

MONITOR KEMALIHIDUPAN
MORFALOGI DAN FISIOLOGI
FAKULTAS KEDOKTERAN
UNIVERSITAS PADJARAN
JURUSAN PATOLOGI ANATOMI
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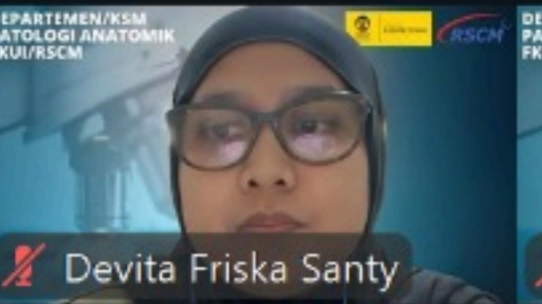
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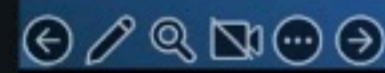
IMUNOFLUORESENS

DEVITA FRISKA SANTY
DIAS ISNANTI
FAIRI CUNNY
JONATHAN ARIEL

MARDIANA FARHALINA
RISTI KHAFIDAH
SABRINA RIZKI ANDRIANI
TRI ADINDA GUSVI MEISYA

Pembimbing: Prof. Dr. Drs. Kusmardi, MS.

The slide features a white background with blue and green abstract shapes. On the left, there are icons of a green apple, a yellow pencil, a yellow alarm clock, and blue puzzle pieces. On the right, there is a blue hand holding a black graduation cap over a globe, with letters A, B, and C floating around. A green horizontal line is drawn across the middle of the slide.



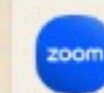
Open recordings >



AI can make mistakes. Review for accuracy.



Search



14:03
23/04/2026



INTERPRETATION - PATTERN

- **CENTROMERE**
ANA WITH CENTROMERE PATTERN IS PARTICULAR MARKER OF SYSTEMIC SCLEROSIS OF LIMITED TYPE, BUT ARE ALSO SEEN IN OTHER RHEUMATIC DISEASES SYSTEM AND IN PRIMARY BILIARY CHOLANGITIS (PBC).
- **NUCLEOLAR**
ANA WITH NUCLEOLAR PATTERN IS PRIMARILY MARKER OF SYSTEMIC SCLEROSIS OF DIFFUSE TYPE, BUT SLIGHTLY POSITIVE FINDINGS CAN ALSO BE SEEN IN OTHER CONDITIONS.
- **PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA)**
ANA WITH PCNA PATTERN IS AN UNUSUAL SUBTYPE OF ANA BUT IS REGARDED TO HAVE HIGH SPECIFICITY FOR SLE.
- **MITOTIC SPINDLE**
ANTIBODIES TO THE MITOTIC SPINDLE IS PRESENT AT MYCOPLASMA PNEUMONIAE INFECTIONS. OTHER ASSOCIATIONS ARE UNCLEAR.
- **NUCLEAR DOTS**
NUCLEAR DOTS PATTERN WITH SPECIFICITY FOR THE SP100 IS STRONGLY ASSOCIATED WITH PRIMARY BILIARY CHOLANGITIS (PBC). CHOLESTASIS?
- **NUCLEAR MEMBRANE**
ANTIBODIES AGAINST NUCLEAR MEMBRANE IS PRIMARILY ASSOCIATED WITH PRIMARY BILIARY CIRRHOSIS. CHOLESTASIS?
- **HOMOGENEOUS**
SAMPLES WITH HOMOGENEOUS ANA WILL FURTHER BE ANALYZED FOR ANTI-DSDNA, ANTI-HISTONES AND ANTI-ENA (ANTI-SM, ANTI-NRNP, ANTI-SSA, ANTI-SSB, ANTI-SCL-70, ANTI-JO1 AND ANTI-RO 52).
- **SPECKLED**
SPECKLED NUCLEAR STAINING COMPRISES ANTIBODIES AGAINST ENA, SSA AND SSB. SAMPLES WITH SPECKLED ANA WILL FURTHER BE ANALYZED FOR ANTI-DSDNA AND ANTI-ENA (ANTI-SM, ANTI-NRNP, ANTI-SSA, ANTI-SSB, ANTI-SCL-70, ANTI-JO1 AND ANTI-RO 52).

<http://www.wieslab.com/diagnostic-services/index.php?headId=72&pageId=116&subId=89&langId=1&productId=153>

> Network

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32°C
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14:52
23/04/2026



ANA-IFA PATTERNS LOOSELY CORRELATE WITH SPECIFIC ANTIBODIES AND DIAGNOSES

Homogeneous

Pattern	Associated Antigens	Clinical Associations
	Double stranded DNA, histones, DNA/histone complexes, others	SLE, drug induced lupus, and other conditions
	"ENA antigens": Sm, RNP, SS-A/Ro, SS-B/La Nuclear matrix antigens: (hnRNP), others	Autoimmune rheumatic diseases: SLE, MCTD, Sjögren's syndrome, and other conditions
	Centromere proteins A, B, C located at the kinetochore plates	Scleroderma (CREST syndrome) primary biliary cirrhosis
	Fibrillarin, RNA polymerase, NOR-90, Scl-70, Pm/Scl, ribosomal antigens, others	Autoimmune rheumatic diseases: scleroderma, myositis, SLE, and other

Speckled

Centromere

Nucleolar



Dapatkan WhatsApp untuk Windows

+ Ketik pesan





ASOSIASI LPPM PTNBH

MONEV KEMAJUAN

PROGRAM RISET KOLABORASI INDONESIA (RKI)

PRN EQUITY DAPT LPDP TAHUN 2025-2026

SKEMA A2, B1 DAN B2

Kamis, 23 April 2026

UNPAD - UNHAS

Kusmardi

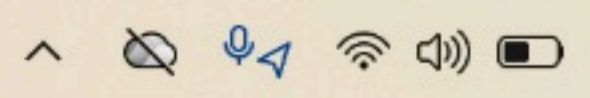
~Dede Moeswir: Selamat ya dr Umar M sukses selalu 5

Dapatkan WhatsApp untuk Windows

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32°C Berawan

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14:58 23/04/2026



IMUNOFLUORESENS

DEVITA FRISKA SANTY
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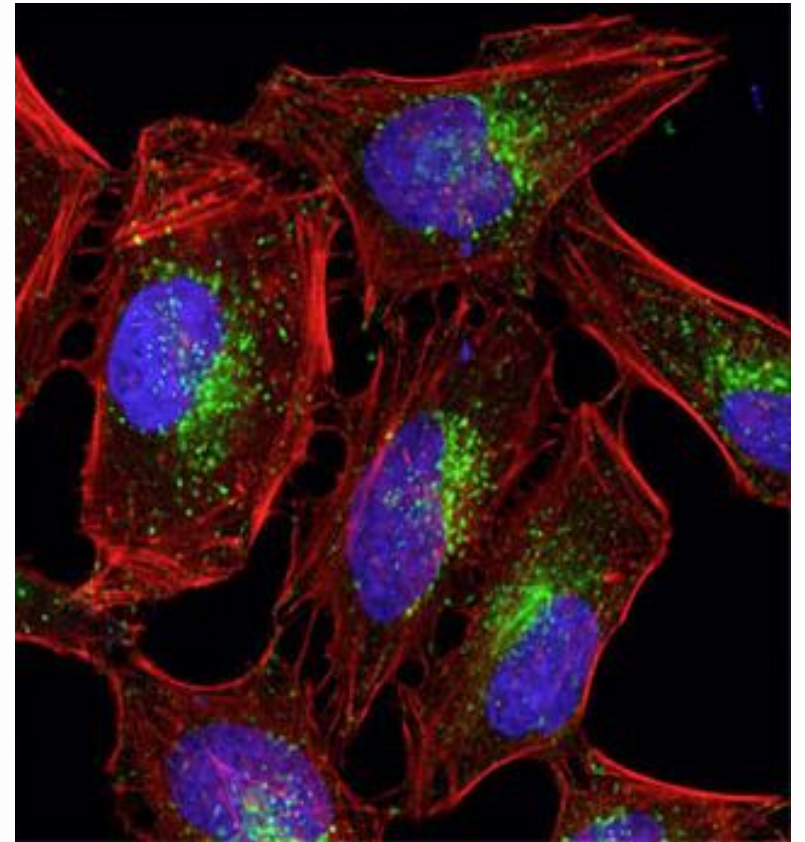
Pembimbing: Prof. Dr. Drs. Kusmardi, MS





PENDAHULUAN

- IMUNOFLUORESENS -> DETEKSI ANTIGEN DENGAN ANTIBODI SPESIFIK YANG TERIKAT DENGAN LABEL FLUORESEN (FLUOROCHROME)
- FLUOROCHROME -> ZAT PEWARNA YANG MEMILIKI KEMAMPUAN UNTUK MENYERAP RADIASI UV PANJANG GELOMBANG PENDEK DAN MEMANCARKAN FLUORESENSI PANJANG GELOMBANG LEBIH PANJANG (LAMPU HIJAU TERLIHAT).

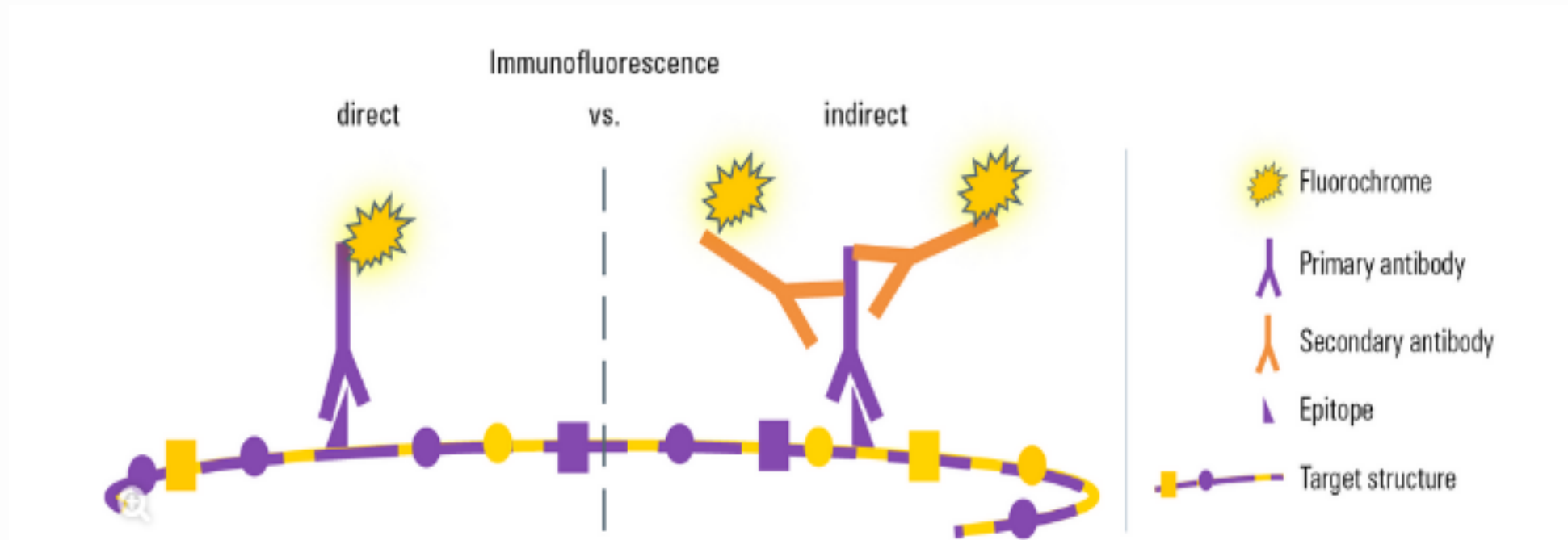


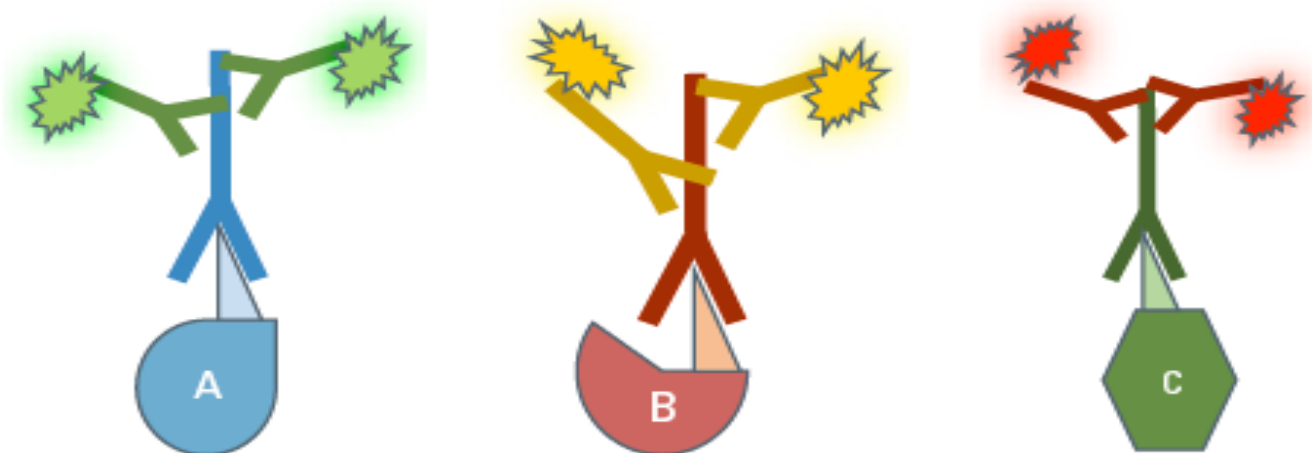


- FLUOROCHROME MENYERAP UV ATAU CAHAYA GELOMBANG TERTENTU -> UNSTABLE EXCITED STATE -> MEMANCARKAN CAHAYA DENGAN PANJANG GELOMBANG BERBEDA -> KEMBALI KE GROUND STATE
 - FLUORESCEIN ISOTHIOCYANATE (FITC), 494 NM -> 518 NM (HIJAU)
 - TETRAMETHYL RHODAMIN ISOTHIOCYANAT , 550 NM -> 580 NM (MERAH)

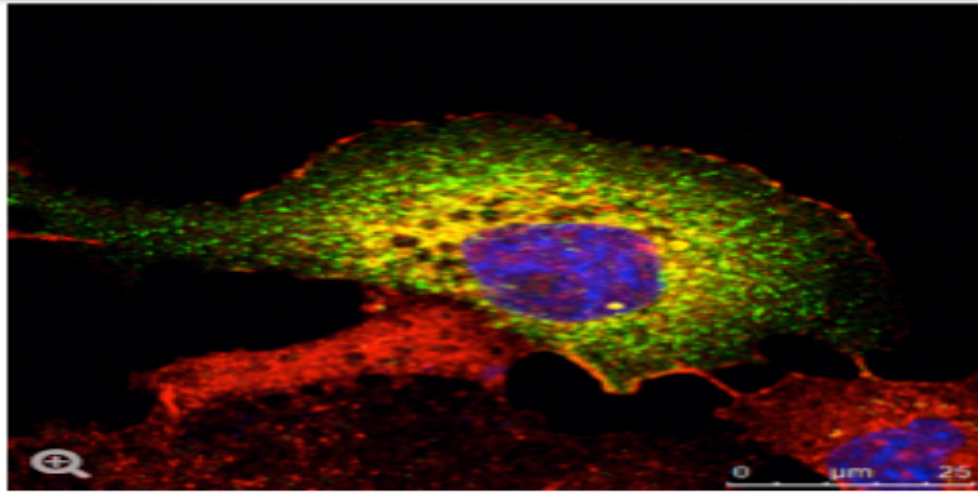
PENDAHULUAN

- TERDAPAT 2 JENIS IF
 - INDIRECT -> FLUOROCHROME TERIKAT PADA ANTIBODI SEKUNDER
 - DIRECT -> FLUOROCHROME TERIKAT PADA ANTIBODI PRIMER

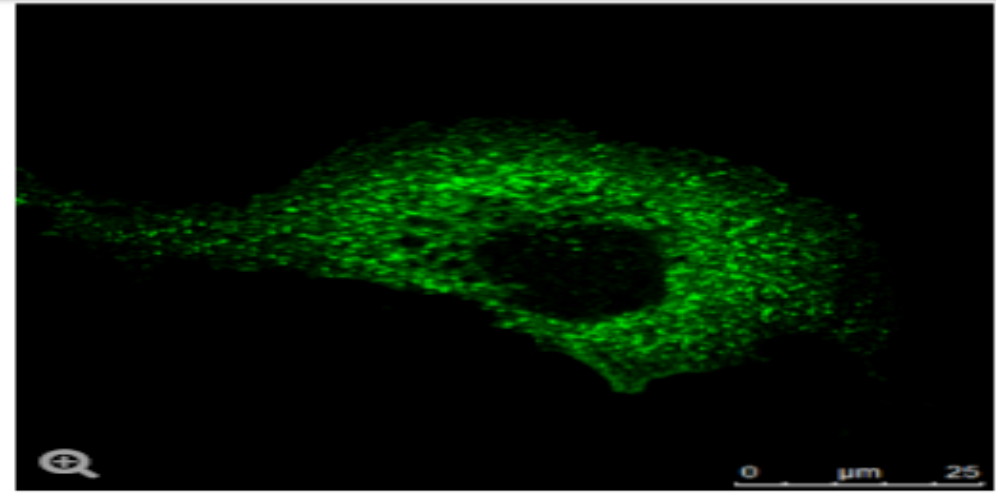




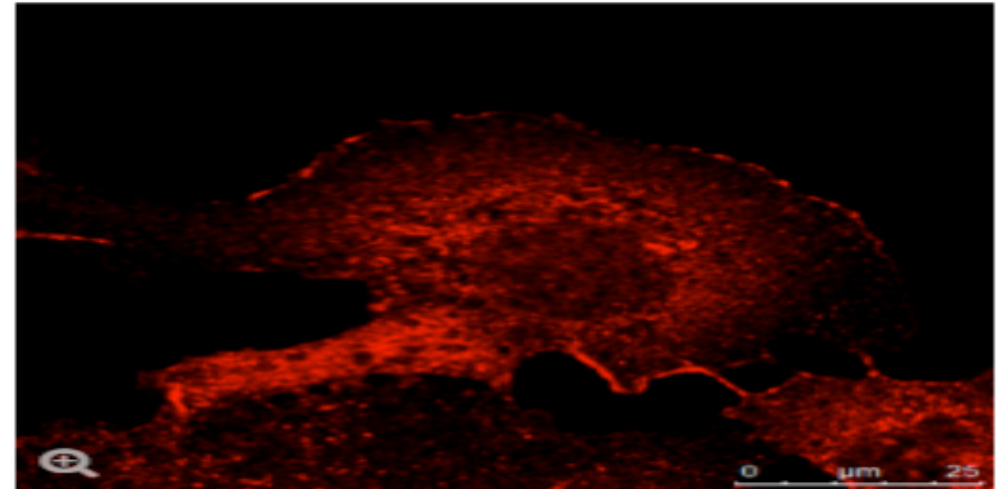
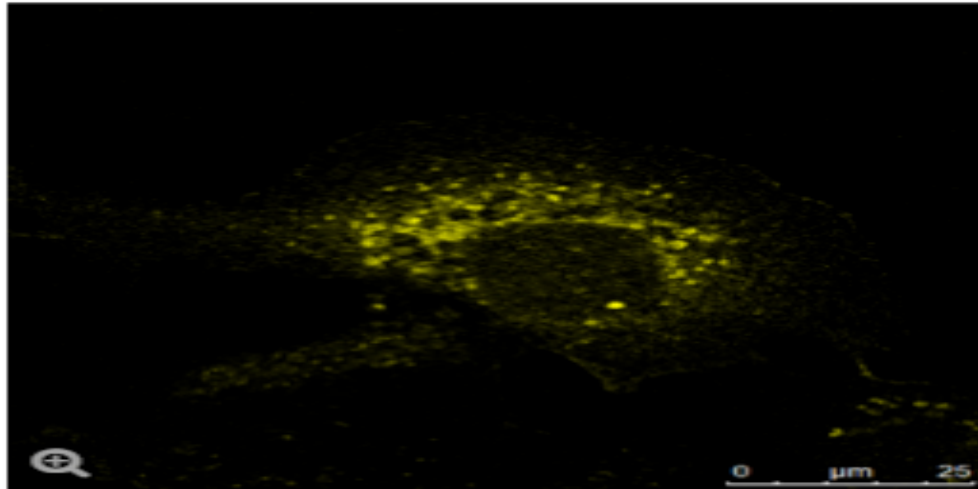
Target protein	Protein A	Protein B	Protein C
Target species	Human	Human	Human
First antibody	Anti-Protein A	Anti-Protein B	Anti-Protein C
First antibody species reactivity	Mouse anti-human	Rabbit anti-human	Rat anti-human
Second antibody species reactivity	Goat anti-mouse	Goat anti-rabbit	Goat anti-rat
Fluorochrome excitation/emission	490/525 nm	556/573 nm	650/665 nm



Overlap



Protein A



Tab. 1: This example of a **multicolor indirect IF** demonstrates how to label simultaneously three different proteins in the same cell. The three first antibodies must derive from different species in order to detect them with three different fluorochrome-coupled secondary antibodies. The secondary antibodies' fluorochromes must differ in their wavelength spectrum for a distinct analysis in the microscope. The cell depicted in the sample image was also treated with Hoechst 33342 for nucleus staining. Microscopy was performed on a Leica TCS SP2.



