Vasculitides
ANCA Testing

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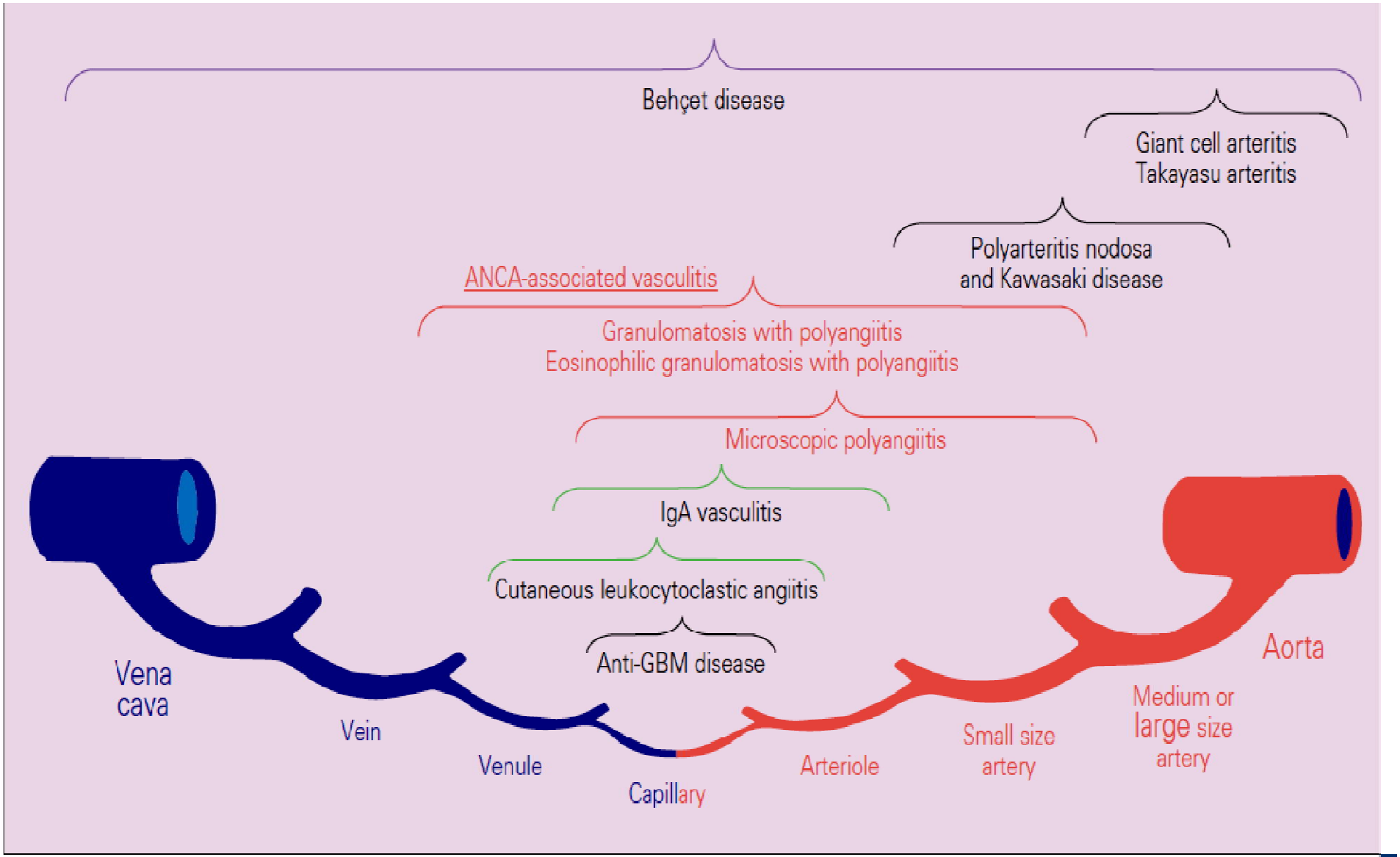
Classification of Vasculitides

