is cut in half each time the cell divides.

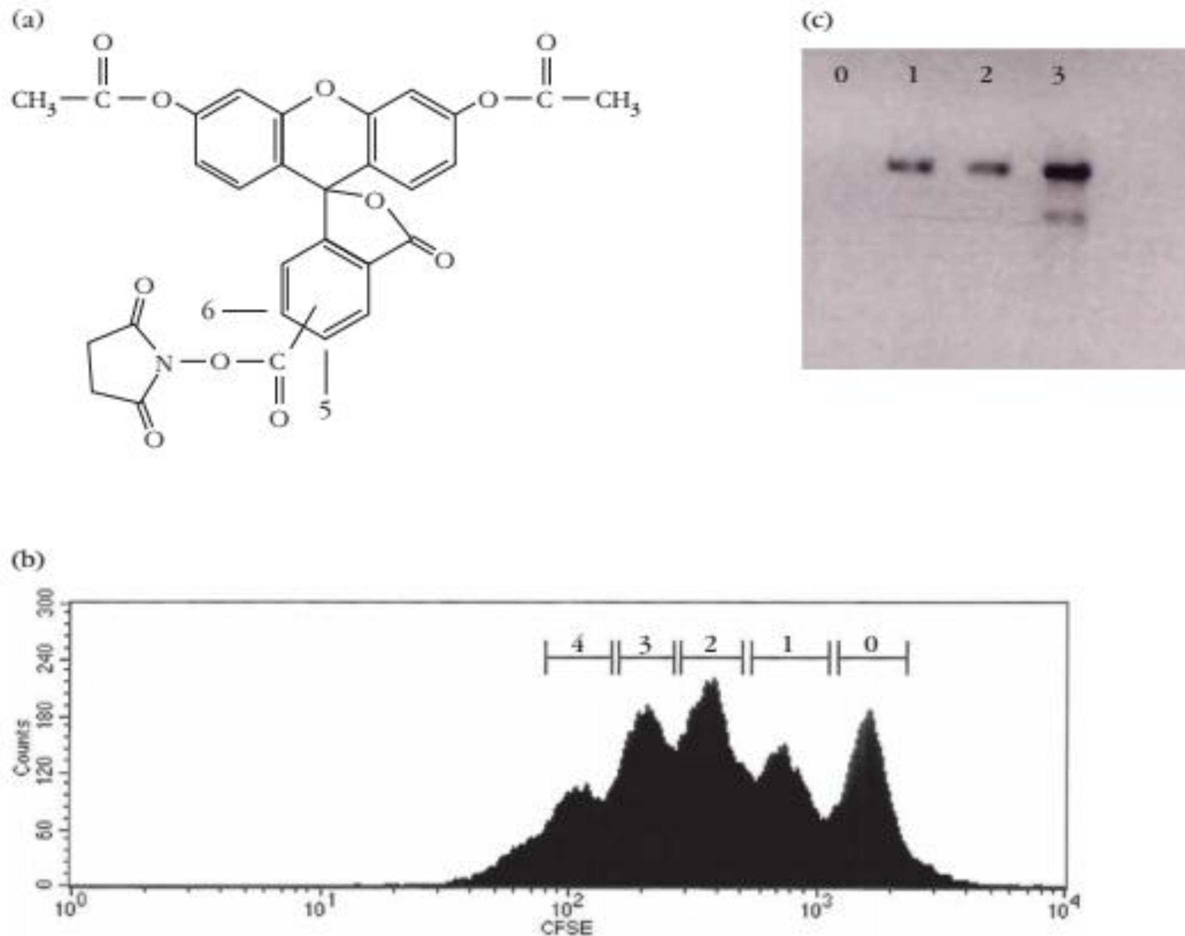


FIGURE 20-22 Staining with carboxyfluorescein succinimidyl ester allows assessment of the number of cell divisions undertaken after staining. (a) Structure of carboxyfluorescein diacetyl succinimidyl ester (CFSE). (b) Fluorescence histogram showing CFSE fluorescence from a population of dividing cells. The peak on the right represents those cells that have not divided since addition of the CFSE. Cells that have divided once have half the fluorescence of the undivided population and are shown in the peak immediately to the left of the undivided population. The other peaks have divided two, three, or four times respectively post stimulation. (c) Cells can be sorted according to how many times they have divided post addition of CFSE and tested for gene expression. In this example, the gene for the protein survivin is not expressed in nondividing T cells, but is expressed following stimulation with anti-CD3 and anti-CD28. After three cell divisions, the expression is up-regulated and two mRNA species (representing the RNA encoding two isoforms of the protein) are evident. *[(a) http://probes.invitrogen.com/media/structure/835.jpg; (b) and (c), Alexander Au, M.D., senior thesis, Haverford College.]*