- KELEBIHAN DIBANDING METODE IMUNOLOGI LAINNYA -> IDENTIFIKASI ANTIGEN DAN LOKASINYA DI DALAM SEL.
- KEBERHASILAN IF DITENTUKAN OLEH :
 - PRESERVASI ANTIGEN SUBSTRAT DAN KUALITAS JARINGAN
 - AFINITAS DAN SPESIFISITAS ANTIBODI DAN KONJUGAT
 - MIKROSKOP
 - QUALITY CONTROL



PRESERVASI ANTIGEN SUBSTRAT (FIKSASI)

- ANTIGEN YG DAPAT DIDETEKSI IF -> VIRUS, PROTOZOA, BAKTERI, ENZIM, HORMON, PROTEIN PLASMA, SEL DAN PENYUSUNNYA.
- SYARAT ANTIGEN DAPAT TERDETEKSI
 - JUMLAH CUKUP
 - TIDAK LARUT IN SITU
 - TIDAK MENGALAMI DENATURASI
- TUJUAN FIKSASI -> MEMPERTAHANKAN STRUKTUR SEL TETAP DALAM BENTUK ASALNYA.
- FIKSASI DAPAT MENUTUP ANTIBODY BINDING SITE



PRESERVASI ANTIGEN SUBSTRAT

- METODE PRESERVASI :
 - AIR-DRIED UNFIXED (POTONG BEKU) -> UNTUK ANTIGEN YG SIFATNYA SENSITIF TERHADAP ZAT KIMIA
 - CHEMICAL CROSSLINKER (FORMALIN BUFFER 10% ATAU GLUTARALDEHIDA) -> DAPAT DIGUNAKAN JIKA ANTIGEN BERSIFAT LARUT/SOLUBLE
 - PELARUT ORGANIK (ETANOL ATAU ASETON) -> PALING TIDAK DISARANKAN



PRESERVASI ANTIGEN SUBSTRAT

AIR-DRIED UNFIXED

- JARINGAN BIOPSI DITRANSPORTASIKAN KE LAB DENGAN MEDIUM TRANSPORT KHUSUS (MICHEL TRANSPOR MEDIUM) PADA PH 7.0
 - DAPAT BERTAHAN BEBERAPA HARI DALAM MICHEL MEDIUM ☐ RISIKO AUTOFLUORESENS DAN BACKGROUND STAINING
 - TERTUTUP DALAM WADAH DAN PH DIPERIKSA SECARA REGULER
- CUCI DENGAN WASH SOLUTION 30 MENIT
- JARINGAN DI SNAP FROZEN (PROSES PEMBEKUAN SECARA CEPAT SAMPAI SUHU DIBAWAH 70 DERAJAT)
- TRIMMING (15 MM) DAN SECTIONING (5 MM) DENGAN CYROSTAT
- AIR-DRYING 30 MENIT
- SLAID DIBUNGKUS DENGAN ALUMINIUM FOIL DAN DISIMPAN DALAM SUHU -20⁰C HINGGA SIAP DI PULAS.



PRESERVASI ANTIGEN SUBSTRAT *CHEMICAL CROSSLINKER*

- PROTEIN CROSSLINKER PADA GUGUS AMINO BEBAS
- KEUNTUNGAN : MORFOLOGI SEL DIPERTAHANKAN DENGAN BAIK
- KELEMAHAN : ANTIGEN JUGA DAPAT MENGALAMI CROSSLINK



PRESERVASI ANTIGEN SUBSTRAT

ORGANIC SOLVENTS

- FIKSASI DENGAN EFEK DEHIDROGENASI DAN PRESIPITASI PROTEIN
- KELEBIHAN :
 - BAIK DALAM MEMPERTAHANKAN ARSITEKTUR SELULAR
 - LEBIH CEPAT
- KELEMAHAN :
 - MELARUTKAN LIPID



ANTIBODI PRIMER & KONJUGAT





- ANTIBODI PRIMER

ANTIBODI YG MENGENALI MOLEKUL TARGET (ANTIGEN) & MENGIKAT PADA AREA SPESIFIK (EPITOPE)

- ANTIBODI TERKONJUGASI

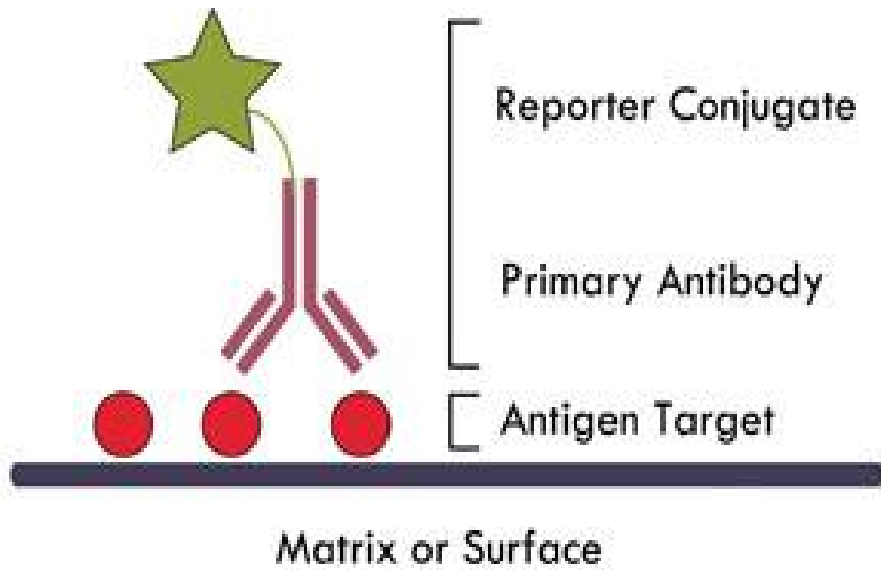
ANTIBODI TERKONJUGASI --> ANTIBODI MONOCLONAL / POLIKLONAL YANG TERIKAT PD LABEL & DIGUNAKAN UNTUK DETEKSI PADA BERBAGAI TEKNIK ASSAY. (EX IMMUNOFLUORESCENT).

- KONJUGAT

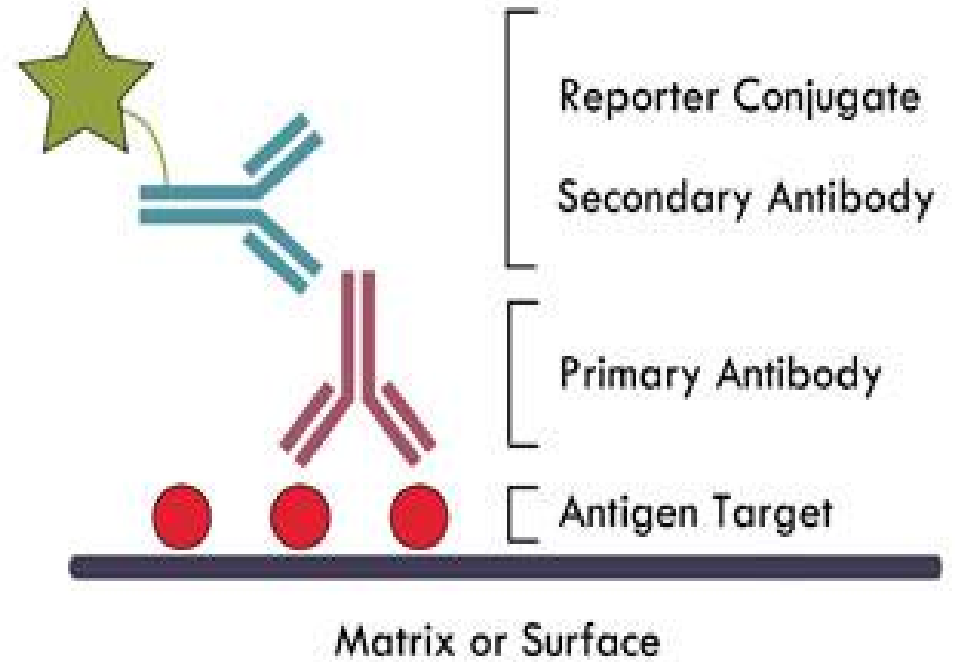
IF --> FLUOROPHORE



Direct Immunoassay using a Conjugated Primary Antibody



Indirect Immunoassay using a Conjugated Secondary Antibody



JENIS IF

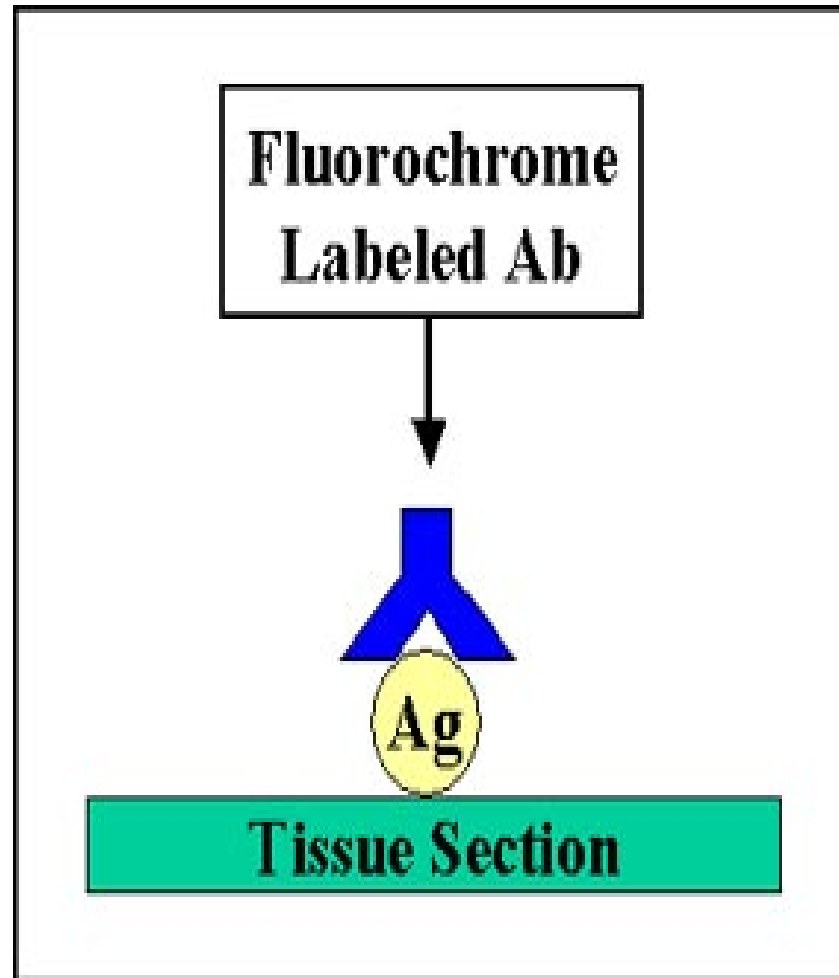
- DIRECT IMMUNOFLUORESCENCE
- INDIRECT IMMUNOFLUORESCENCE



DIRECT IMMUNOFLUORESCENCE

- DETEKSI SECARA DIREK PATHOGEN / ANTIGENNYA PADA JARINGAN ATAU SAMPEL PATOLOGIS
- AG DIFIKSASI PADA SLIDE
- ANTIBODI YANG SUDAH DILABEL *FLUORESCIN* BERADA DIATASNYA
- SLIDE DIBERSIHKAN UNTUK MENYINGKIRKAN ANTIBODI YANG TIDAK TERTEMPEL
- PERIKSA DI BAWAH SINAR UV PADA MIKROSKOP FLUORESCENT
- LOKASI DIMANA ANTIBODI MENEMPEL PADA ANTIGEN SPESIFIK --> MENUNJUKKAN *APPLE GREEN FLUORESCENCE*

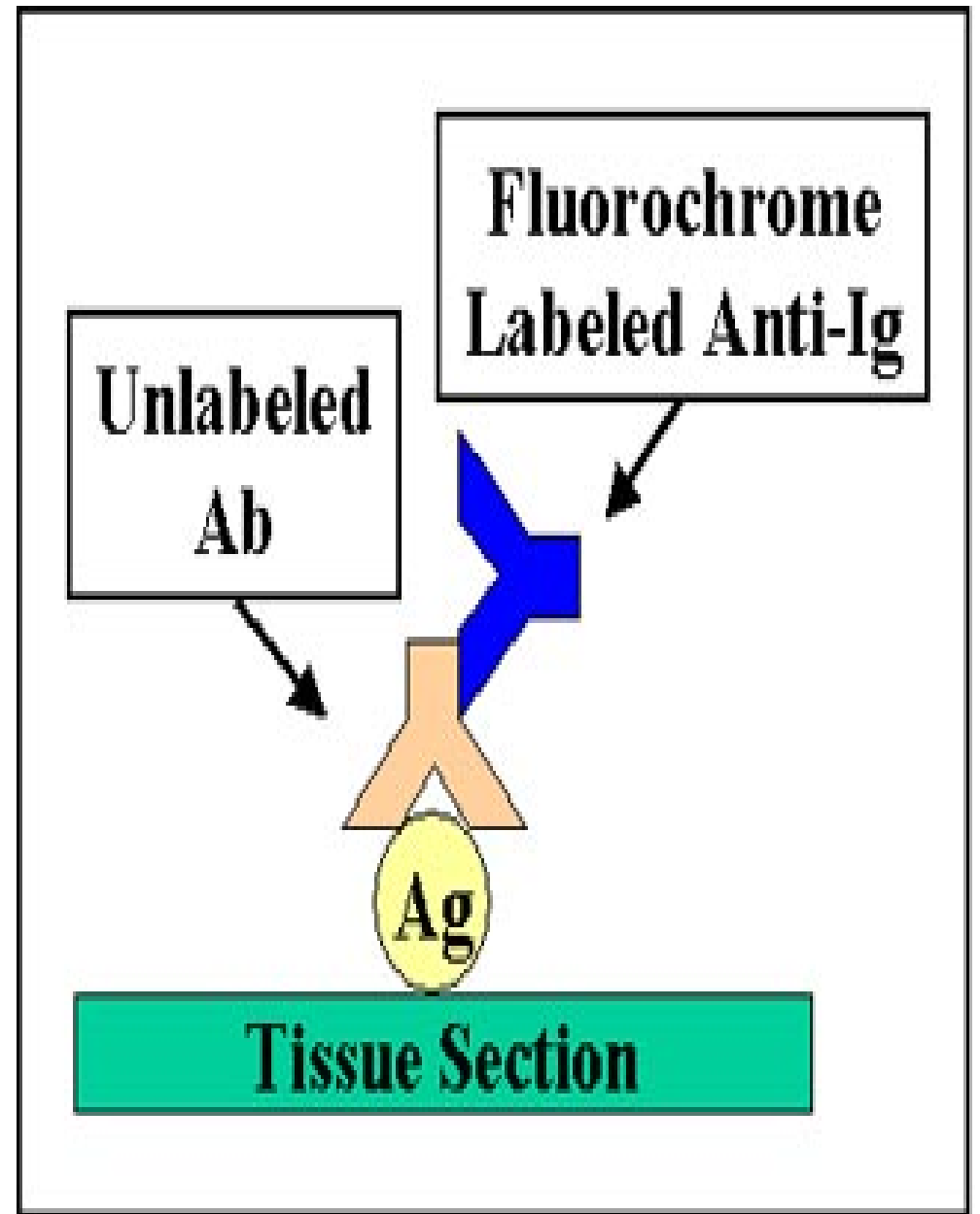
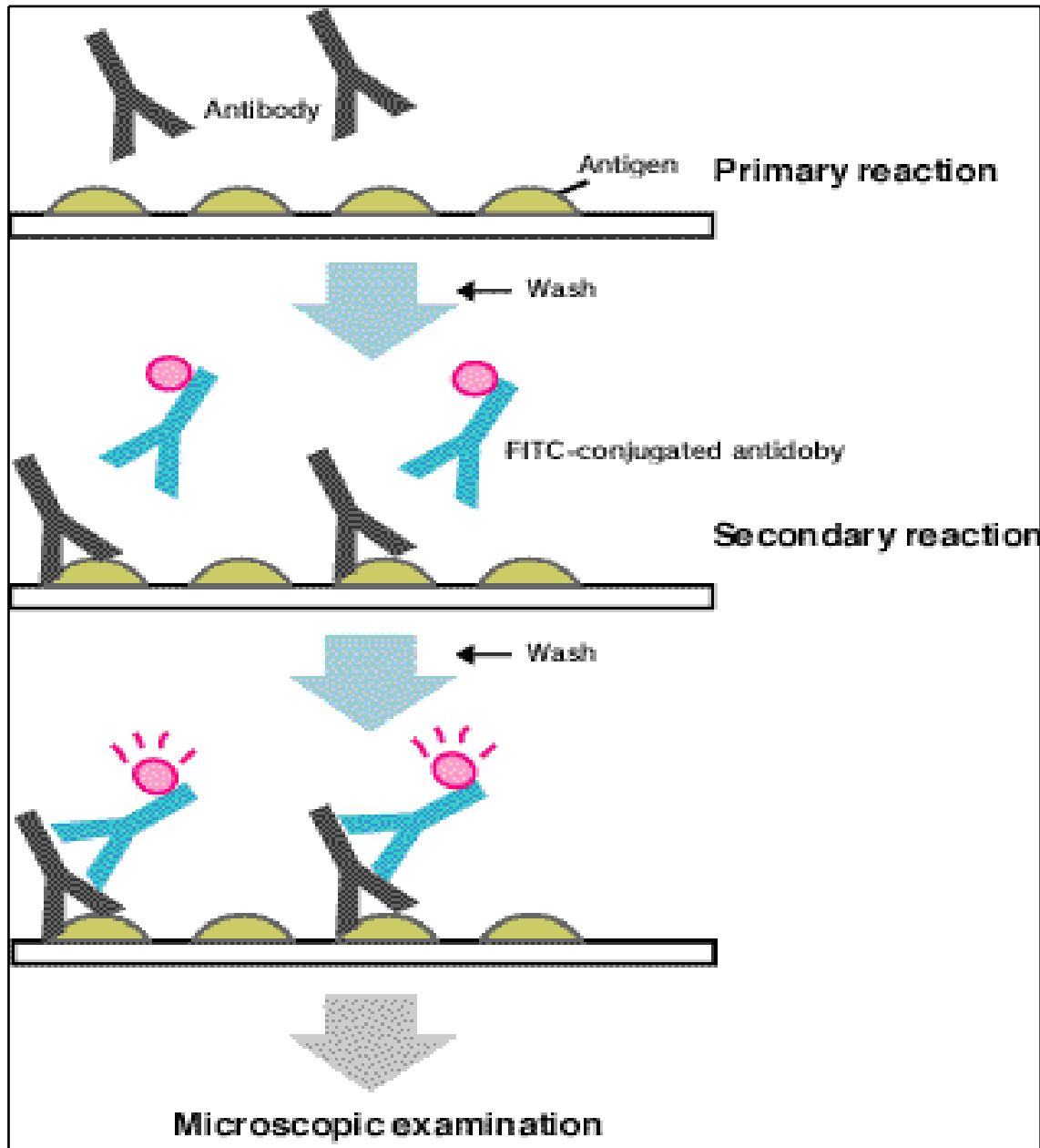
DIRECT IMMUNOFLUORESCENCE