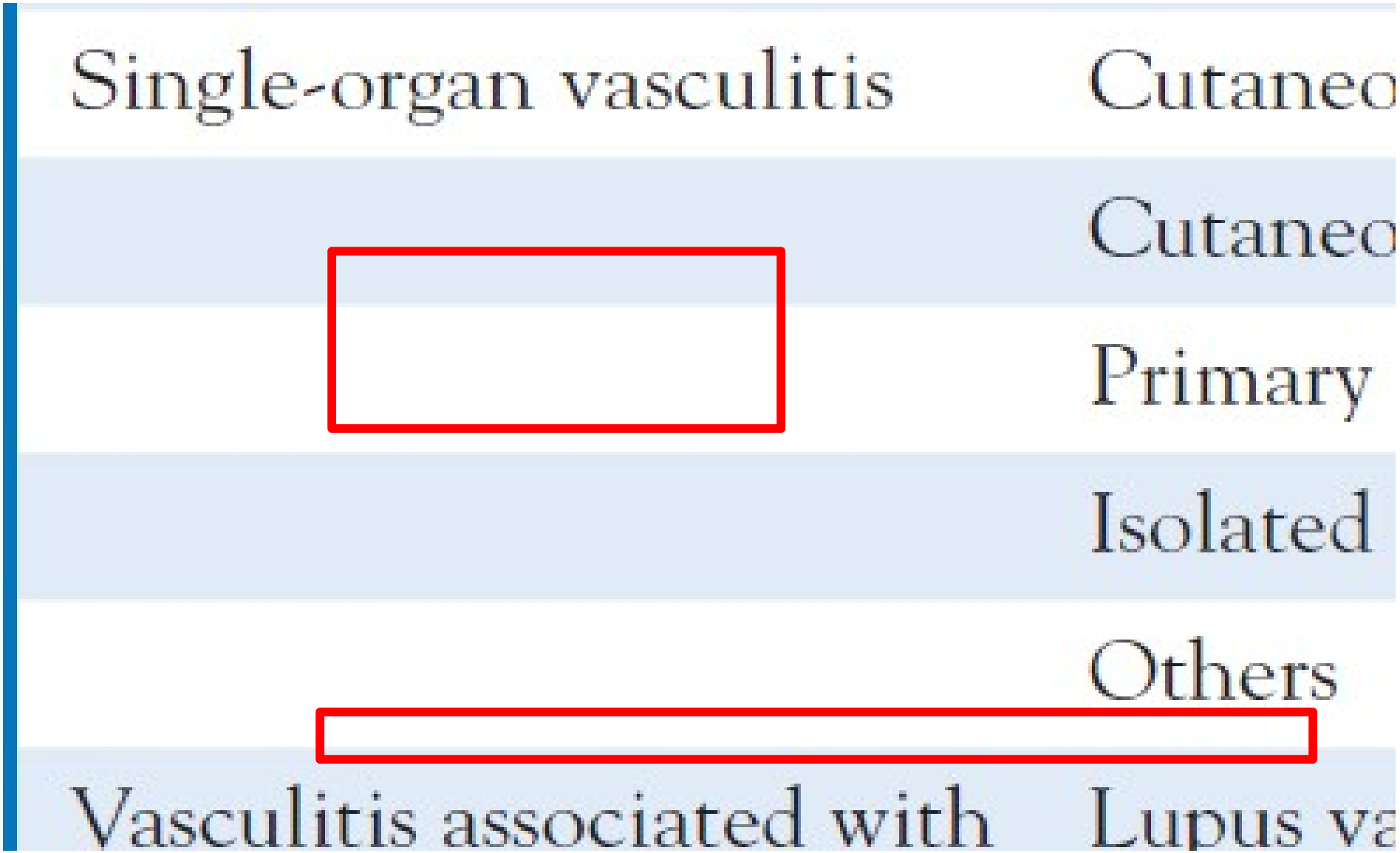


Classification of Vasculitides

Table 26-1. Vasculitis Nomenclat Conference

NOMENCLATURE	VASCUL
Large-vessel vasculitis	Takayasu
	Giant ce
Medium-vessel vasculitis	Polyarter

Classification of Vasculitides



ANCA associated Vasculitides

Small vessel vasculitis	Vasculitis predominantly affecting small vessels, defined as small intraparenchymal arteries, arterioles, capillaries, and venules. Medium arteries and veins may be affected
ANCA – associated vasculitis	Necrotizing vasculitis, with few or no immune deposits, predominantly affecting small vessels (i.e., capillaries, venules, arterioles, and small arteries). Associated with myeloperoxidase (MPO) ANCA or proteinase 3 (PR3) ANCA. Not all patients have ANCA. Add a prefix indicating ANCA reactivity (e.g., MPO-ANCA, PR3-ANCA, ANCA-negative)

ANCA associated Vasculitides

Granulomatousis with Polyangiitis (GPA) Wegener's Granulomatosis	•Necrotizing granulomatous inflammation •usually involving the upper and lower respiratory tract & •necrotizing vasculitis affecting predominantly small to medium vessels (e.g., capillaries, venules, arterioles, arteries, and veins) • Necrotizing glomerulonephritis is common.
Microscopic Polyangiitis (MPA)	•Necrotizing vasculitis, •with few or no immune deposits •predominantly affecting small vessels (i.e., capillaries, venules, or arterioles) •Necrotizing glomerulonephritis is very common •Pulmonary capillaritis often occurs •Granulomatous inflammation is absent
Eosinophilic Granulomatousis with Polyangiitis (EGPA)	•Eosinophil-rich and necrotizing granulomatous inflammation •Often involving the respiratory tract and •Necrotizing vasculitis predominantly affecting small to medium vessels and •Associated with asthma and eosinophilia. •ANCA is more frequent when glomerulonephritis is present