Cell Death Assays

The ^{51}Cr Release Assay Was the First Assay Used to Measure Cell Death

^{51}Cr release assay: for measuring cytotoxic T cell- and natural killer cell-mediated killing.

Target cells incubated with sodium ^{51}chromate and then the killer cell.

Modern Alternative: CFSE (carboxyfl uorescein succinimidyl ester) release upon killing.

Fluorescently Labeled Annexin V Measures Phosphatidyl Serine in the Outer Lipid Envelope of Apoptotic Cells

Phosphatidyl serine flips from the interior to the exterior side of the plasma membrane phospholipid bilayer.

Annexin V is a protein that binds to phosphatidyl serine in calcium dependent manner

Caspase Assays Measure the Activity of Enzymes Involved in Apoptosis

Caspase 8, Assay kits available (fluorescence or western blot based).

The TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) Assay Measures Apoptotically Generated DNA Fragmentation

Assay is based on terminal deoxynucleotidyl *t*ransferase (TdT) to add bases onto the broken ends of DNA sequences in a non templated manner.

The classic variation of the TUNEL method uses TdT to add BrdU to fixed and permeabilized cells.

BrdU is incorporated into the newly synthesized DNA, and is then detected with fluorescently labeled anti-BrdU antibodies

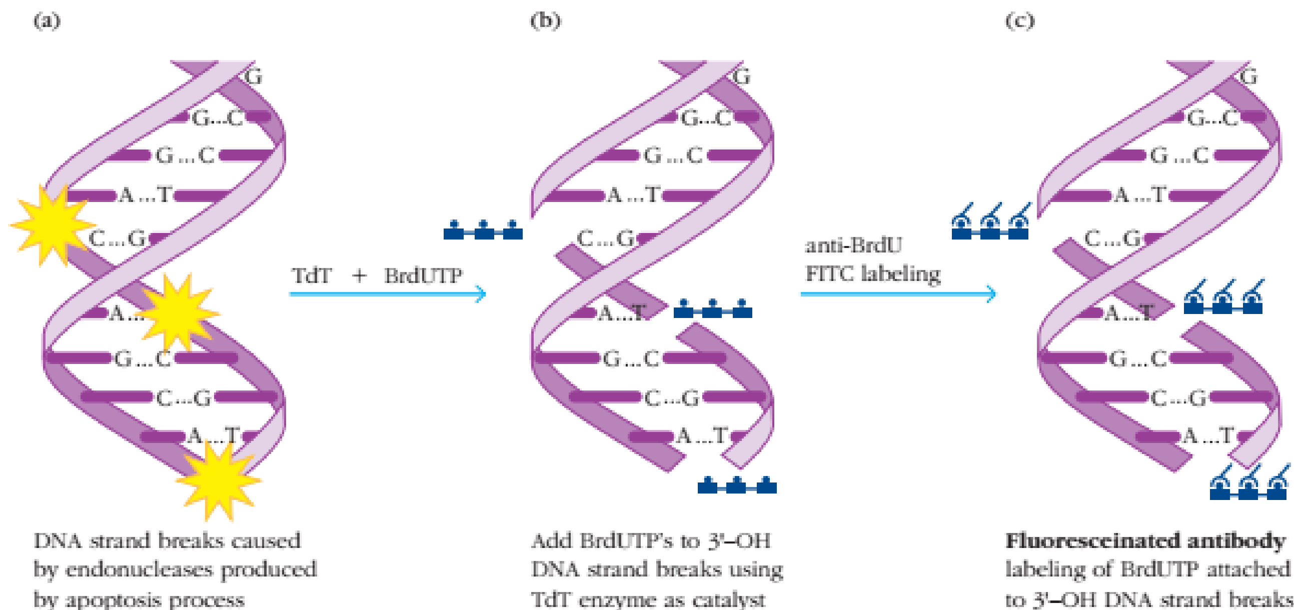


FIGURE 20-23 Assessment of apoptosis using a TUNEL assay. (a) Apoptosis results in DNA fragmentation by intracellular nucleases. (b) BrdU nucleotide triphosphates are added onto the broken ends of fragmented DNA in fixed and permeabilized apoptotic cells using the enzyme TdT. (c). Fluoresceinated antibodies specific for BrdU can then be used to detect apoptotic cells. *[www.phnxflow.com/images/DNA.gif.]*