INDIRECT IMMUNOFLUORESCENCE

- TEKNIK 2 LAPIS / 2 LANGKAH
- *UNLABELLED ANTIBODY* DIGUNAKAN SECARA LANGSUNG PADA SUBSTRAT JARINGAN
- DIBERIKAN *FLUOROCHROME-CONJUGATED ANTI-IMMUNOGLOBULIN SERUM*

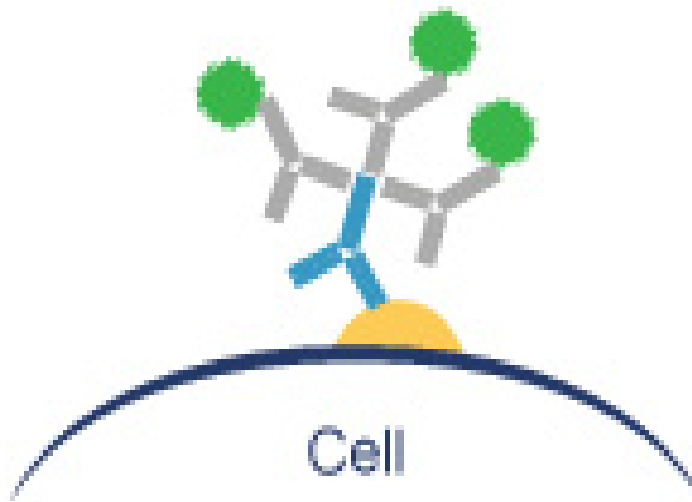








Direct

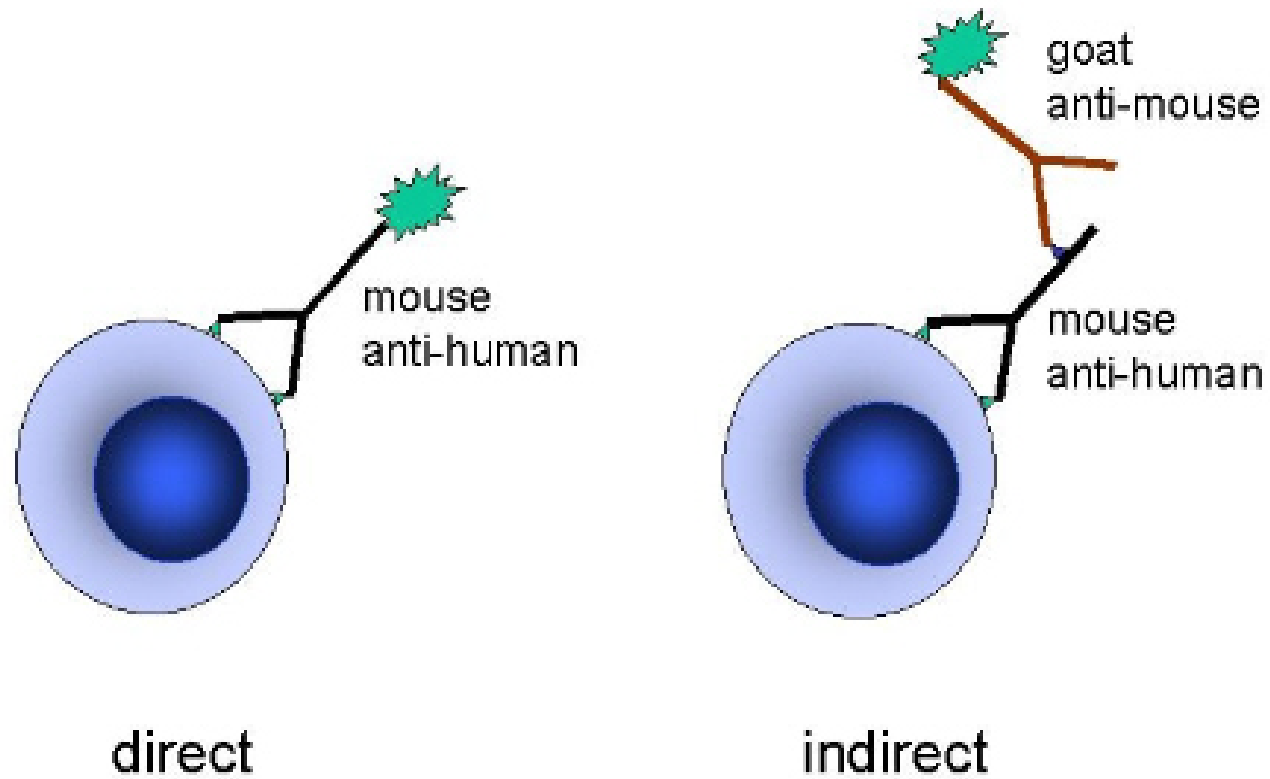


Indirect



-  Antigen
-  Primary Antibody
-  Secondary Antibody
-  Fluorophore

Immunofluorescent Methods





FLUOROCHROME

- PEWARNA YANG MEMILIKI KEMAMPUAN UNTUK MENYERAP RADIASI UV PANJANG GELOMBANG PENDEK DAN MEMANCARKAN FLUORESENSI PANJANG GELOMBANG LEBIH PANJANG (LAMPU HIJAU TERLIHAT).

Table 19.1 Spectral characteristics of commonly used fluorochromes

Fluorochrome	Absorption maximum (nm)	Emission maximum (nm)	Observed color
Fluorescein (FITC)	494	518	green
Rhodamine (TRITC)	550	580	red
Texas Red™	595	615	red
R-Phycoerythrin (PE)	565	575	orange/red

Allan, V.J., 2000. Protein localization by fluorescence microscopy. Oxford University Press, Oxford.



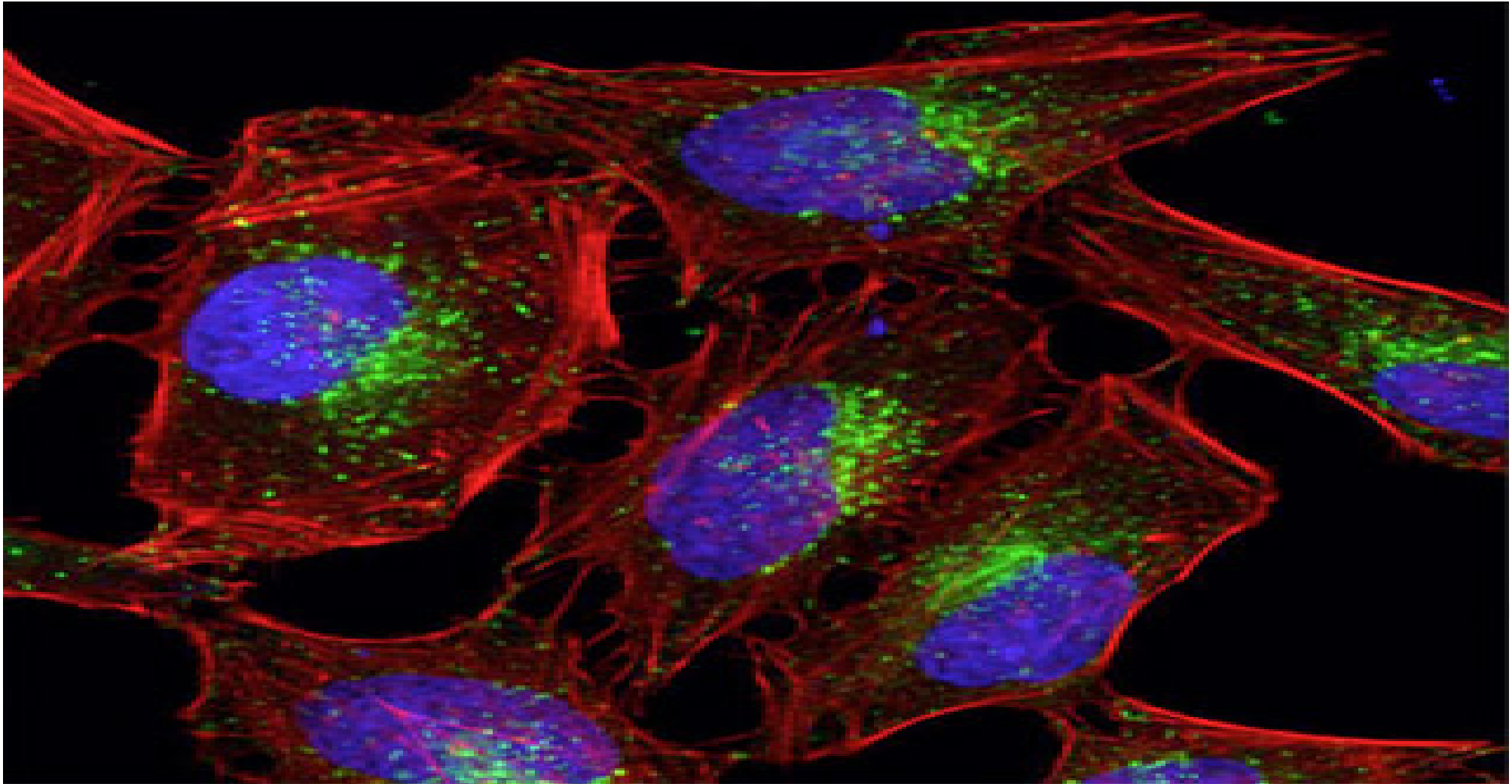
- PEWARNA FLUORESCENT YANG PALING UMUM DIGUNAKAN : FLUORESCEIN DAN RHODAMINE, TETAPI ZAT-ZAT LAIN YANG SANGAT BERFLUORESENSI (PHYCOERYTHRIN DAN PHYCOBILIPROTEINS) JUGA MULAI DIGUNAKAN.
- FLUORESCEIN (FITC): MENYERAP CAHAYA BIRU & MEMANCARKAN FLUORESENSI KUNING-HIJAU YANG INTENS (APEL HIJAU)
- RHODAMINE: MENYERAP KUNING-HIJAU DAN MEMANCARKAN FLUORESENSI MERAH GELAP.
- PHYCOERYTHRIN: PEREDAM CAHAYA YANG EFISIEN DAN PEMANCAR CAHAYA MERAH YANG CEMERLANG



KONJUGASI

- TERGANTUNG PD TIPE FLUOROKROM
- OVER CONJUGATION --> BACKGROUND STAINING TINGGI
- UNDER CONJUGATION --> LEVEL FLUORESCENCE RENDAH
- FREE CHROMOPHORE --> DIHILANGKAN DENGAN DIALISIS TERHADAP 0,15M NATRIUM KLORIDA / KROMATOGRAFI KOLOM MENGGUNAKAN SEPHADEX
- OPTIMASI

Staining Procedures





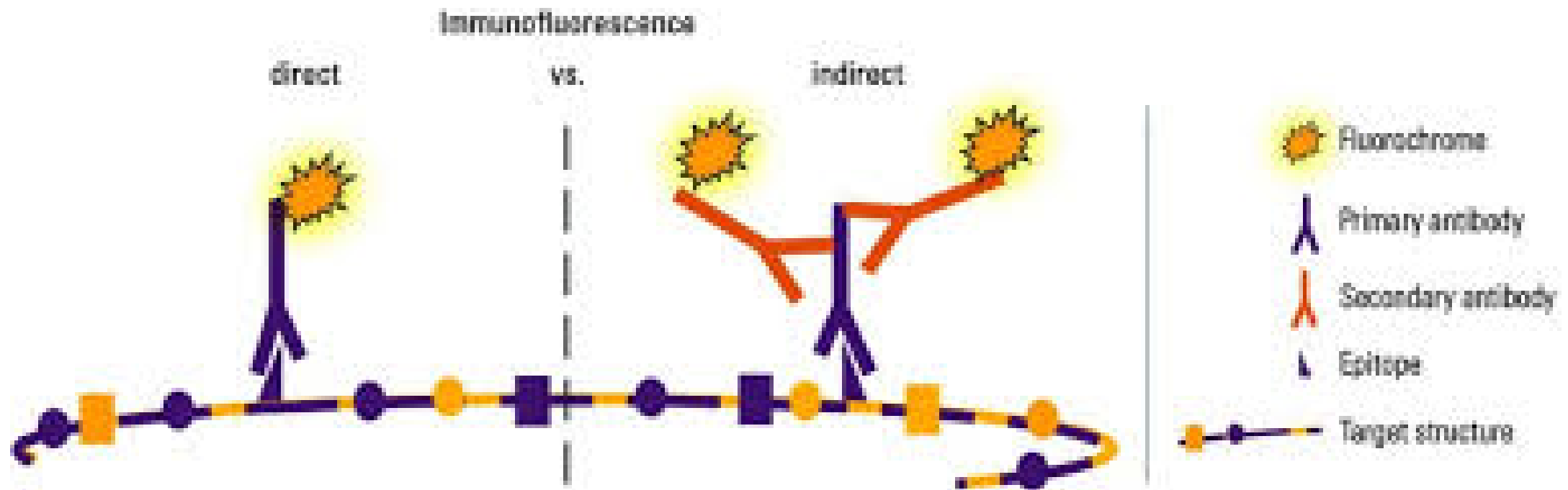
Cara Pewarnaan IF

- ◆ *Direct immunofluorescence*
- ◆ *Indirect immunofluorescence*

Direct Immunofluorescence:

- Antibodi yang berlabel fluorescein diteteskan pada spesimen.
- Slaid dicuci untuk membersihkan antibodi yang tidak berikatan.
- Diperiksa di bawah mikroskop dengan sinar UV.
- Antibodi yang berikatan dengan antigen spesifik akan menunjukkan warna hijau apel berpendar.
- Fungsi: Deteksi langsung patogen/antigen pada jaringan atau sampel patologi.

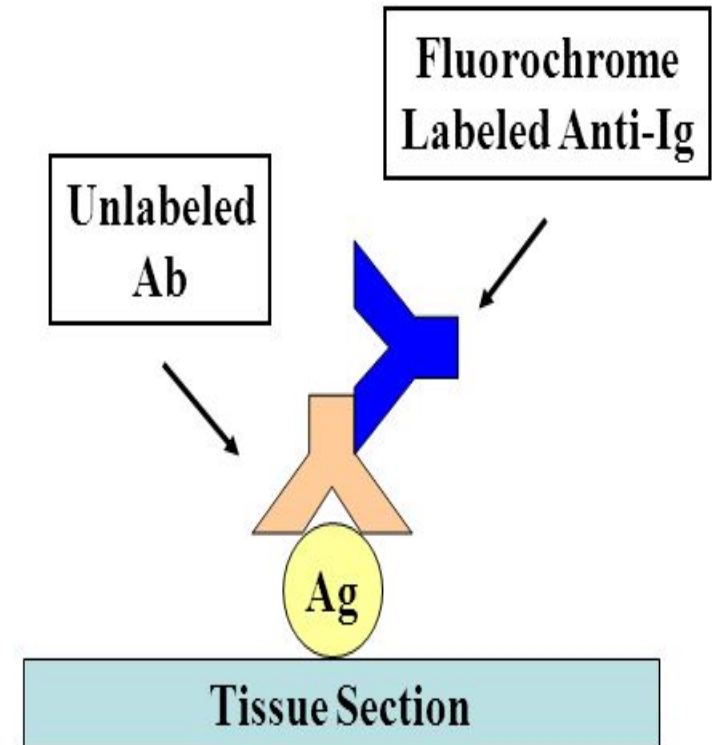
DIRECT IMMUNOFLUORESCENCE





Indirect Immunofluorescence

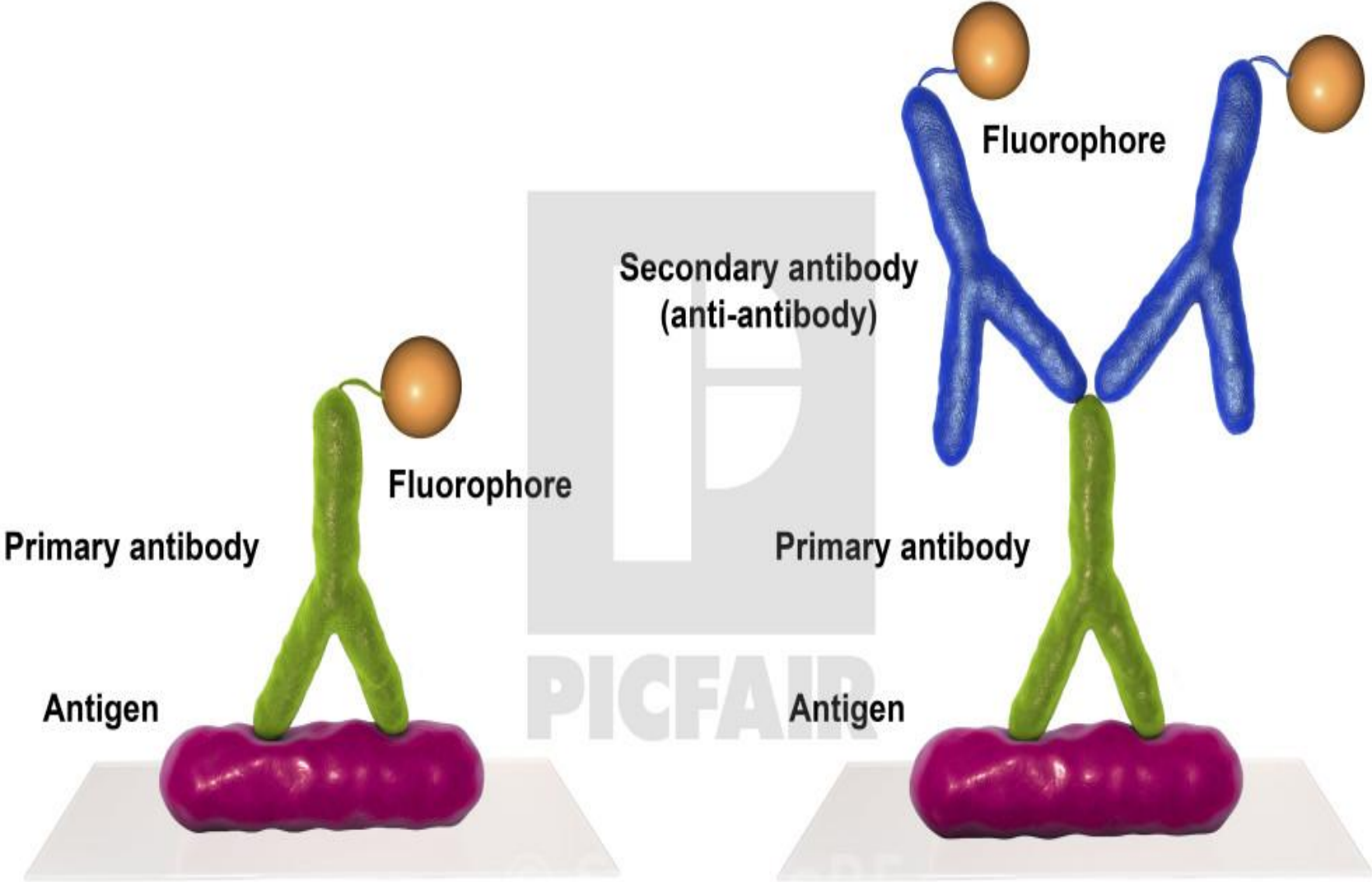
- Teknik double-layer
- Antibodi yang tidak berlabel diberikan pada substrat jaringan.
- Diberikan serum anti-Ig yang terkonjugasi dengan fluorokrom