Granulomatousis with Polyangiitis (GPA) Wegener's Granulomatosis

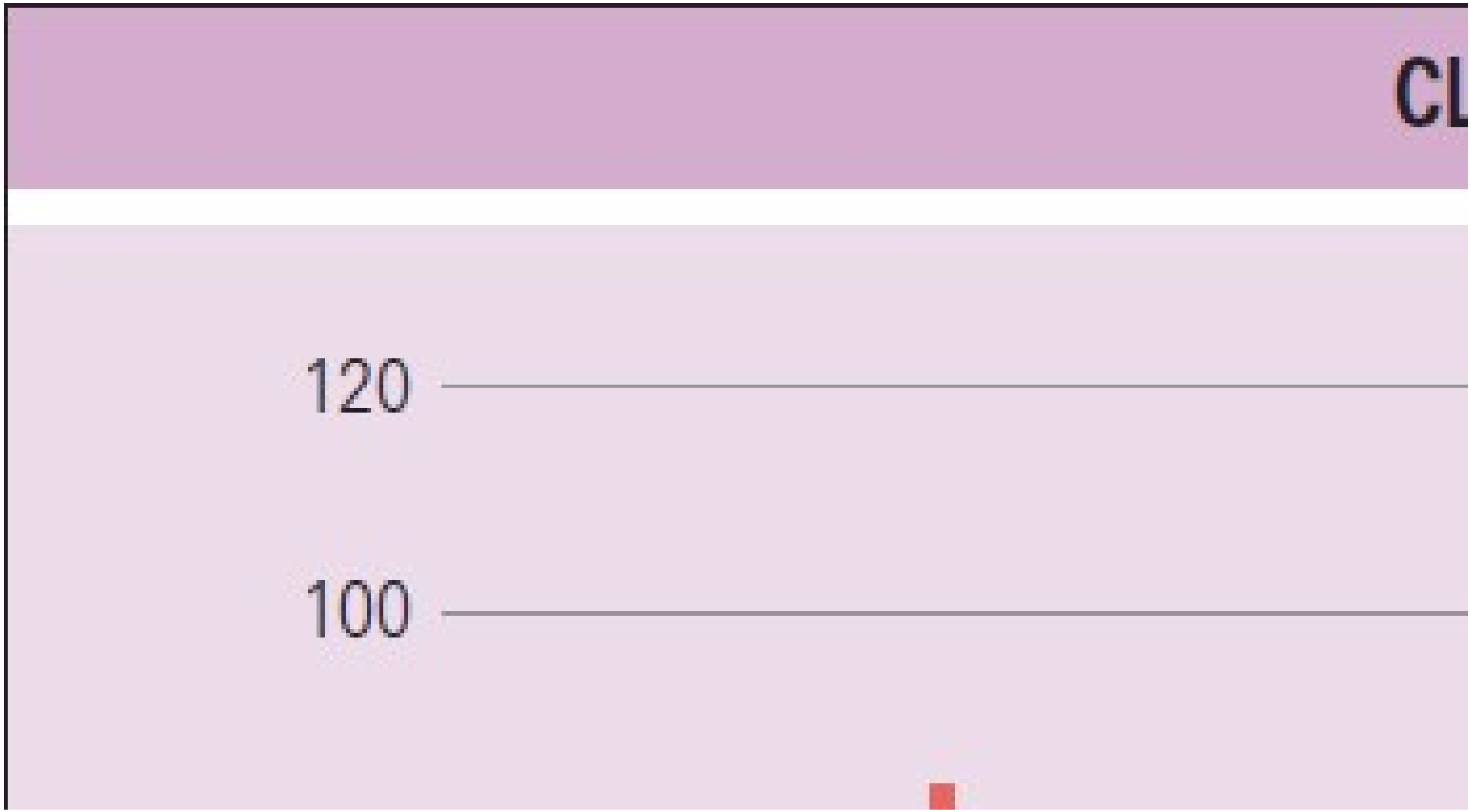
- GPA is primary vaculitis characterized by
  - Upper and lower respiratory tract involvement with granulomatous vasculitis of mostly small vessels along with extravascular granulomatous inflammation and necrosis
  - Glomerulitis – that is pauci-immune, focal and segmental, necrotizing and often crescentric
  - Strong association with cANCA and anti – Proteinase 3 antibodies (PR3)

- Generalized GPA – Involve all three sites – Upper resp tract, Lung & Kidneys

- Limited GPA - defined as absence of Renal involvement
  - Present as granulomatous disorder without vasculitic features
  - Only 10 % evolve to Generalized GPA

- Pulmonary symptoms at presentation – seen in 50% of patients
- 85 – 90% of patients have pulmonary involvemet

Organ involvement in GPA / WA



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Microscopic Polyangiitis (MPA)

- 30 -50 yrs
- No sex prediliction

- Presentation
  - Patient typically presents with Glomerulonephritis (100%)
  - 50% have Pulmonary Infiltrate and / or effusions
  - 30% have diffuse alveolar hemorrage with hemoptysis
  - Fever (50-70%)
  - Arthralgia (30 -65%)
  - Gastointestinal Tract (50%)
  - Purpura (40%)
  - Ear/Nose/Throat involvement (30%)
  - Peripherl or CNS involvemet (25 – 30%)

Microscopic Polyangiitis (MPA)