Biochemical Approaches Used to Elucidate Signal Transduction Pathways

TABLE 20-2 Some common inhibitors used in the dissection of signal transduction pathways

Inhibitor	Protein or organelle affected
Ly294002	PI3 kinase
Wortmannin	PI3 kinase
Rapamycin	mTOR
Chloroquine	Integrity of lysosomal compartments—pH gradient is collapsed
BX795	Inhibits IKKα
PepinhMyD	MyD88
PD98059	MAPKKK
Cyclosporine	Works with immunophilin to inhibit calcineurin
zVAD fmk	Members of the caspase family

Recombinant DNA Technology

- Restriction enzymes cleave DNA at precise sequences
- DNA sequences are cloned into vectors
  - Virus
    - If it's a bacteriophage, it can then infect bacteria and the bacteria will express inserted gene
  - Plasmid
    - Gene of interest is inserted into plasmid containing antibiotic resistance gene, incubated with bacterial cells, if bacteria uptake plasmid they will be able to grow on medium with antibiotic

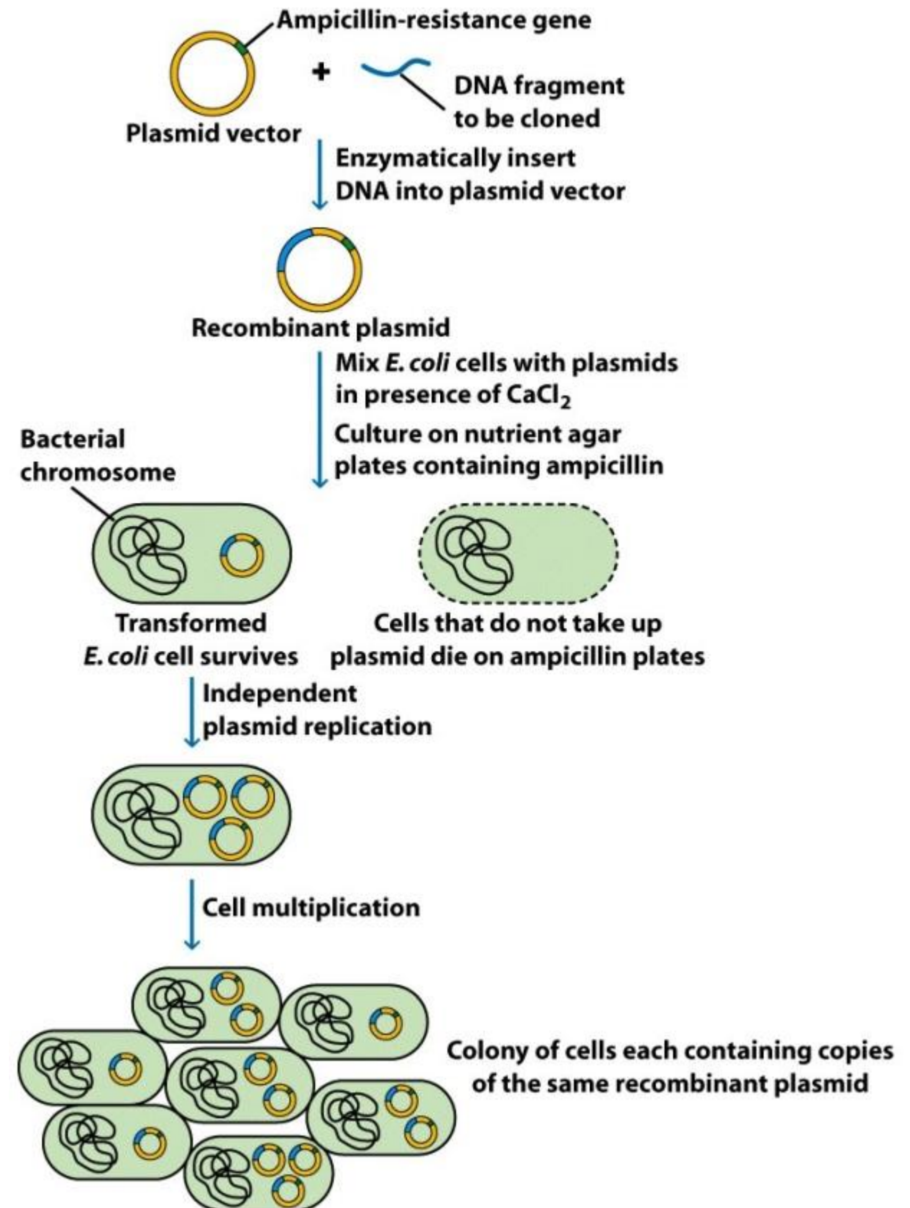


Figure 22-6
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Recombinant DNA Technology

- Cloning of cDNA and genomic DNA
  - Messenger RNA isolated from cells can be transcribed into complementary DNA
  - This can be inserted into vector and then expressed
  - cDNA library
    - Expressed genes of cell

- Recombinant DNA Technology
  - Southern Blotting

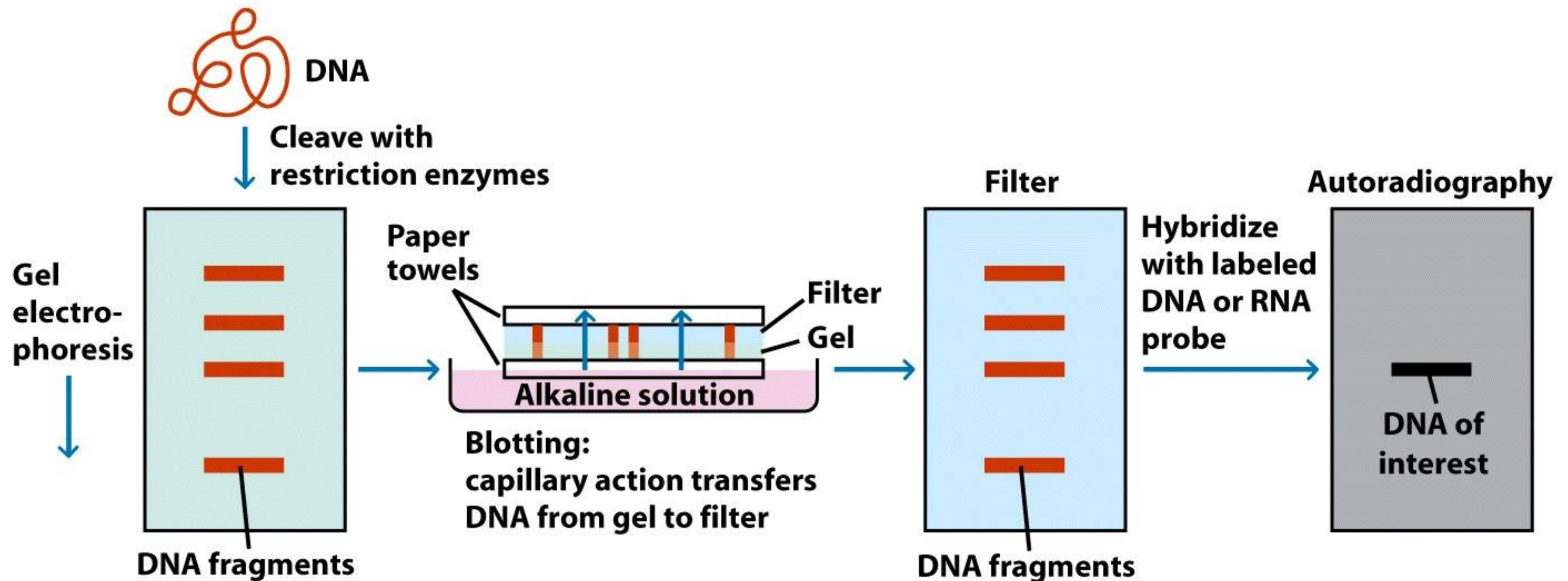

Figure 22-9
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- Gene Transfer
  - Common technique
    - Retrovirus – replace viral structural gene with clone gene to be transfected
    - Virus is now used as vector to insert new gene into cultured cells
    - Inserting these transgenes into mouse embryos allows researchers to study effects of immune system genes in vivo
- Gene Transfer
  - Knockout mice
    - Replace normal gene with mutant allele