Immunofluorescence

Direct

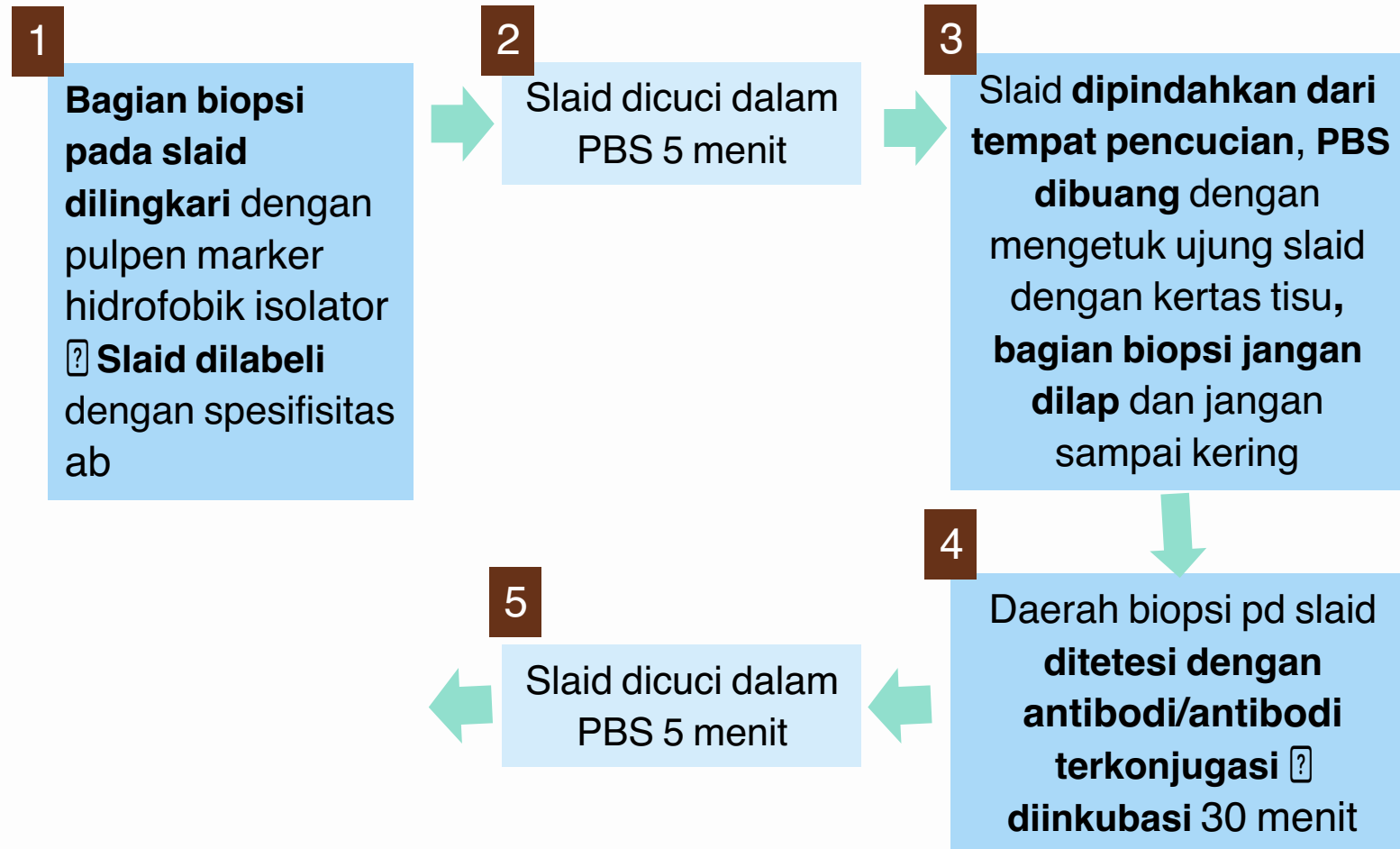
Indirect





(untuk *direct* dan *indirect immunofluorescence*)

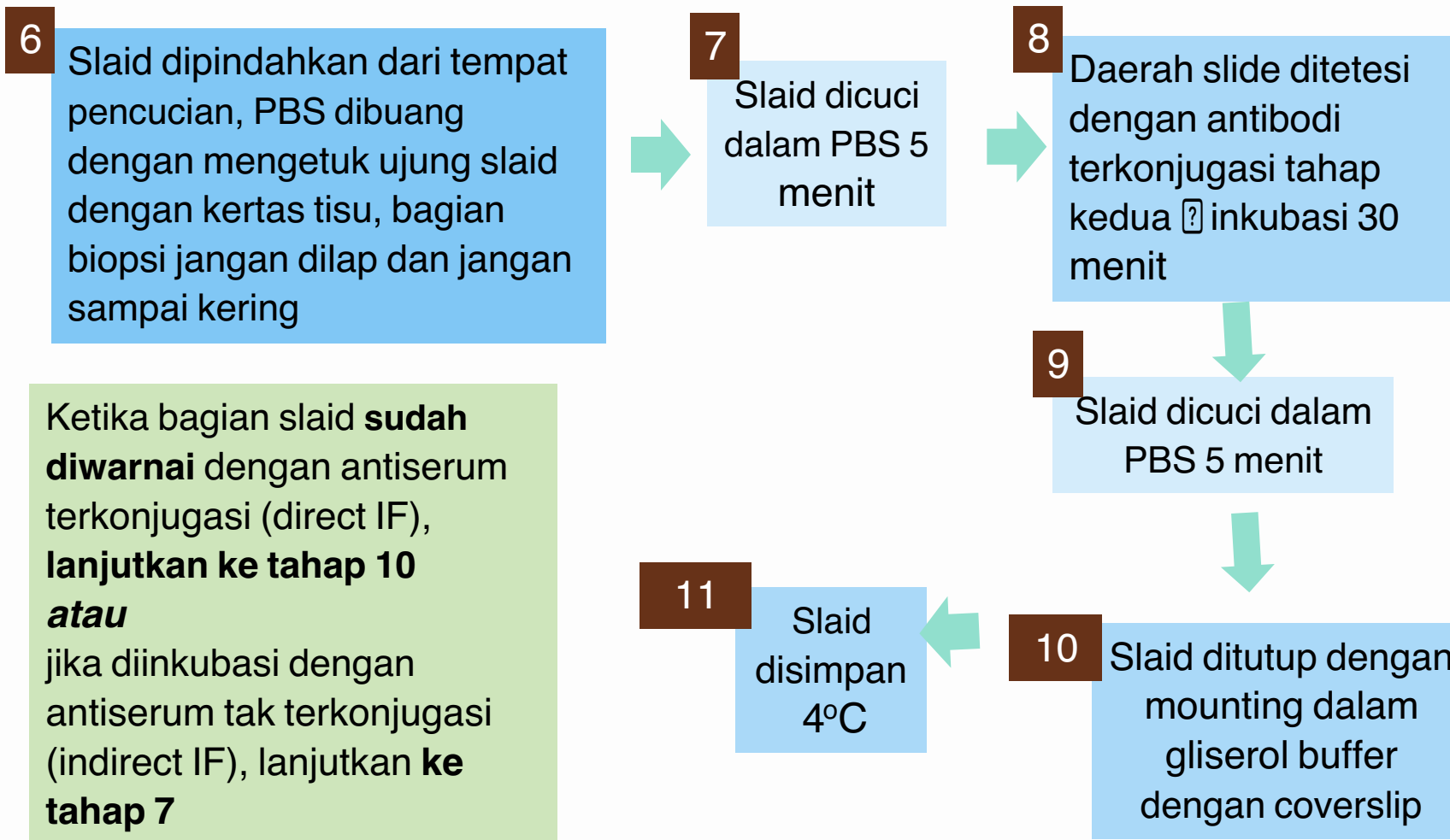
PROSEDUR PEWARNAAN BIOPSI





Prosedur Pewarnaan Biopsi

(untuk *direct* dan *indirect immunofluorescence*)

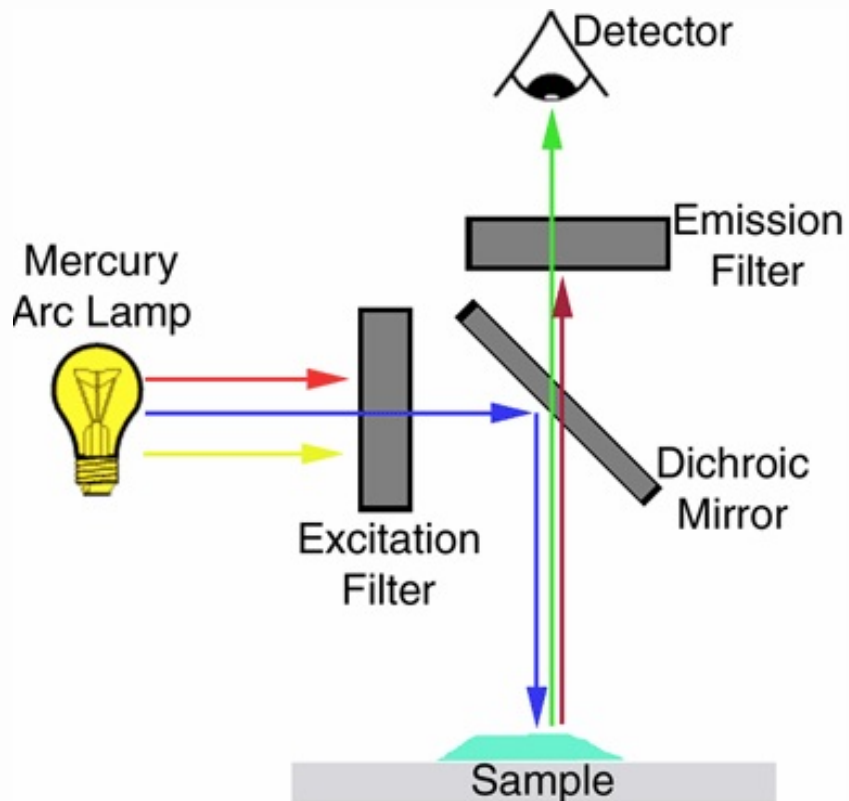




Mikroskop Fluorescens

- Mikroskop fluoresens mengirimkan sinar dari sumber cahaya/sinar (*mercury vapor* atau *xenon*) ke slaid (merangsang *fluorochrome*) dan kemudian dipancarkan ke detektor.
- Penyerapan energi oleh *fluorochrome* seiring dengan energi yang dipancarkan.
- Sumber cahaya/sinar dan pengaturan filter mikroskop → hasil yang baik.

Mikroskop Fluorescens



- Fungsi *excitation filter* : menyaring cahaya/sinar (yang diteruskan yaitu yang sesuai dengan *fluorochrome*).
- Fungsi *dichroic mirror* : memantulkan sinar eksitasi yang terseleksi dan kemudian mentransmisikan sinar ke mata.



Mikroskop Fluorescens

- *Light-emitting diode* (LED) diperkenalkan sebagai sumber cahaya/sinar dari mikroskop fluoresens.
- Keuntungan:
 - Tidak menghasilkan panas yang berlebihan.
 - Bertahan 8000-10000 jam.
 - Tidak berisiko meledak.
 - Tidak perlu waktu untuk warm-up atau cool-down.
 - Menghasilkan cahaya monokrom dengan intensitas tinggi.



Mikroskop Fluorescens

- Kekurangan mikroskop fluorescens:
 - Chromophobe dapat pudar, terutama jika terlalu banyak terpapar dengan sinar/cahaya.
 - Slaid tidak dapat digunakan permanen → dokumentasi.



Quality Control

- *QUALITY CONTROL* MERUPAKAN KOMPONEN PENTING UNTUK MENJAMIN KONSISTENSI DAN RELIABILITAS DARI SUATU HASIL.
- EVALUASI SENSITIVITAS DAN SPESIFISITAS ANTISERUM DAN *CONJUGATES*.



Quality Control

- Setiap slaid immunofluoresens harus memiliki slaid dengan deposit antigen yang sudah dikenal (*slaid quality control*).
- Hasil slaid quality control menyimpang → rejected.



Quality Control

- Warna latar belakang yang kuat atau *false-positive* terjadi karena :
 - Proses washing slaid tidak adekuat setelah inkubasi antibodi atau conjugate.
 - Rasio fluorochrome dengan protein besar.
 - Inkubasi jaringan dengan reagen terlalu lama.



BIOPSI GINJAL & KULIT

SAMPEL BIOPSI KULIT DIPERIKSA UNTUK MELIHAT KEBERADAAN **IGG, IGA, IGM, KAPPA (K), LAMBDA (Λ), KOMPLEMEN C3** DAN **KOMPLEMEN C1Q**.

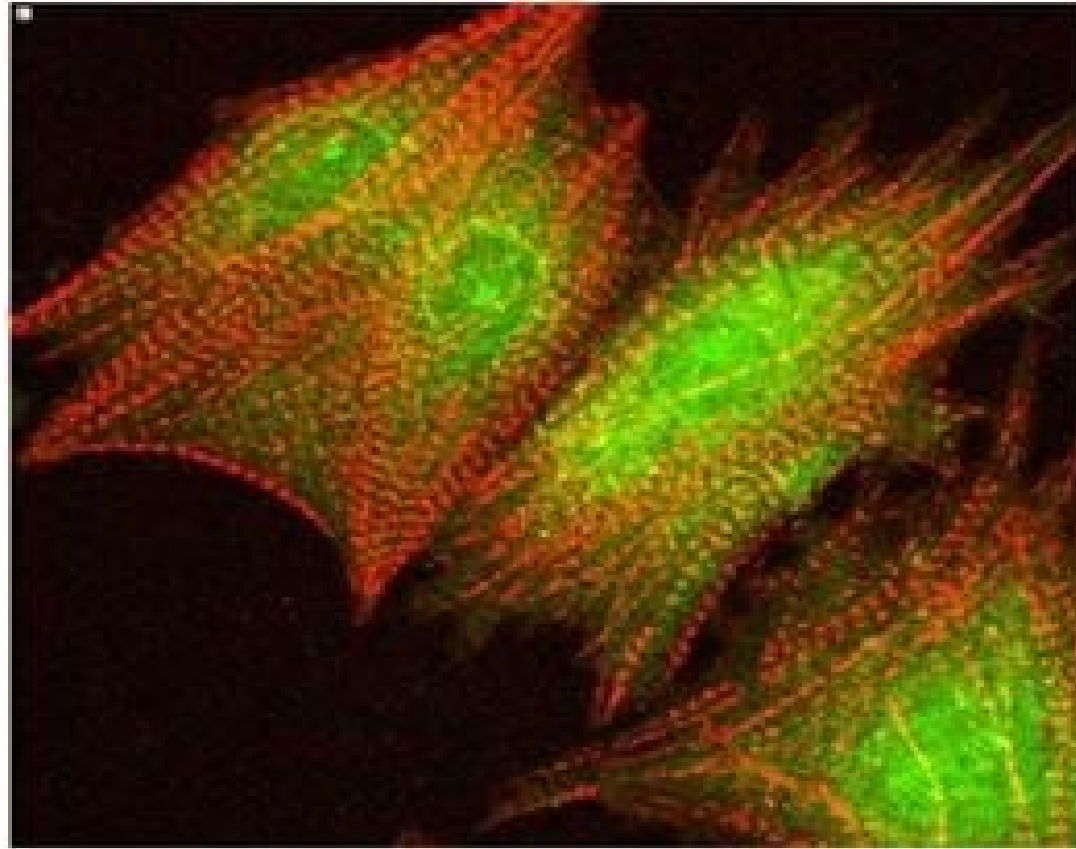
SAMPEL BIOPSI GINJAL DIWARNAI UNTUK **IGG, IGA, K, Λ, C3, C1Q, C4D** DAN **AMYLOID P**