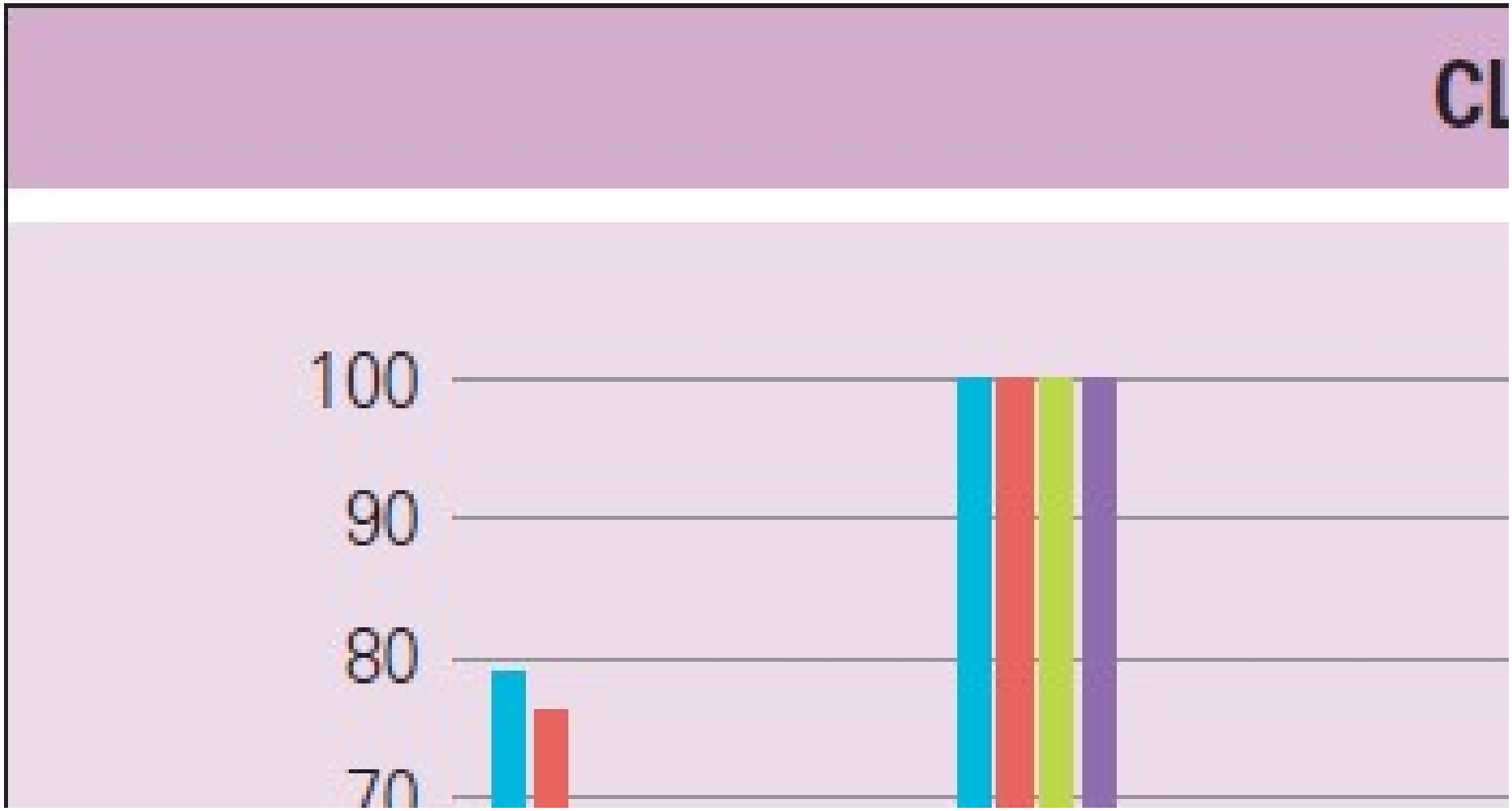

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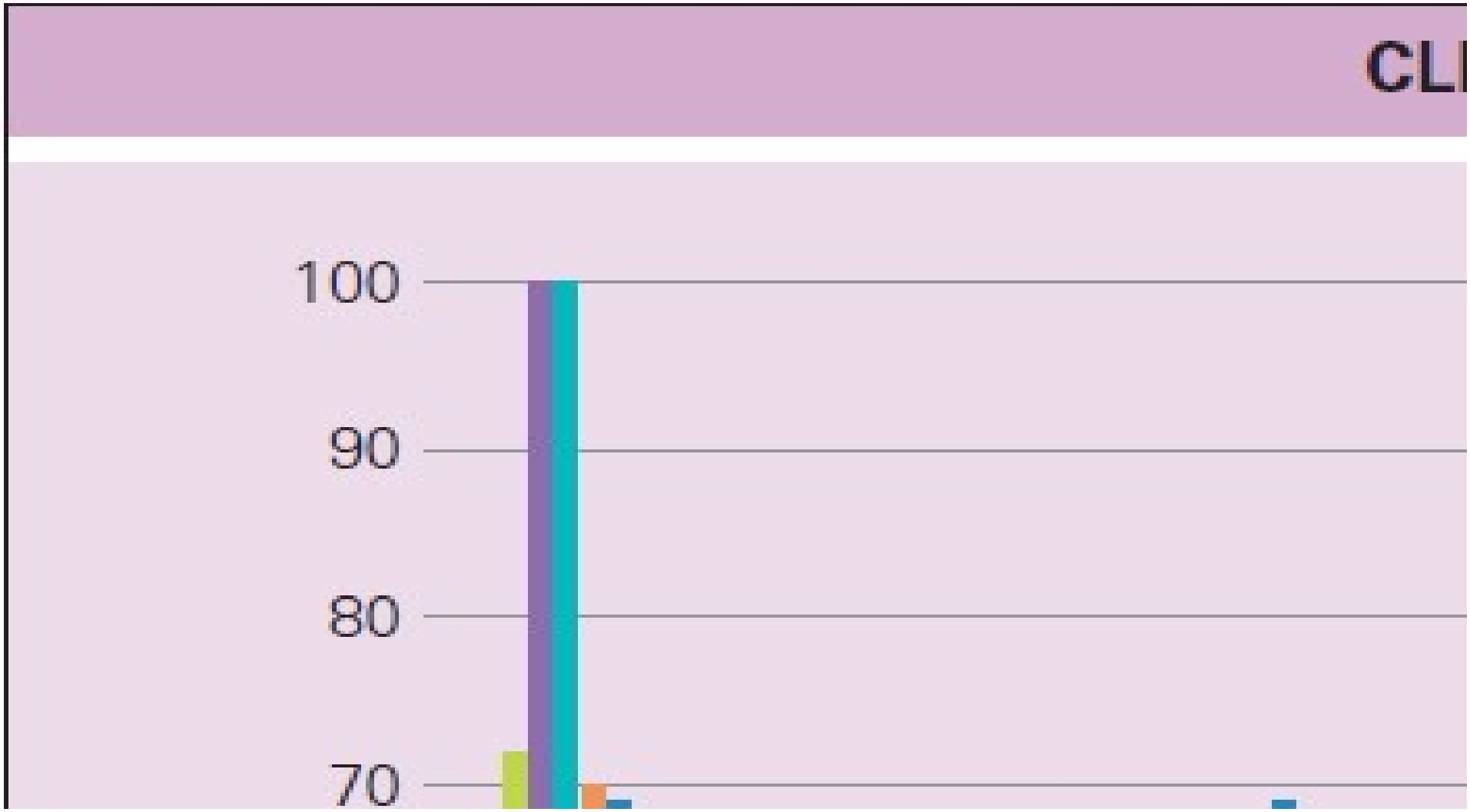
Table 29-3. Clinical Features of F
Granulomatosis With