Animal models

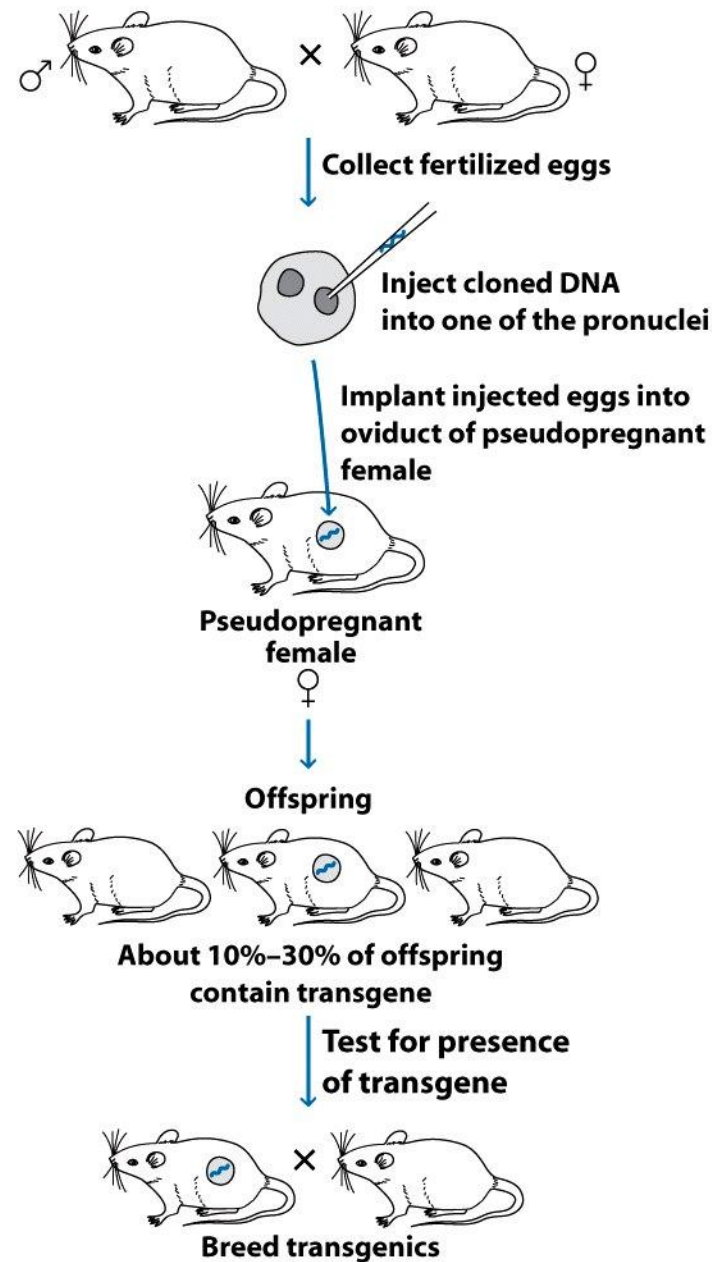

Figure 22-13
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TABLE 22-7 Comparison of transgenic and knockout mice

Characteristic	Transgenic Mice	Knockout Mice
Cells receiving DNA	Zygote	Embryonic stem (ES) cells
Means of delivery	Microinjection into zygote and implantation into foster mother	Transfer of ES cells to blastocyst and implantation into foster mother
Outcome	Gain of a gene	Loss of gene

Table 22-7
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- Microarrays
  - Assess differences in gene expression between cell types
  - Can scan large #'s of mRNAs
  - Procedure
    - mRNA isolated, cDNA synthesis is initiated
    - First strand of cDNA is labeled with tag
    - Labeled cDNA is then hybridized with nucleic acid affixed in microarray