BIOPSI TRANSPLANTASI GINJAL DIPERIKSA UNTUK MELIHAT INFILTRASI LIMFOSIT MENGGUNAKAN **CD3, CD4, CD8, DAN CD19**

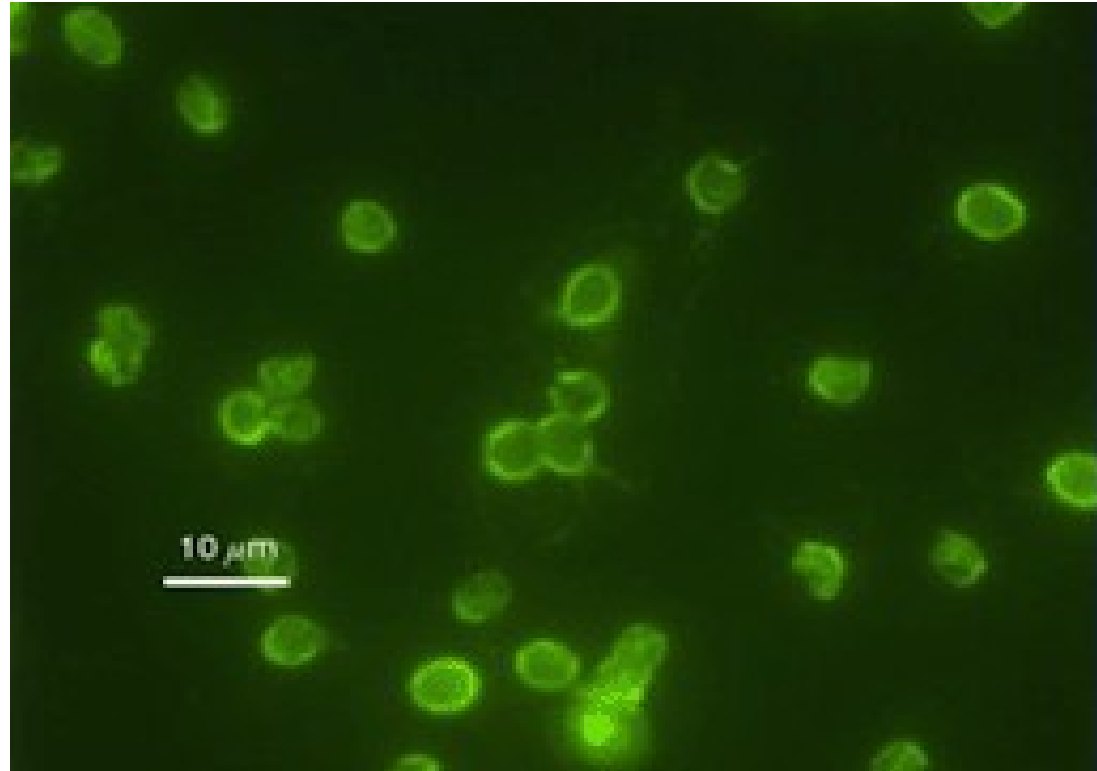


BIOPSI GINJAL & KULIT

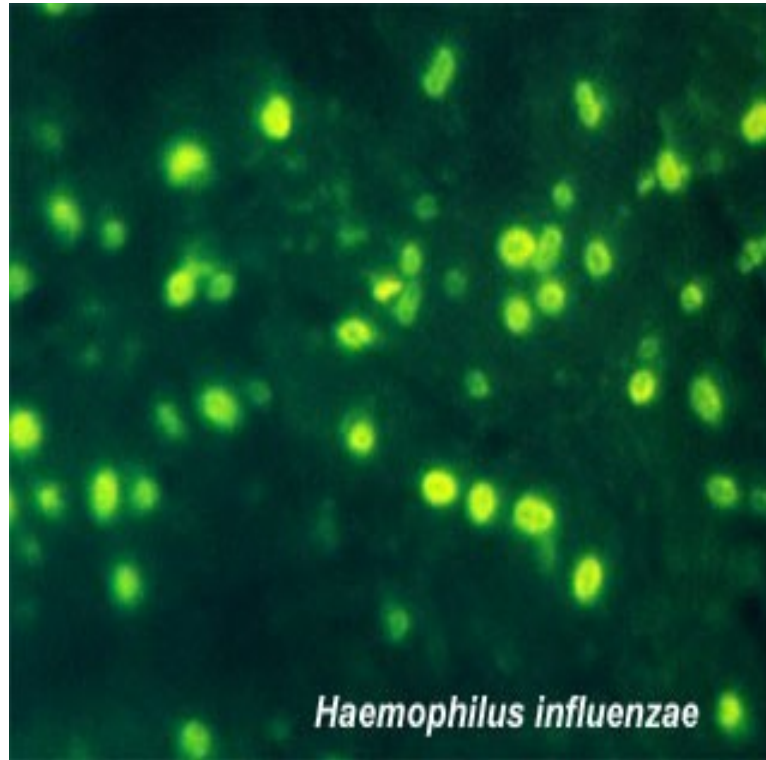
- STAINING RENAL & SKIN BIOPSIES [?] **DIRECT & INDIRECT**
TERGANTUNG AVAILABILITAS DARI ANTISERUM MONOSPESIFIK YANG COCOK TERHADAP PROTEIN MANUSIA
- KETIKA REAGEN TIDAK TERSEDIA/ANTIGEN TERDAPAT DLM JUMLAH KECIL & SENSITIVITAS BESAR DIBUTUHKAN [?] **INDIRECT STAINING/SANDWICH**
 - AB PRIMER TDK BERLABEL DINKUBASI DENGAN JARINGAN DAN ANTIBODI TERKONJUGASI YANG DIBERIKAN PADA HEWAN AB PRIMER DIGUNAKAN UNTUK MENDETEKSI ADANYA IKATAN



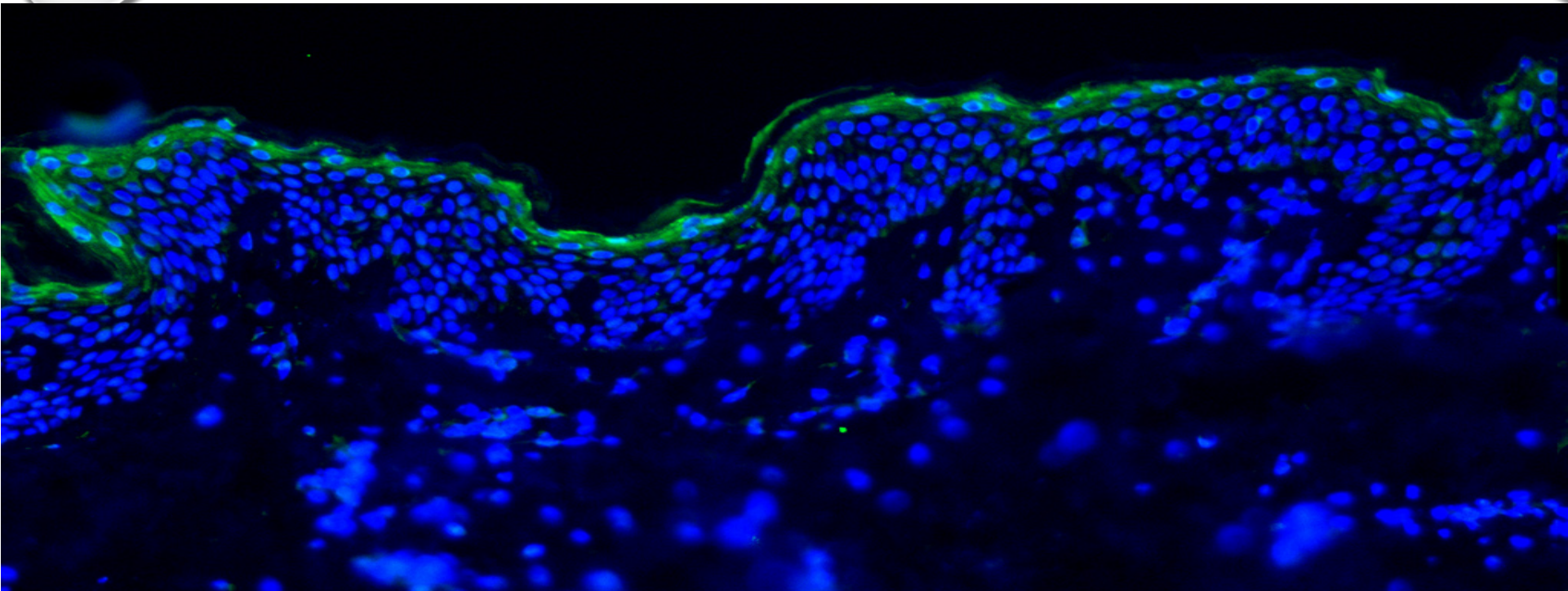
**Confocal image to detect phosphorylated AKT
(green) in cardiomyocytes infected with
adenovirus**



Ookista Cryptosporidium parvum



Haemophilus influenzae

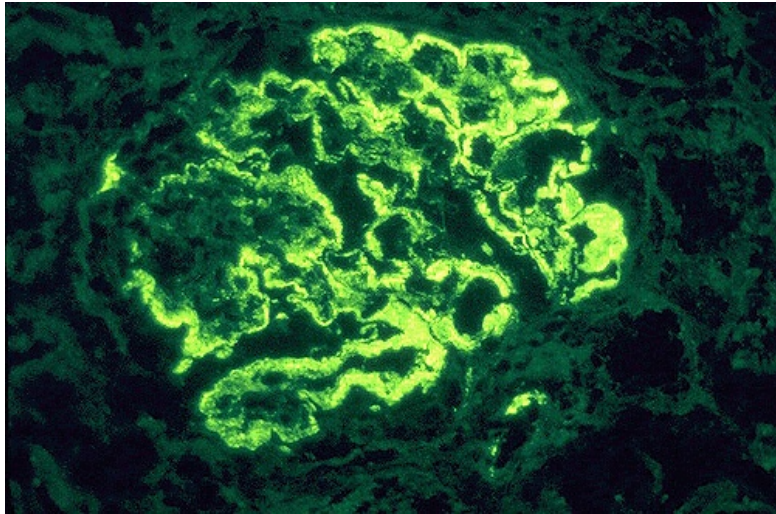


PENERAPAN IMMUNOFLUORESENS

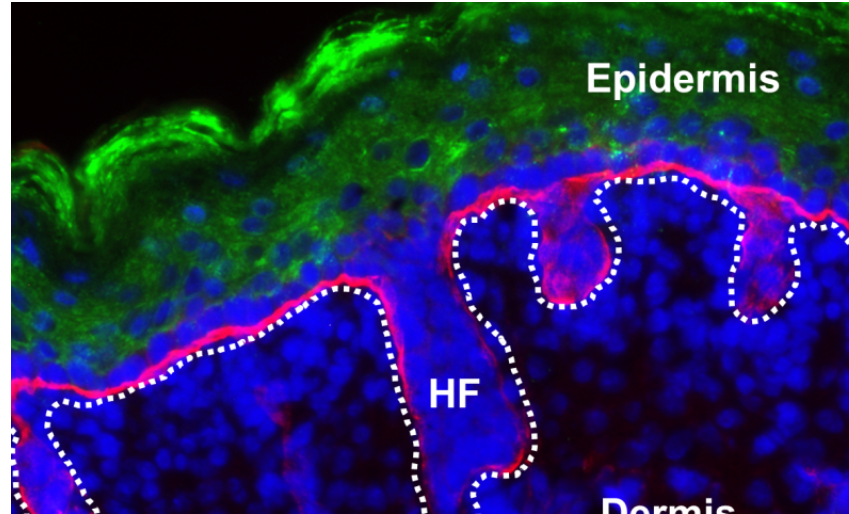
HISTOPATOLOGI DIAGNOSTIK

PENERAPAN IMMUNOFLUORESENS

Ginjal



Kulit





GINJAL

- DETEKSI & DISTRIBUSI ANTIGEN -> POLA DISTRIBUSI
- KELAINAN GLOMERULUS

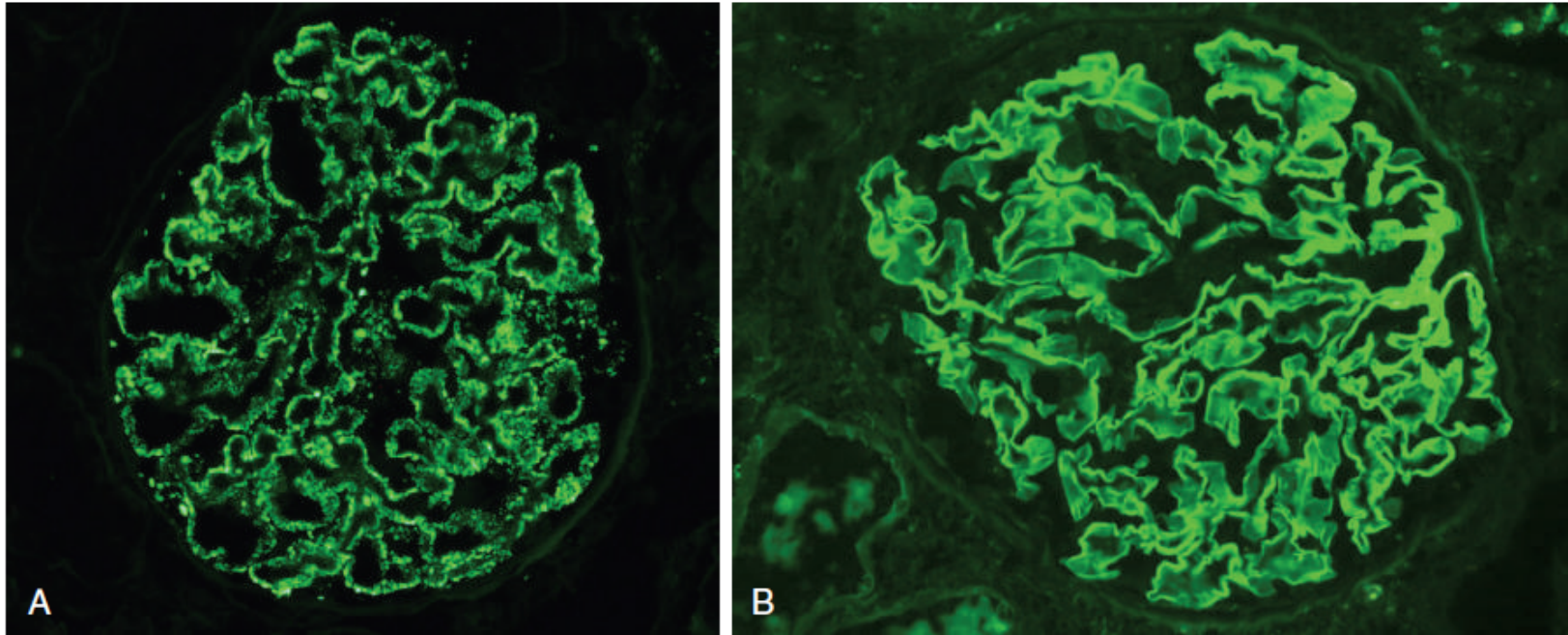


Fig. 14.4 Two patterns of deposition of immune complexes as seen by immunofluorescence microscopy. (A) Granular, characteristic of circulating and in situ immune complex deposition. (B) Linear, characteristic of classic anti-glomerular basement membrane (anti-GBM) antibody glomerulonephritis. (A, Courtesy of Dr. J. Kowalewska, Department of Pathology, University of Washington, Seattle, Washington.)

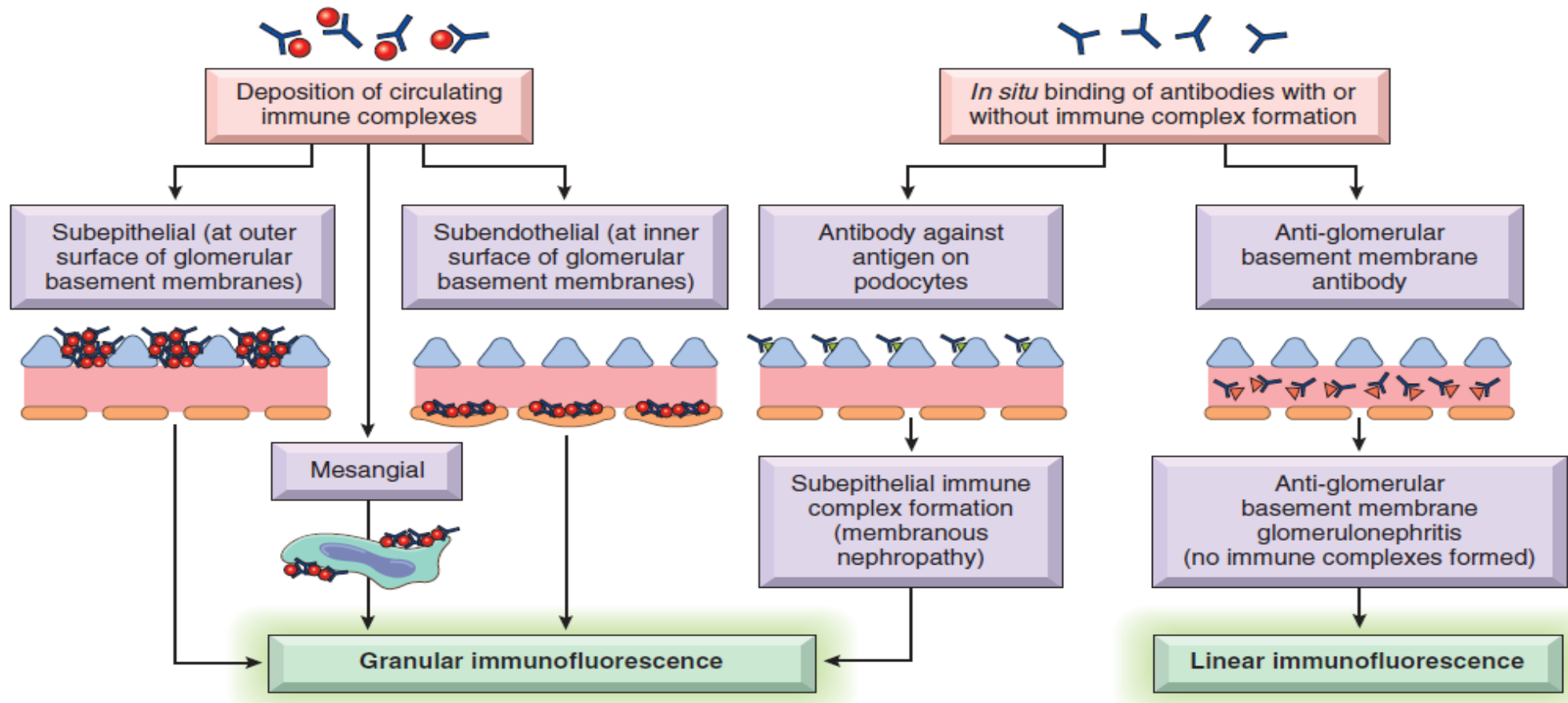


Fig. 14.3 Antibody-mediated glomerular injury. Injury can result either from the deposition of circulating immune complexes or from antibody-binding to glomerular components followed by formation of complexes in situ. Deposition of circulating immune complexes gives a granular immunofluorescence pattern. Anti-glomerular basement membrane (anti-GBM) antibody glomerulonephritis is characterized by a linear immunofluorescence pattern; there is no immune deposit formation in this disease.