CLINICAL FEATURES
Kidney involvement
Renal vasculitis with infarcts and microaneurysms

Eosinophilic Granulomatousis with Polyangiitis (EGPA)

- Diagnosed on basis of Clinical & Pathological feature
- Suspicion in cases with allergy and asthma with absolute eosinophil count - >1500 cells/ul
- **Prodromal Stage**
  - Average 28 months but may persists wfor years (2-7 yesrs)
  - Allergic manifestation – rhinitis, polyposis and asthma (80-90%)
  - Recurrent fevers (50%)
- **Peripheral blood and Tissue Eosinophilia**
  - Chronic eosinophilic pneumonia
  - Eosinophilic gastroentritis
  - Myocarditis may develop
  - Fever with flares
- **Life threattening Systemic Vasculitis**
  - Avg 3 years after onset of prodromal stage
  - Severe asthma, myocarditis, valvular insufficiency, eosinophilic gastroentritis, pupura and testicular pain

Eosinophilic Granulomatousis with Polyangiitis (EGPA)



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Eosinophilic Granulomatousis with Polyangiitis (EGPA)

Table 29-4. Major Clinical Featur

ORGAN	Cl
Paranasal sinus	A
Lungs	A

GPA vs MPA vs EGPA

Feature	GPA
Incidence	0.4–11.9 cases per 1 million person-years
Prevalence	2.3–146.0 cases per 1 millio
Typical age of onset (years)	45–65
Male: female ratio	1:1

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ANCA Testing

Antineutrophil Cytoplasmic Antibodies

- Useful in early diagnosis of ANCA-associated vasculitides
- Antibodies to cytoplasmic antigens in neutrophils
- Main patterns by IFA:
  - P-ANCA (myeloperoxidase, MPO)
  - C-ANCA (Proteinase-3, PR-3)
  - Atypical C-ANCA
  - Atypical P-ANCA

ANCA-associated Vasculitic Diseases

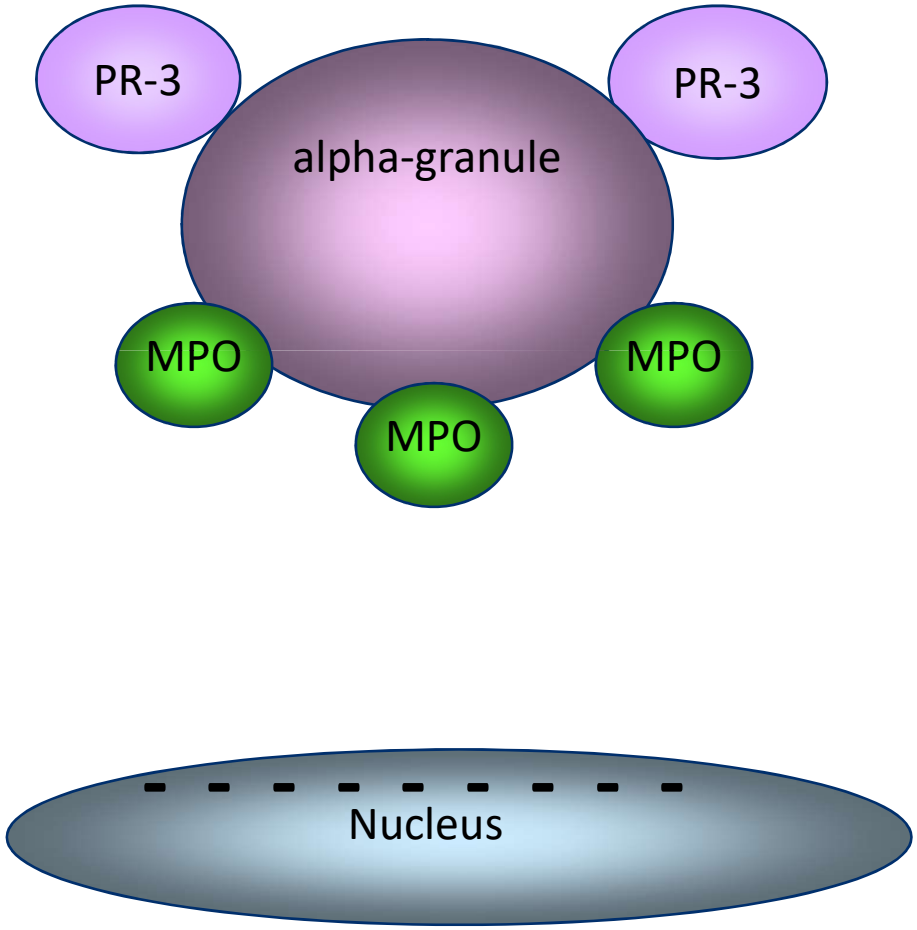
- **Wegener's granulomatosis (Now G with PA)**
  - Lungs and kidneys
  - C-ANCA
- **Microscopic polyangitis**
  - Small vessel vasculitis
  - P-ANCA and C-ANCA about equal
- **Pauci-immune crescentic glomerulonephritis (Renal-limited microscopic polyangiitis)**
  - Rapidly progressive
  - Usually limited to just kidneys & without systemic vasculitis
  - P-ANCA

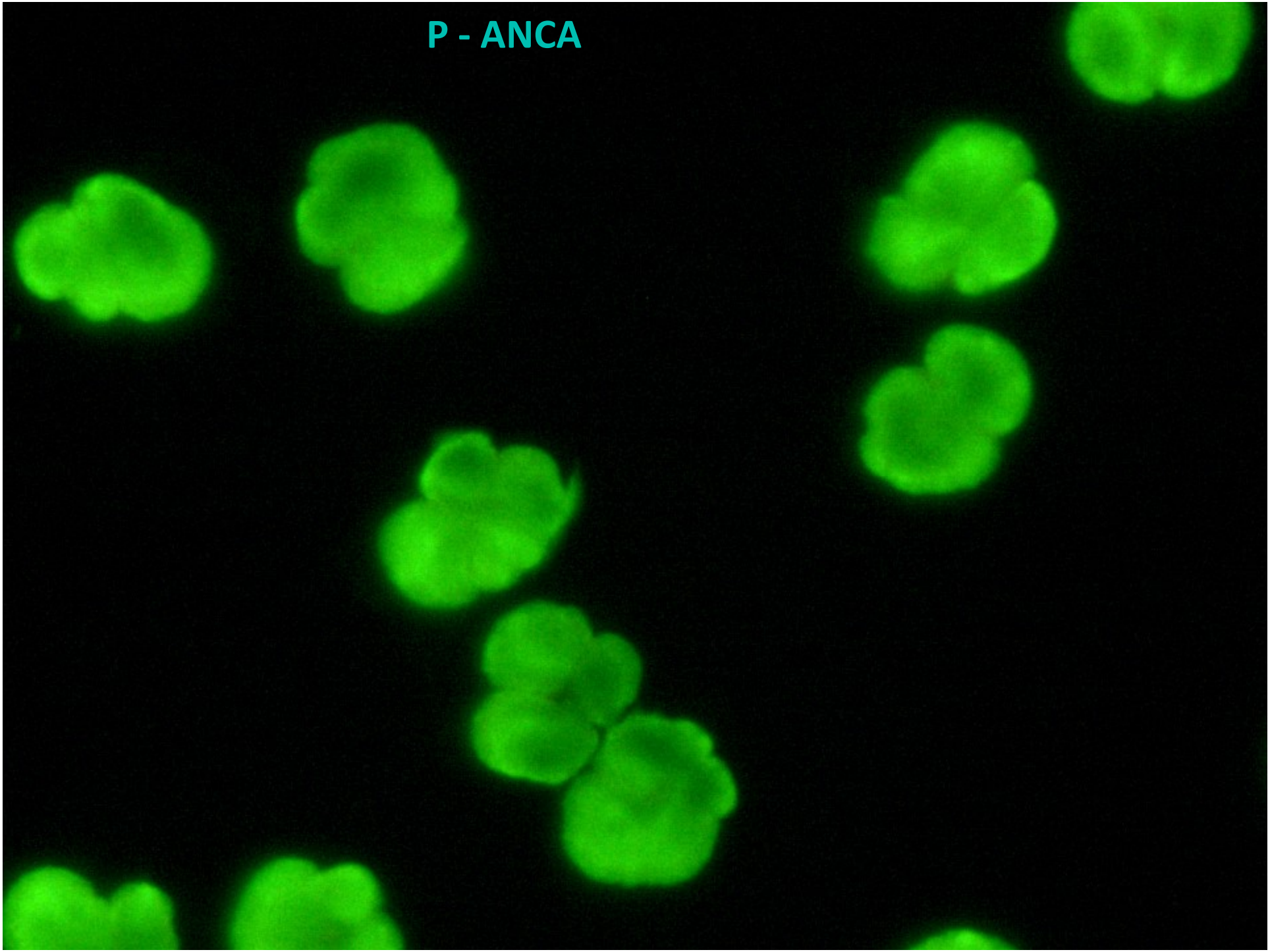
P-ANCA (perinuclear)

- **Myeloperoxidase (MPO) is the most common target antigen**
  - **Perinuclear staining with ethanol fixed cells**
  - Cytoplasmic staining with formalin fixed cells
  - Caused by artifact of ethanol fixation

- **Other P-ANCA antigens:**
  - Lysozyme
  - Catalase
  - Lactoferrin
  - others