Table 14.2 Summary of Major Primary Glomerular Diseases

Disease	Most Frequent Clinical Presentation	Pathogenesis	Glomerular Pathology		
			Light Microscopy	Fluorescence Microscopy	Electron Microscopy
Minimal-change disease	Nephrotic syndrome	Unknown; podocyte injury	Normal	Negative	Effacement of foot processes; no deposits
Focal segmental glomerulosclerosis	Nephrotic syndrome; nonnephrotic range proteinuria	Unknown: reaction to loss of renal mass; plasma factor?	Focal and segmental sclerosis and hyalinosis	Usually negative; IgM and C3 may be present in areas of scarring	Effacement of foot processes; epithelial denudation
Membranous nephropathy	Nephrotic syndrome	In situ immune complex formation; PLA2R antigen in most cases of primary disease	Diffuse capillary wall thickening and subepithelial "spike" formation	Granular IgG and C3 along GBM	Subepithelial deposits
Membranoproliferative glomerulonephritis (MPGN) type I	Nephrotic/nephritic syndrome	Immune complex	Membranoproliferative pattern; GBM splitting	Granular IgG, C3, C1q and C4 along GBM and mesangium	Subendothelial deposits
C3 glomerulopathy (dense deposit disease and C3 glomerulonephritis)	Nephrotic/nephritic syndrome; nonnephrotic proteinuria	Activation of alternative complement pathway; antibody-mediated or hereditary defect in regulation	Mesangial proliferative or membranoproliferative patterns	C3	Mesangial, intramembranous and subendothelial electron-dense or "waxy" deposits
Acute postinfectious glomerulonephritis	Nephritic syndrome	Immune complex mediated; circulating or planted antigen	Diffuse endocapillary proliferation; leukocytic infiltration	Granular IgG and C3 along GBM and mesangium	Primarily subepithelial humps
IgA nephropathy	Recurrent hematuria or proteinuria	Immune complexes containing IgA	Mesangial or focal endocapillary proliferative glomerulonephritis	IgA ± IgG, IgM, and C3 in mesangium	Mesangial and paramesangial dense deposits
Anti-GBM disease (e.g. Goodpasture syndrome)	Rapidly progressive glomerulonephritis	Autoantibodies against collagen type IV α 3 chain	Extracapillary proliferation with crescents; necrosis	Linear IgG and C3; fibrin in crescents	No deposits; GBM disruptions; fibrin
Pauci-immune glomerulonephritis	Rapidly progressive glomerulonephritis	Anti-neutrophil cytoplasmic antibody	Extracapillary proliferation with crescents; necrosis	Fibrin in crescents	No deposits; GBM disruptions; fibrin

GBM, Glomerular basement membrane; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M.

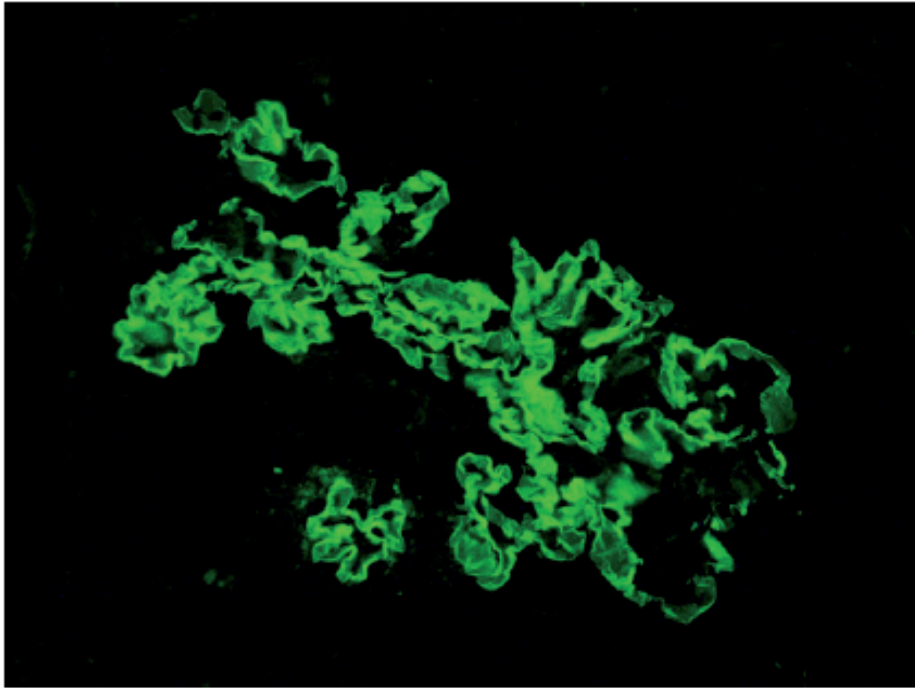


Figure 19.1 Renal biopsy, demonstrating glomerular basement membrane staining pattern, as seen in Goodpasture's syndrome.

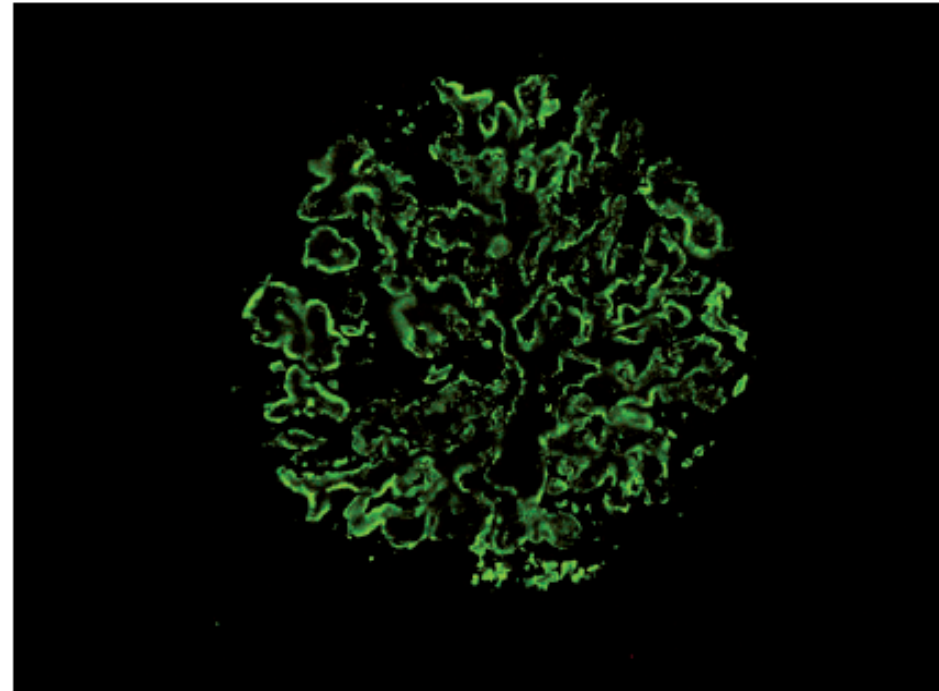


Figure 19.2 Renal biopsy, demonstrating membranous staining pattern with antibody to C3.

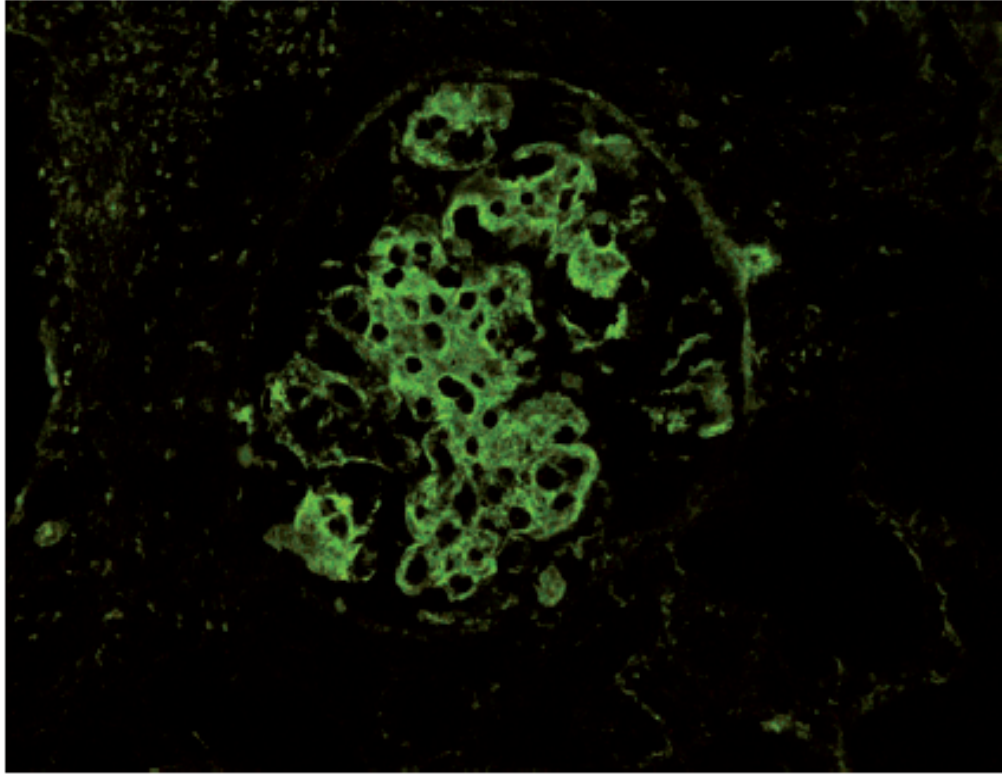


Figure 19.3 Renal biopsy in lupus, demonstrating glomerular staining for C1q.

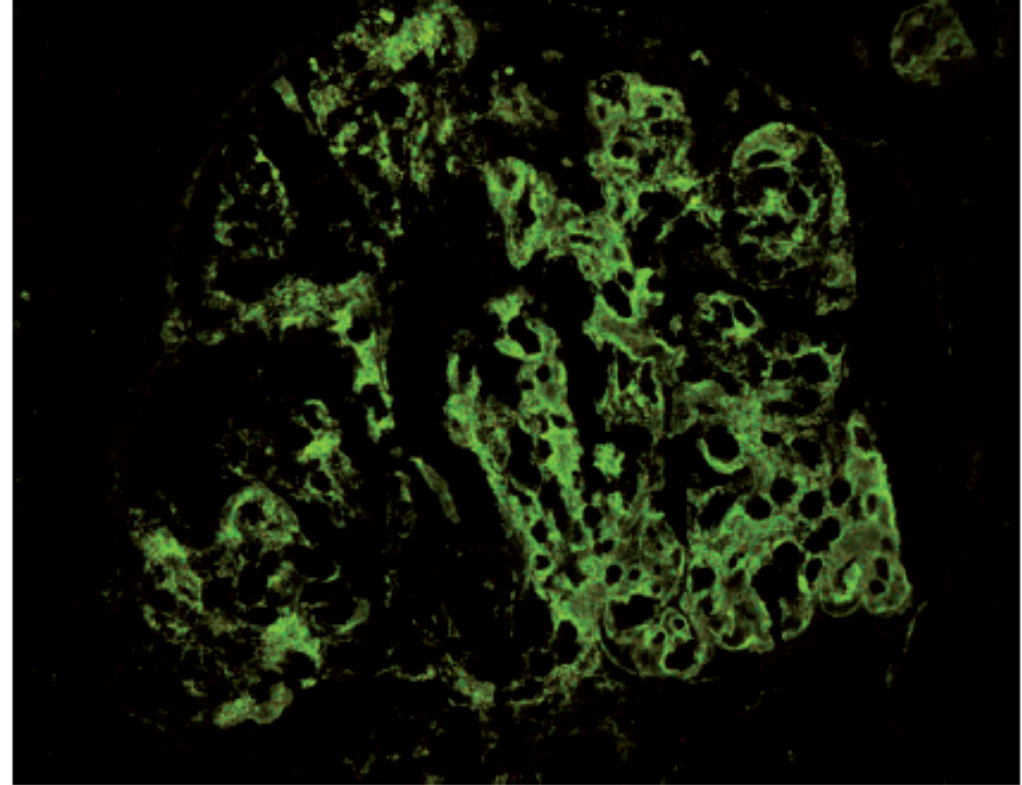


Figure 19.4 Renal biopsy in lupus, demonstrating glomerular staining for IgG.

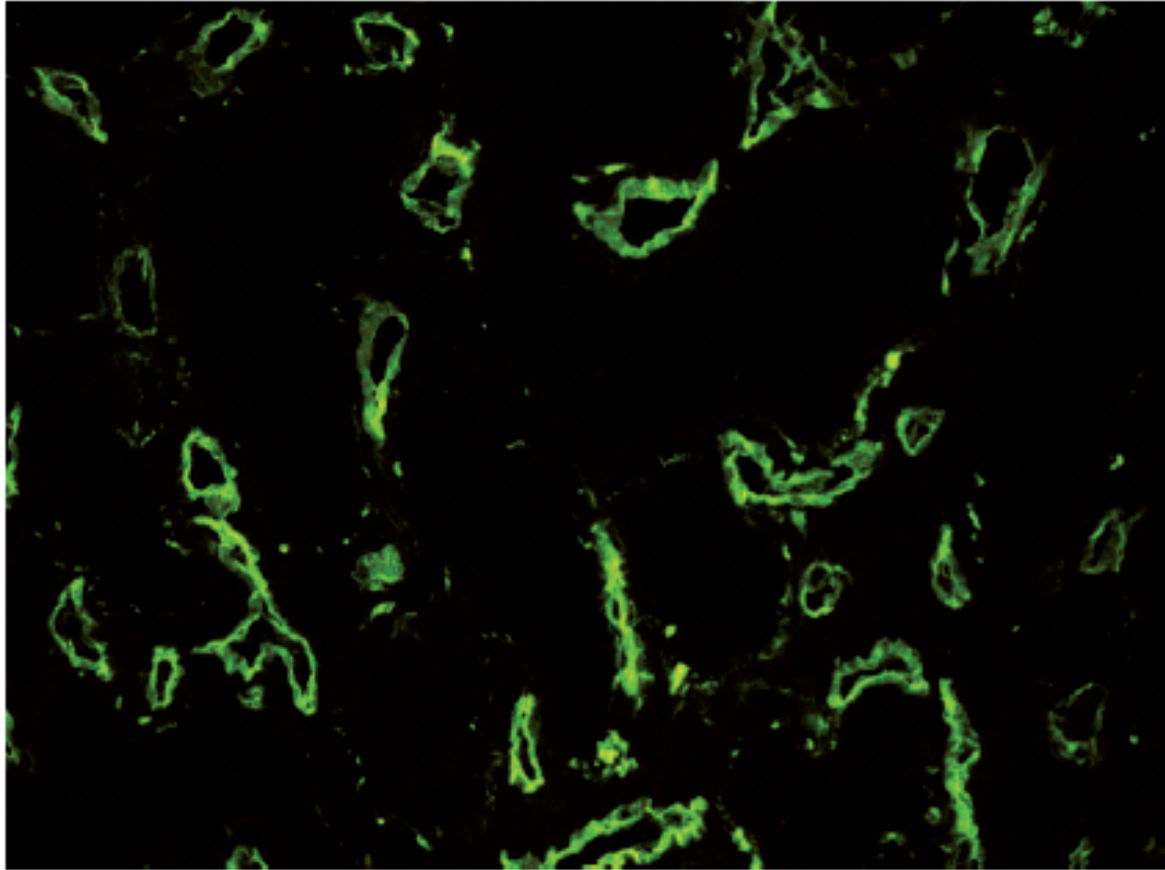


Figure 19.5 Renal biopsy from a patient with kidney transplant rejection, demonstrating staining of peritubular capillaries for C4d.



PELAPORAN

1. TIPE ANTIGEN (IGG, C3, K, Λ , CD3, DLL)
2. INTENSITAS (+, ++, +++)
3. LOKASI (MESANGIAL, PERIPHERAL, BASEMENT MEMBRANE, DLL)
4. POLA (LINEAR, GRANULAR)
5. EKSTENSI (FOCAL, DIFFUSE, GLOBAL, SEGMENTAL)



KULIT

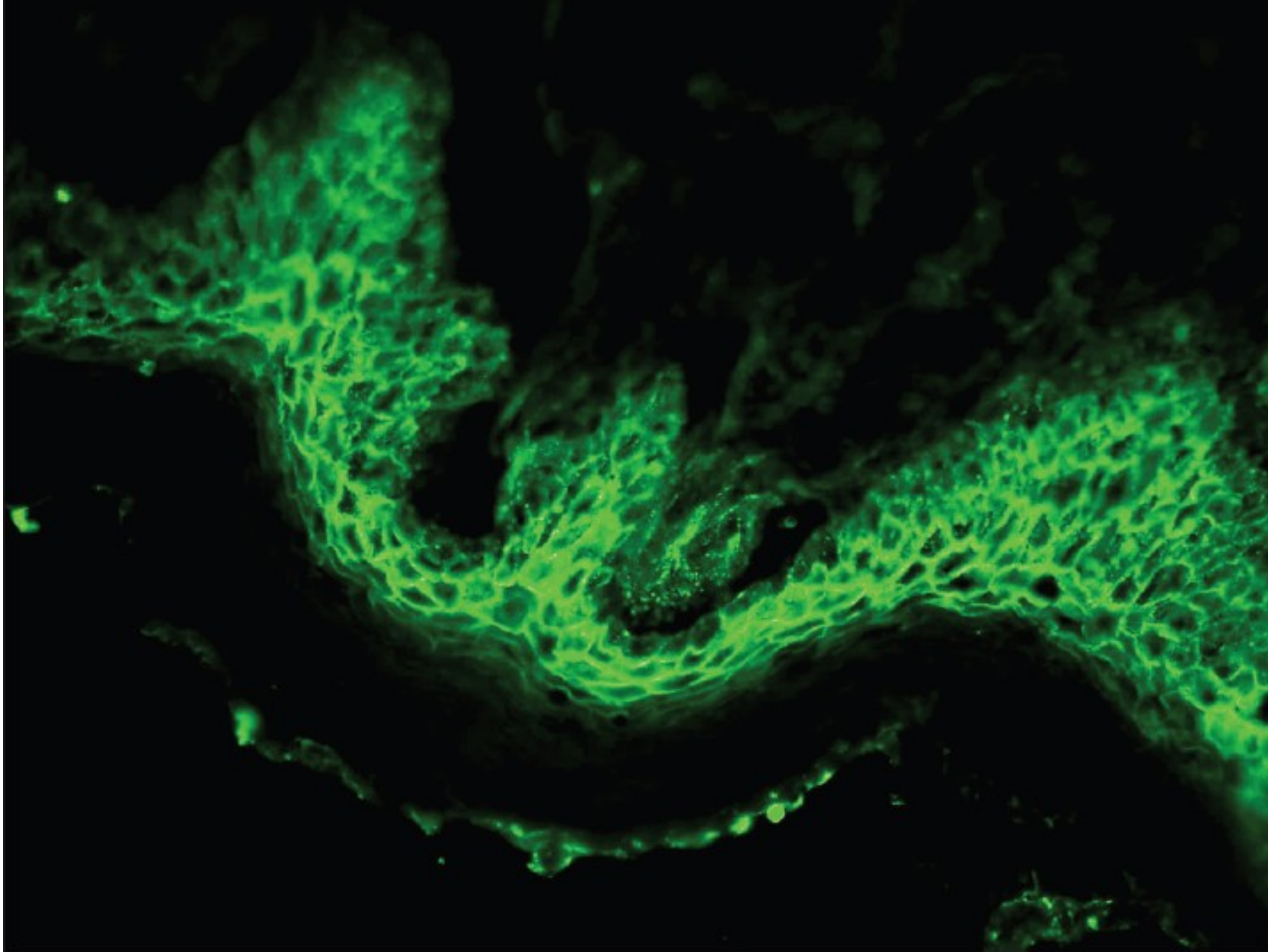
- POLA DEPOSISI IMMUNOGLOBULIN
 - UPPER DERMIS
 - DERMO-EPIDERMAL JUNCTION

- BULLOUS DISORDERS, SYSTEMIC LUPUS ERYTHEMATOSUS, VASCULITIS

Table 19.2 Immunofluorescent patterns in selected skin diseases

Skin disease	Substances present	Location of deposits
Pemphigus	IgG, C3	Intercellular epidermis
Pemphigoid	C3, IgG	Basement membrane zone (linear pattern)
Bullous systemic lupus erythematosus	IgG, C3 (\pm IgM, IgA) (granular)	Basement membrane zone
Dermatitis herpetiformis	IgA and C3 (granular)	Dermal papillae of perilesional skin (granular pattern)

Farmer, E.R., Hood, A.F., 2000. Pathology of the skin, second ed. McGraw-Hill, New York.