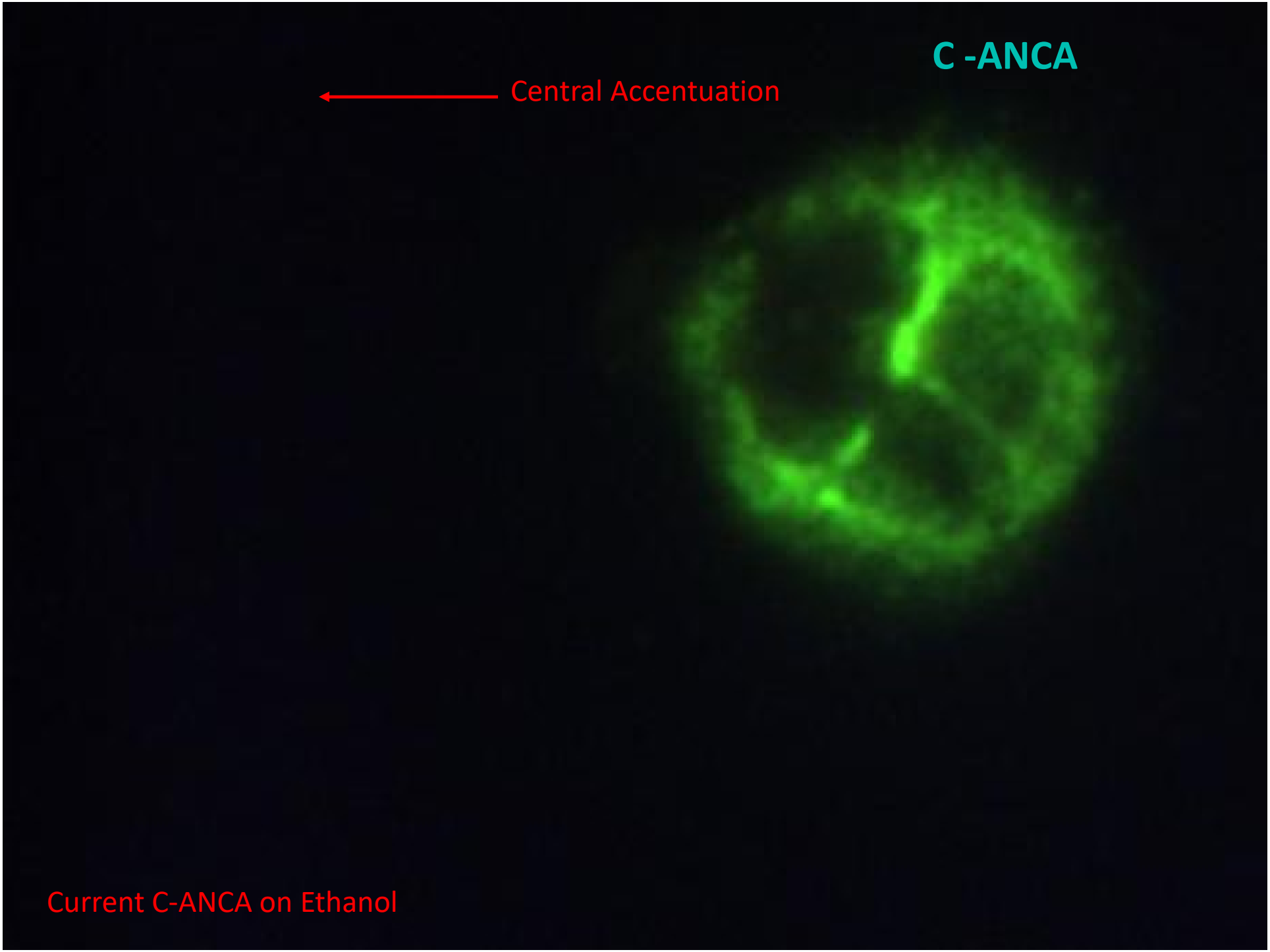
Effects of EtOH fixation on P-ANCA




P - ANCA

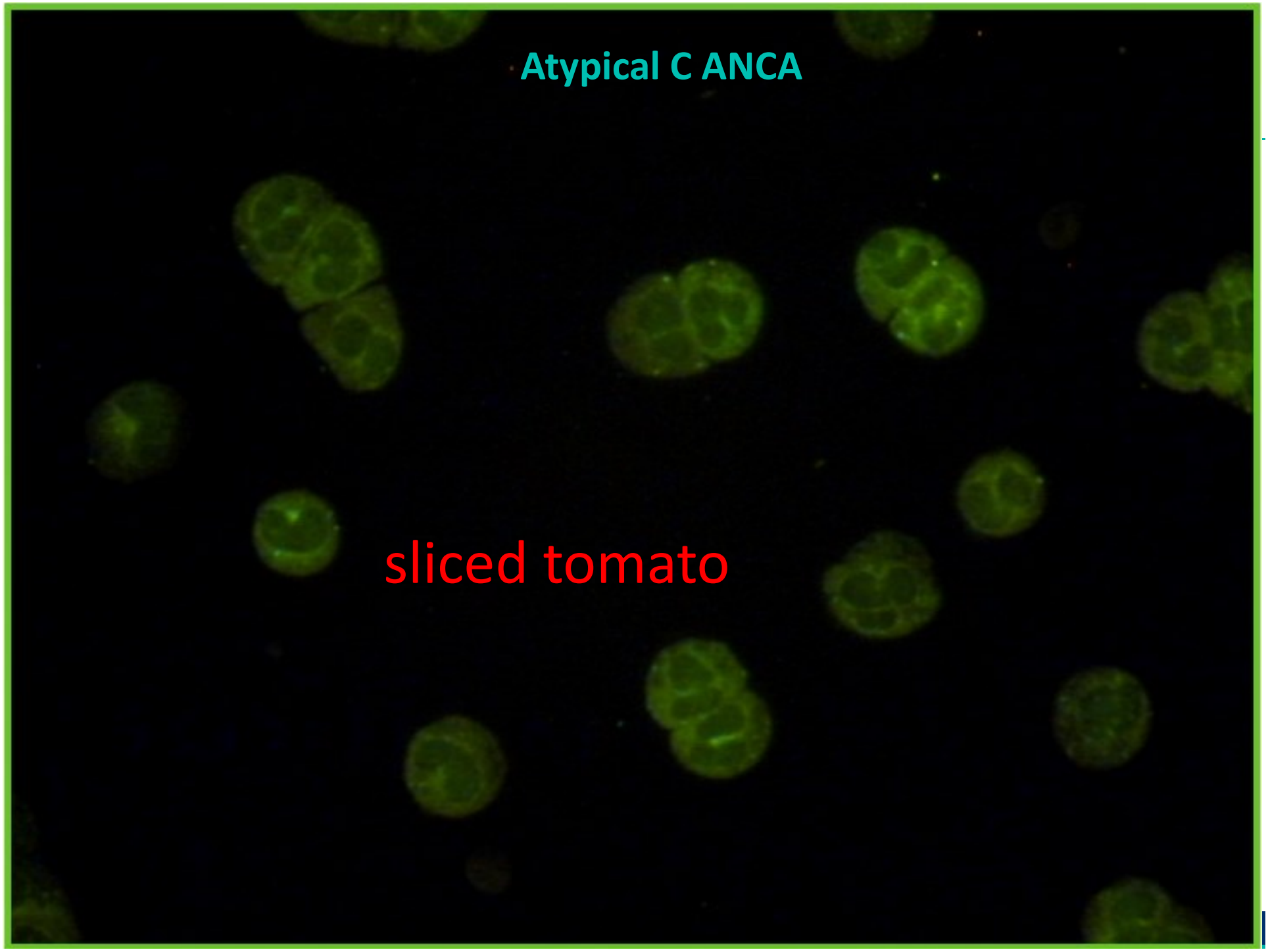
C-ANCA (cytoplasmic)