DIF photomicrograph of a case of PV showing IgG reactive granular (+++) deposits at the intercellular spaces in the epidermis (×400)

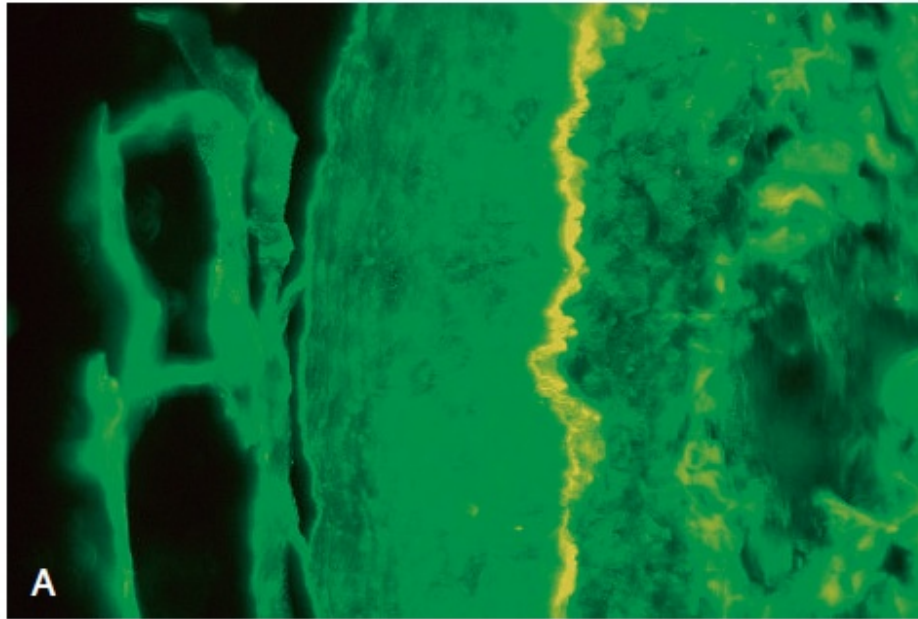
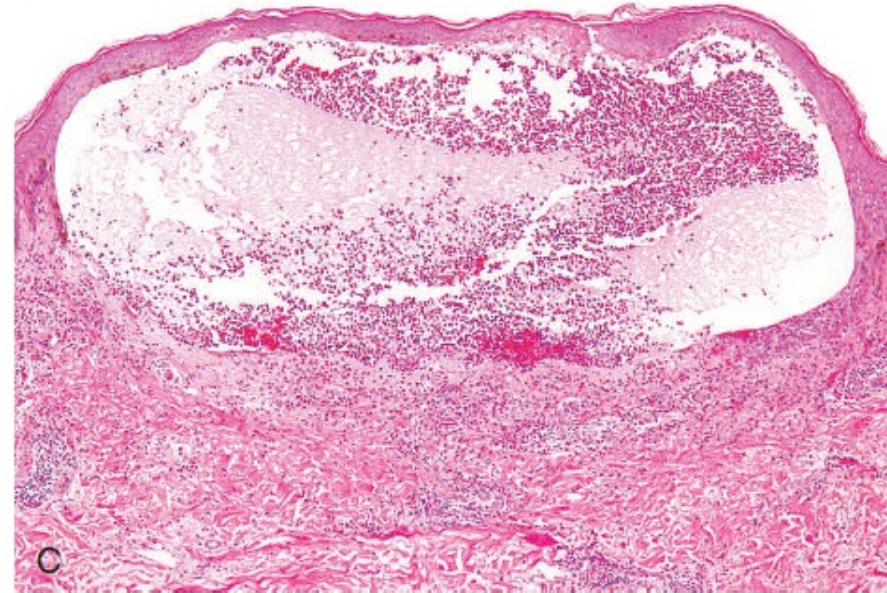
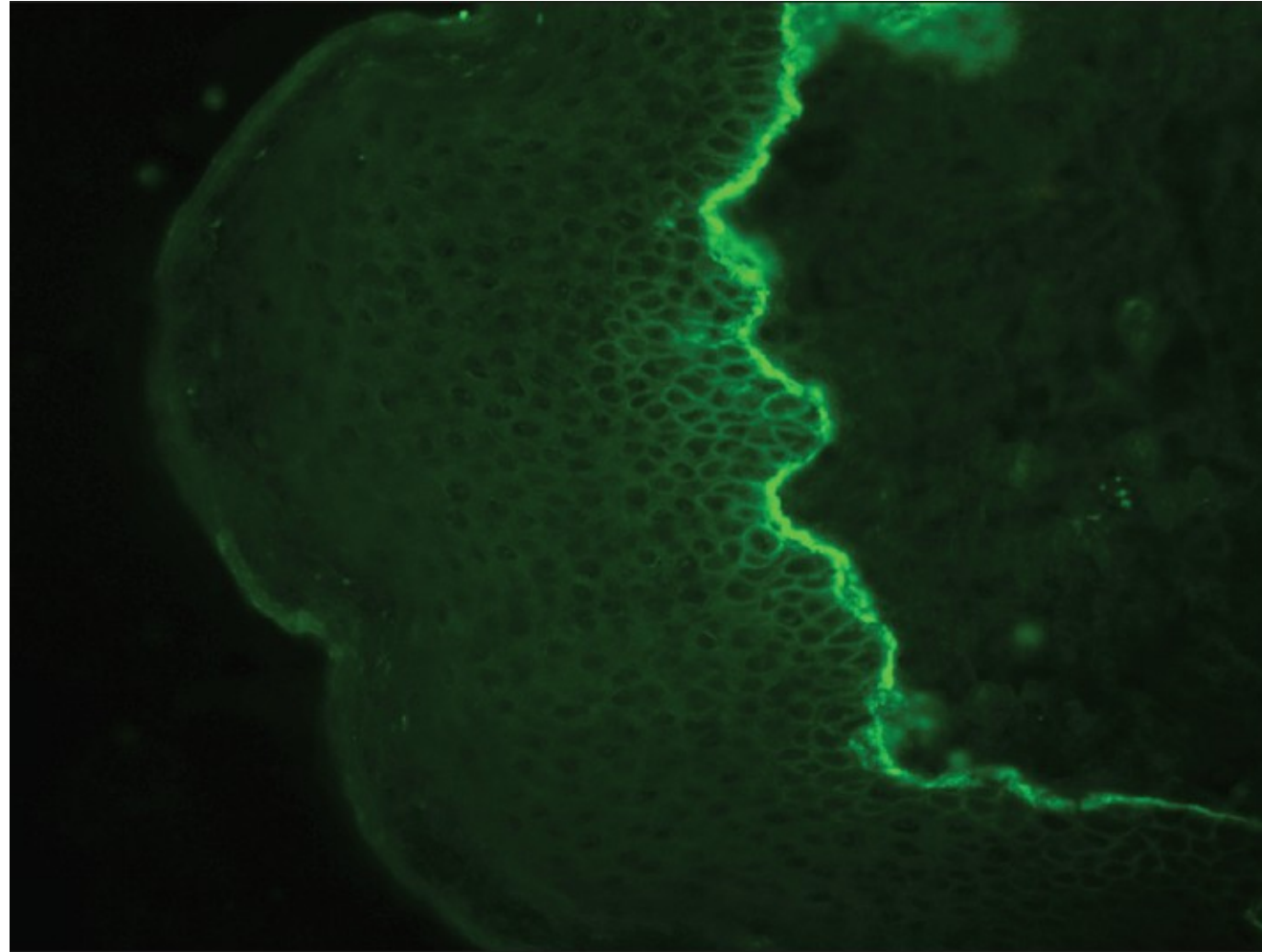


Fig. 24.12 Bullous pemphigoid. (A) Deposition of IgG antibody (detected by direct immunofluorescence) in the subepidermal basement membrane (epidermis is on the left side of the fluorescent band). (B) Gross appearance of characteristic tense, fluid-filled blisters. (C) Subepidermal vesicle with an eosinophil-rich inflammatory infiltrate. (C, Courtesy of Dr. Victor G. Prieto, Houston, Texas.)





DIF photomicrograph of a case of BP showing IgG reactive linear (+++) deposits at the dermo-epidermal junction (×400)

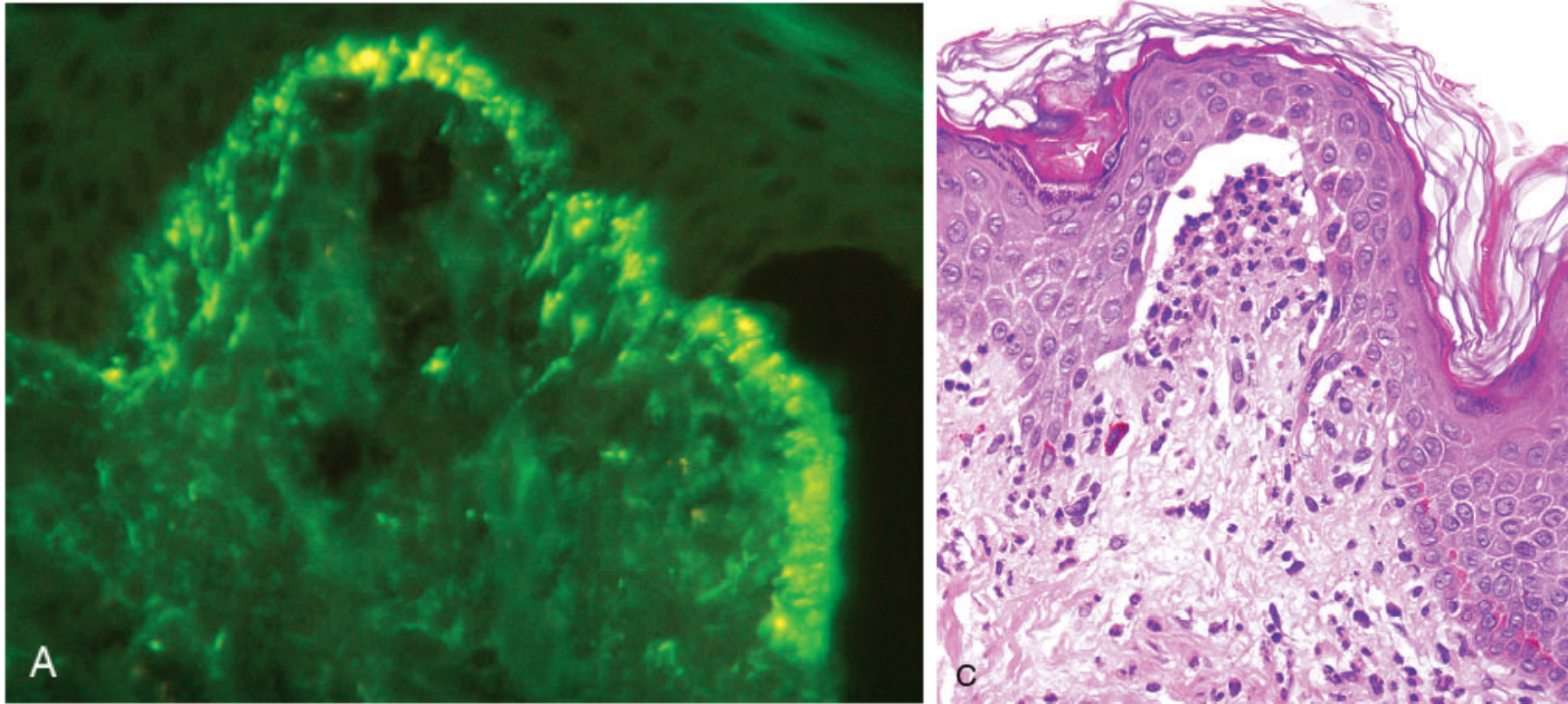
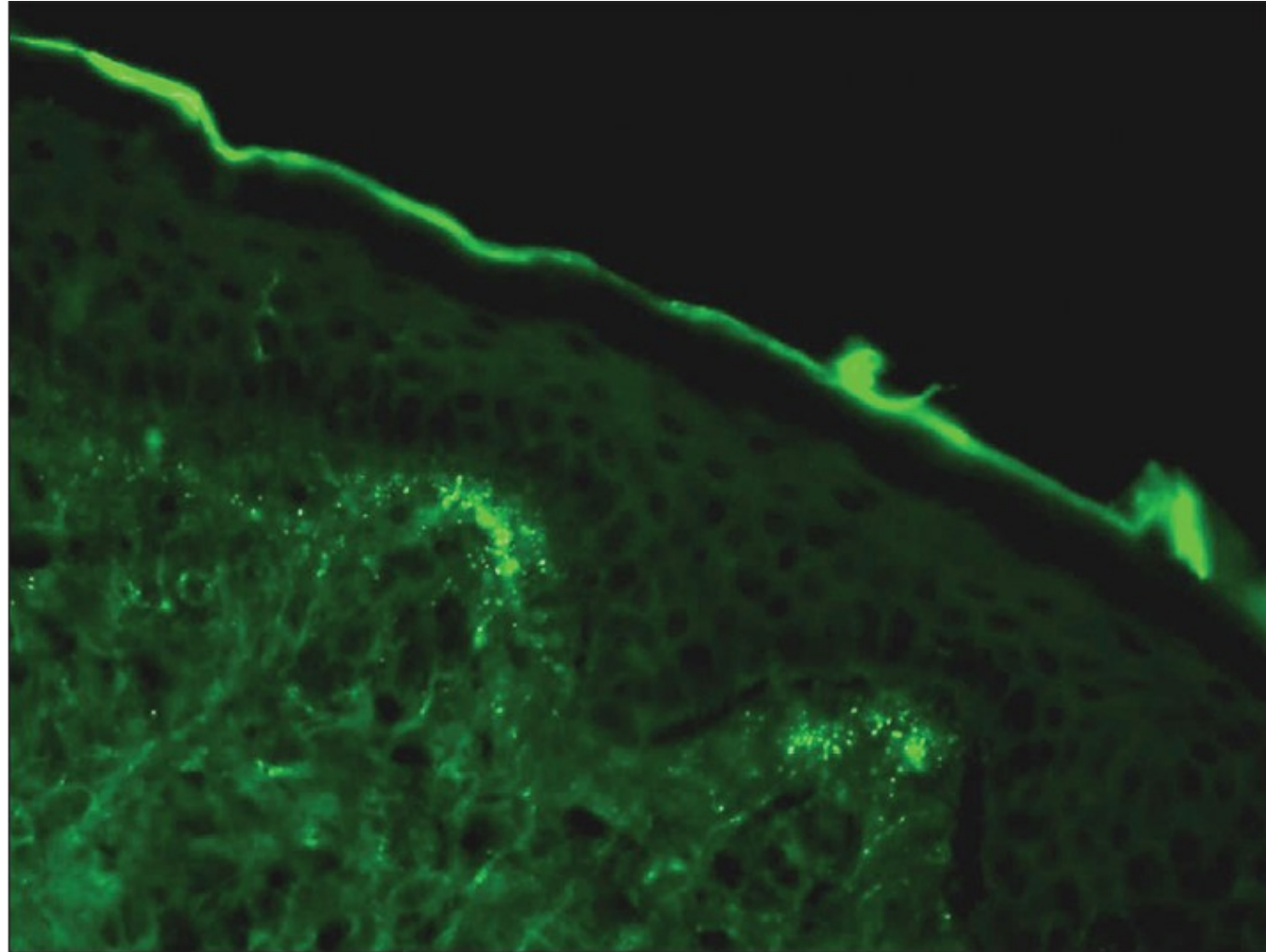
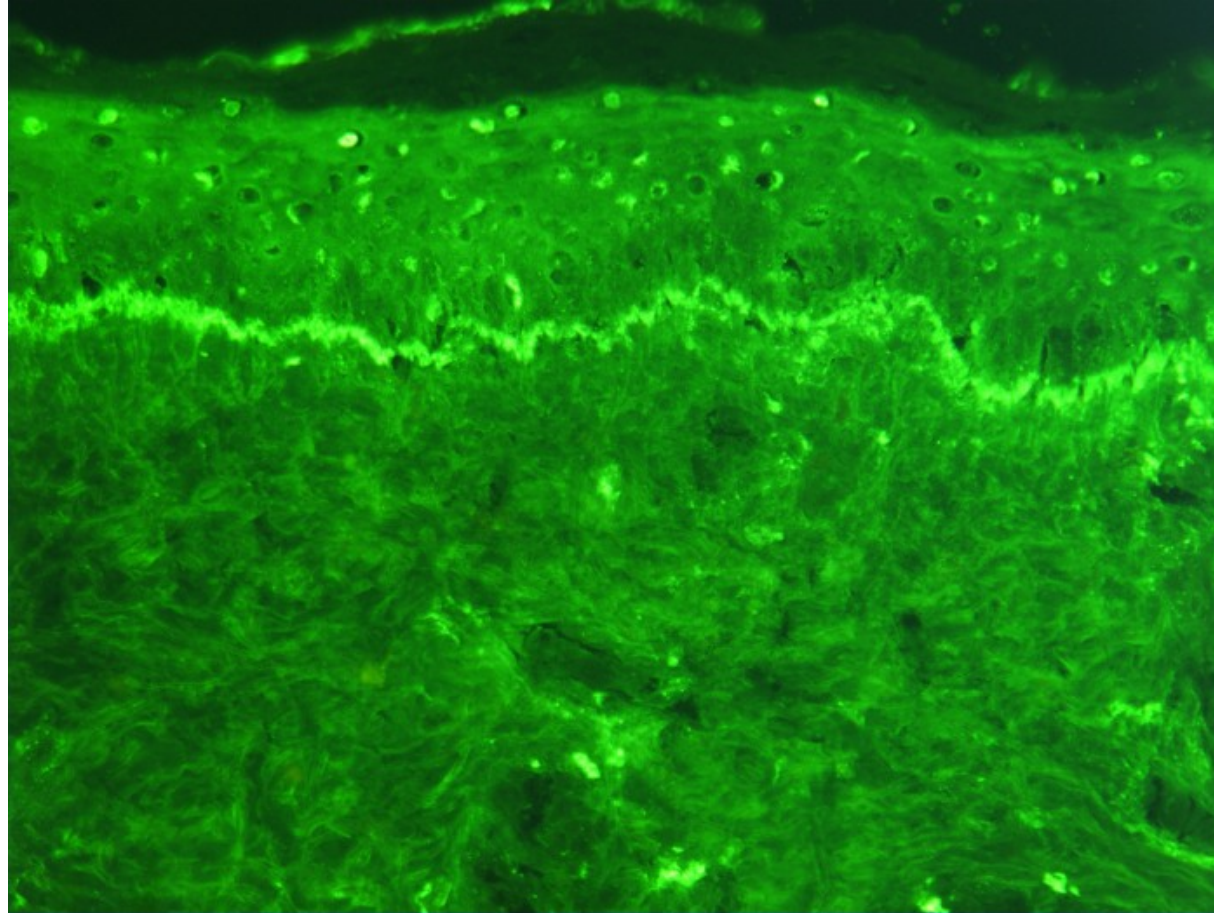


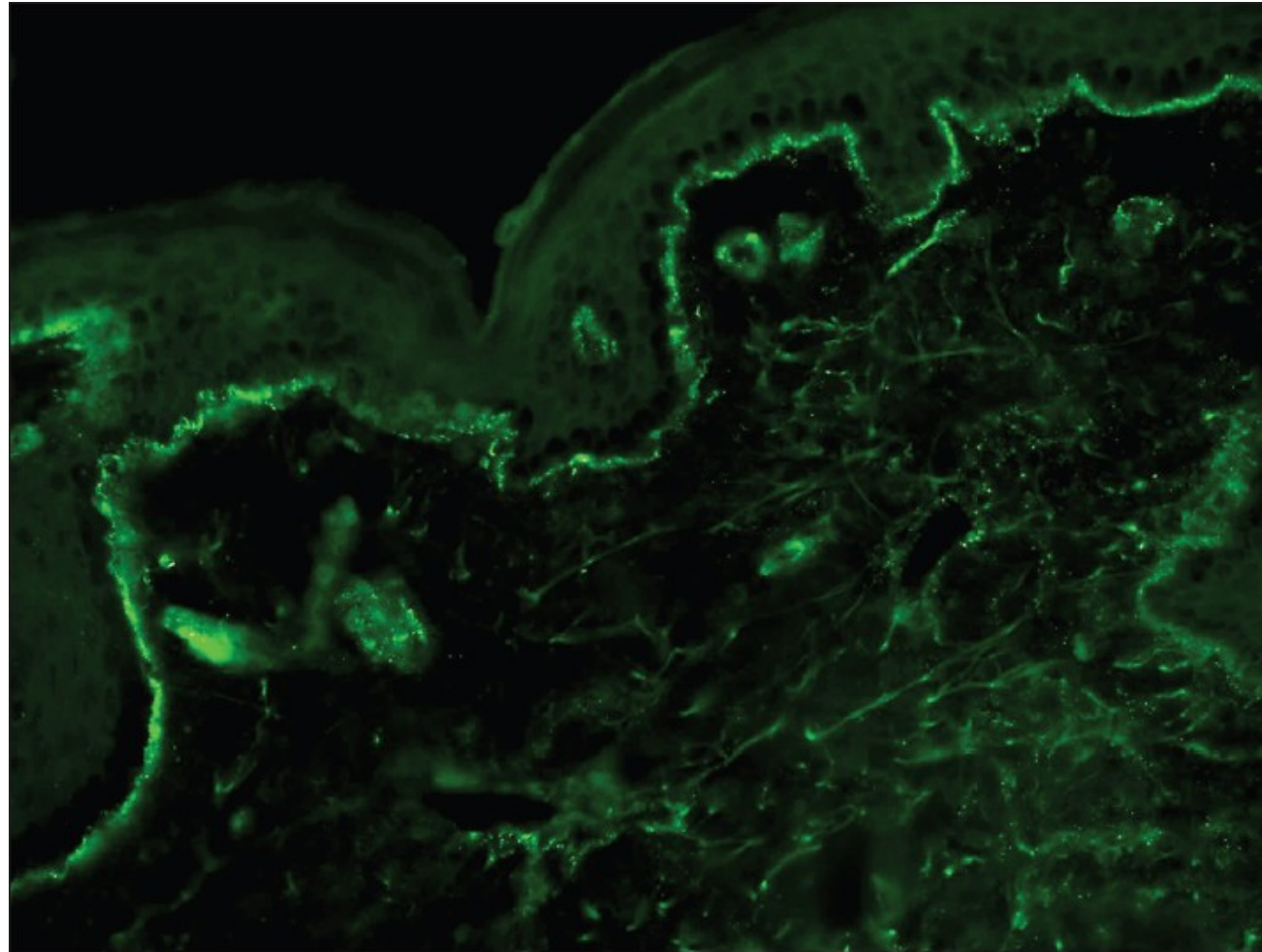
Fig. 24.13 Dermatitis herpetiformis. (A) Characteristic selective deposition of IgA autoantibody at the tips of the dermal papillae. (B) Lesions consist of intact and eroded (usually scratched) erythematous blisters, often grouped (seen here on elbows and arms). (C) Blisters associated with basal cell layer injury, initially caused by accumulation of neutrophils (microabscesses) at the tips of the dermal papillae. (A, Courtesy of Dr. Victor G. Prieto, Houston, Texas.)



DIF photomicrograph of a case of DH showing IgA reactive granular (+++) deposits at the tips of dermal papillae (×400)



Photomicrograph of a histological section of human skin prepared for direct immunofluorescence using an anti-IgG antibody. The skin is from a patient with **systemic lupus erythematosus** and shows IgG deposit at two different places: The first is a band-like deposit along the epidermal basement membrane ("lupus band test" is positive). The second is within the nuclei of the epidermal cells (anti-nuclear antibodies).



DIF photomicrograph of a case of SLE showing IgG reactive granular (+++) deposits at the dermo-epidermal junction and superficial dermal blood vessels (×400)

INTERPRETATION - PATTERN

- **CENTROMERE**

ANA WITH CENTROMERE PATTERN IS PARTICULAR MARKER OF SYSTEMIC SCLEROSIS OF LIMITED TYPE, BUT ARE ALSO SEEN IN OTHER RHEUMATIC DISEASES SYSTEM AND IN PRIMARY BILIARY CHOLANGITIS (PBC).

- **NUCLEOLAR**

ANA WITH NUCLEOLAR PATTERN IS PRIMARILY MARKER OF SYSTEMIC SCLEROSIS OF DIFFUSE TYPE, BUT SLIGHTLY POSITIVE FINDINGS CAN ALSO BE SEEN IN OTHER CONDITIONS.

- **PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA)**

ANA WITH PCNA PATTERN IS AN UNUSUAL SUBTYPE OF ANA BUT IS REGARDED TO HAVE HIGH SPECIFICITY FOR SLE.

- **MITOTIC SPINDLE**

ANTIBODIES TO THE MITOTIC SPINDLE IS PRESENT AT MYCOPLASMA PNEUMONIAE INFECTIONS. OTHER ASSOCIATIONS ARE UNCLEAR.

- **NUCLEAR DOTS**

NUCLEAR DOTS PATTERN WITH SPECIFICITY FOR THE SP100 IS STRONGLY ASSOCIATED WITH PRIMARY BILIARY CHOLANGITIS (PBC). CHOLESTASIS?

- **NUCLEAR MEMBRANE**

ANTIBODIES AGAINST NUCLEAR MEMBRANE IS PRIMARILY ASSOCIATED WITH PRIMARY BILIARY CIRRHOSIS. CHOLESTASIS?

- **HOMOGENEOUS**

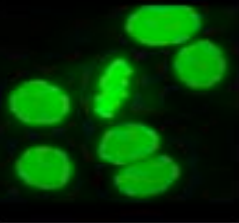
SAMPLES WITH HOMOGENEOUS ANA WILL FURTHER BE ANALYZED FOR ANTI-DSDNA, ANTI-HISTONES AND ANTI-ENA (ANTI-SM, ANTI-NRNP, ANTI-SSA, ANTI-SSB, ANTI-SCL-70, ANTI-JO1 AND ANTI-RO 52).

- **SPECKLED**

SPECKLED NUCLEAR STAINING COMPRISES ANTIBODIES AGAINST ENA, SSA AND SSB. SAMPLES WITH SPECKLED ANA WILL FURTHER BE ANALYZED FOR ANTI-DSDNA AND ANTI-ENA (ANTI-SM, ANTI-NRNP, ANTI-SSA, ANTI-SSB, ANTI-SCL-70, ANTI-JO1 AND ANTI-RO 52).

ANA-IFA PATTERNS LOOSELY CORRELATE WITH SPECIFIC ANTIBODIES AND DIAGNOSES

Homogeneous



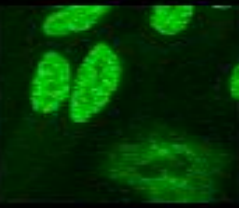
Pattern

Associated Antigens
Double stranded DNA,
histones, DNA/histone
complexes, others

Clinical Associations

SLE, drug induced lupus,
and other conditions

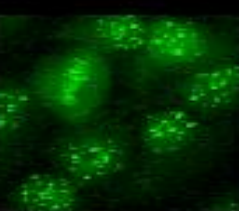
Speckled



“ENA antigens”: Sm, RNP,
SS-A/Ro, SS-B/La
Nuclear matrix antigens:
(hnRNP), others

Autoimmune rheumatic
diseases: SLE, MCTD,
Sjögren’s syndrome, and
other conditions

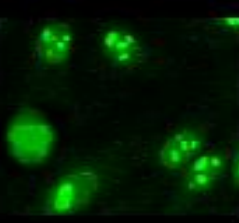
Centromere



Centromere proteins A, B, C
located at the kinetochore
plates

Scleroderma
(CREST syndrome)
primary biliary cirrhosis

Nucleolar

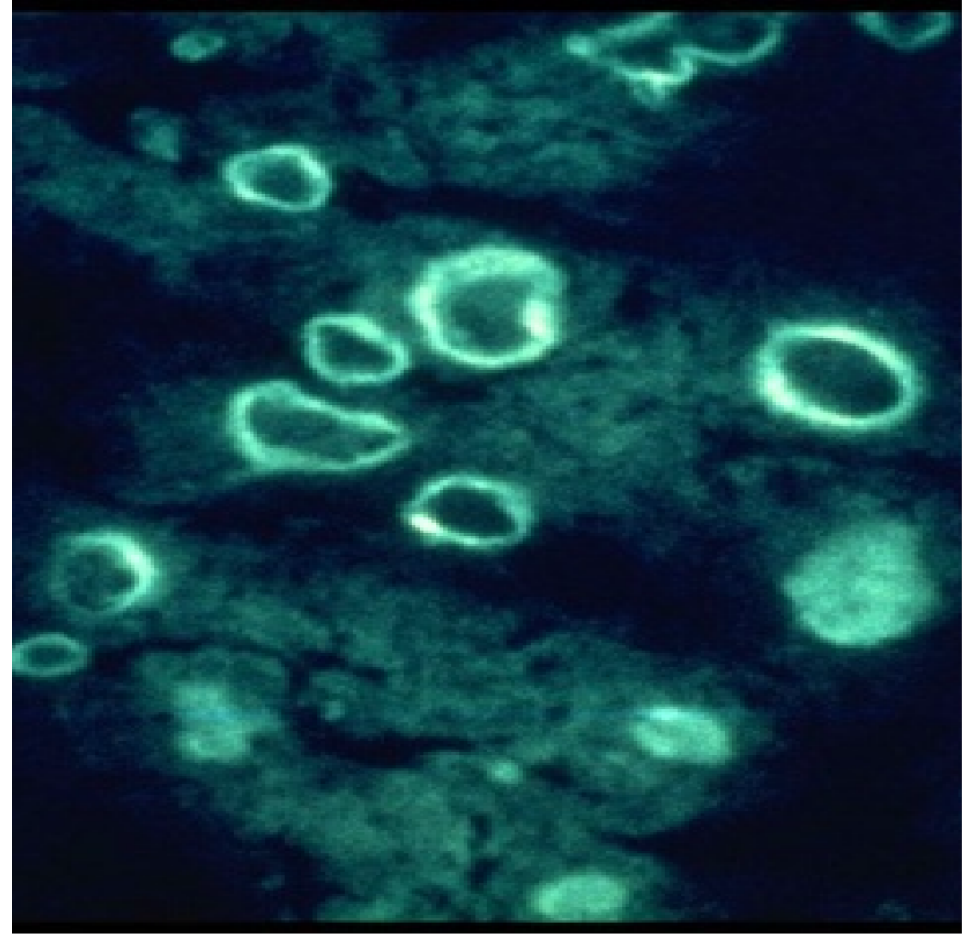


Fibrillarin, RNA polymerase,
NOR-90, Scl-70, Pm/Scl,
ribosomal antigens, others

Autoimmune rheumatic
diseases: scleroderma,
myositis, SLE, and other

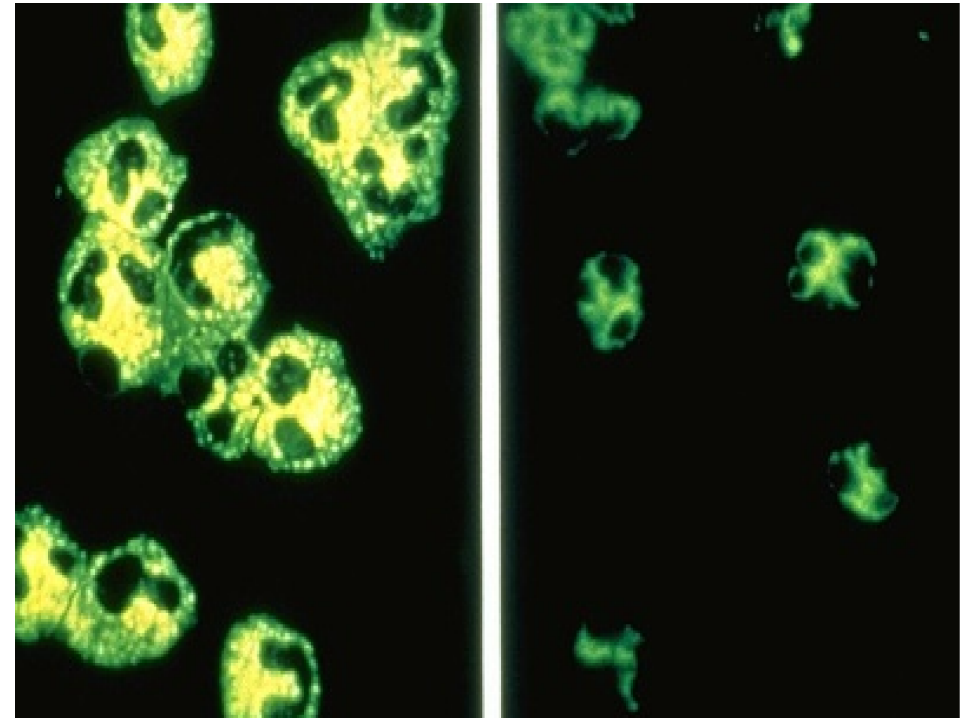
AUTOANTIBODIES IN SLE

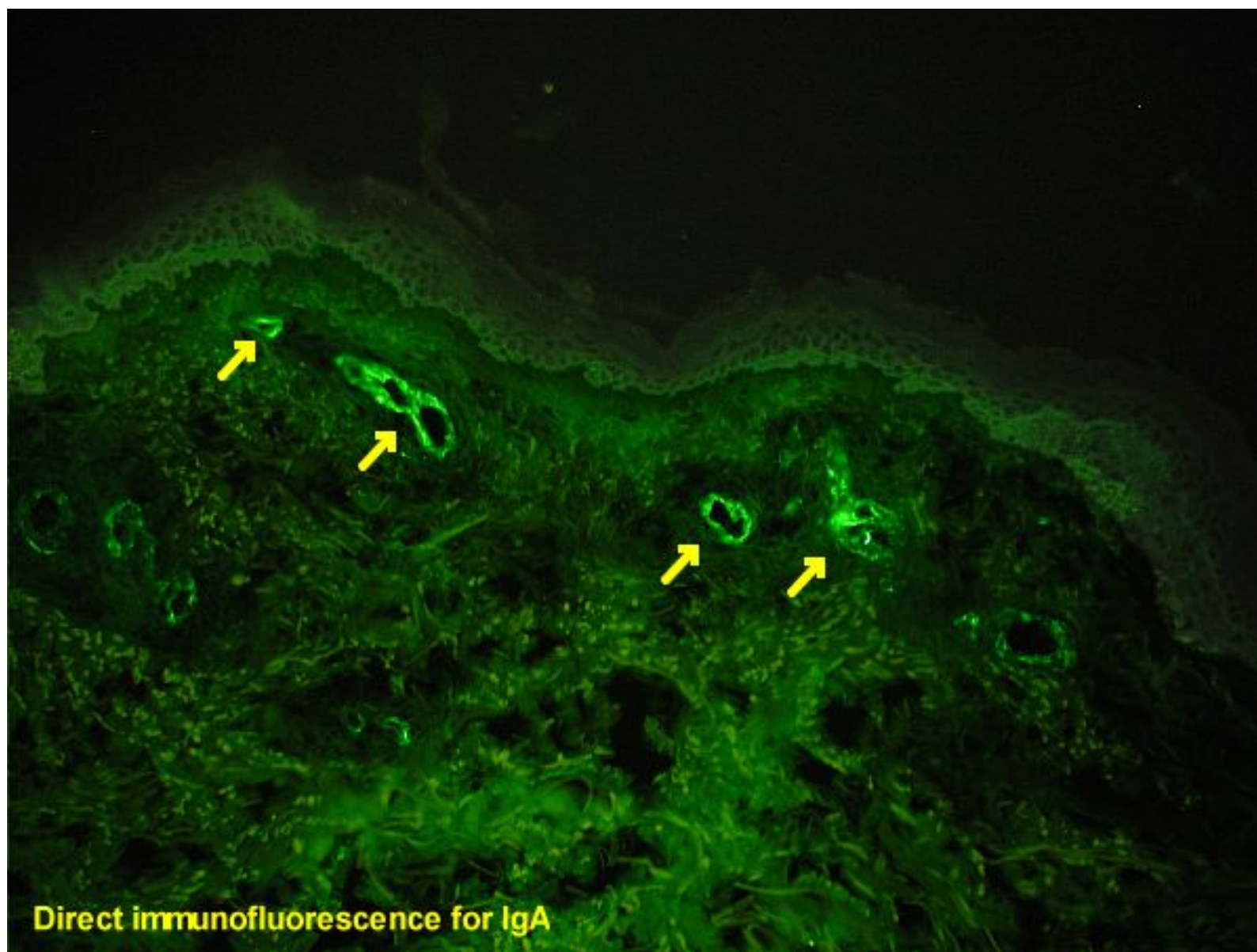
- ANA
 - SEEN IN 95% OF SLE
 - NOT SPECIFIC FOR SLE
 - SEEN IN MANY INFLAMMATORY, INFECTIOUS, AND NEOPLASTIC DISEASES
 - SEEN IN 5% TO 15% OF NORMAL PERSONS



ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES

- ANCA BY IMMUNOFLUORESCENCE METHODS
 - C-ANCA = WEGENER'S DISEASE (60% TO 90%)
 - P-ANCA = MICROSCOPIC POLYANGIITIS (MPA) (50% TO 80%), UC (40% TO 80%), CROHN'S (10% TO 40%)





Photomicrograph of a histological section of human skin prepared for direct immunofluorescence using an anti-IgA antibody. The skin is from a patient with **Henoch–Schönlein purpura**: IgA deposits are found in the walls of small superficial capillaries (yellow arrows). The pale wavy green area on top is the epidermis, the bottom fibrous area is the dermis.



THANK YOU