- Proteinase-3 (PR-3) is target antigen (rarely others)
- Remains attached to alpha-granule during ethanol fixation
- Brighter staining around the lobes of the nucleus is called “central accentuation”


C -ANCA
Central Accentuation
Current C-ANCA on Ethanol

C-ANCA (atypical)

- Flat cytoplasmic staining without central accentuation
- Can account for half of ANCA positives in some labs!
- NOT specific for vasculitic disease
- Possible antigens:
  - Bactericidal Permeating Inhibitor (BPI)
  - Mixture of others including cathepsin G

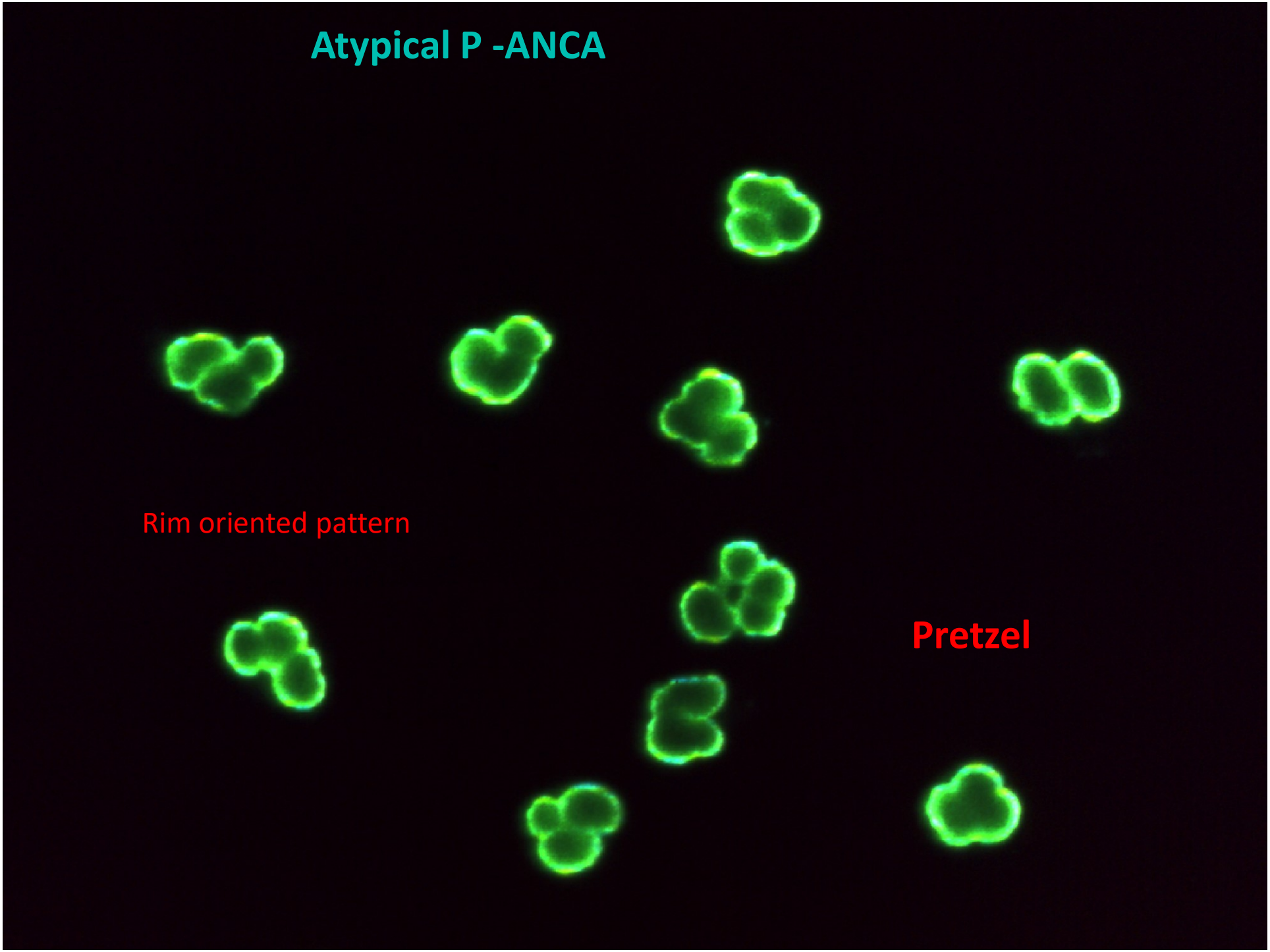

Atypical C ANCA
sliced tomato

“Atypical” P-ANCA

- Associated with various bowel diseases
  - Ulcerative colitis (75%)
  - Primary sclerosing cholangitis (80%)

- P-ANCA staining on EtOH fixed slides, negative on formalin fixed slides

- Target antigen is unknown
  - Could be combination of various antigens(Lactoferin/Beta glucorunidase/ Elastage/ Alpha Enolase etc)


Atypical P -ANCA
Rim oriented pattern
Pretzel

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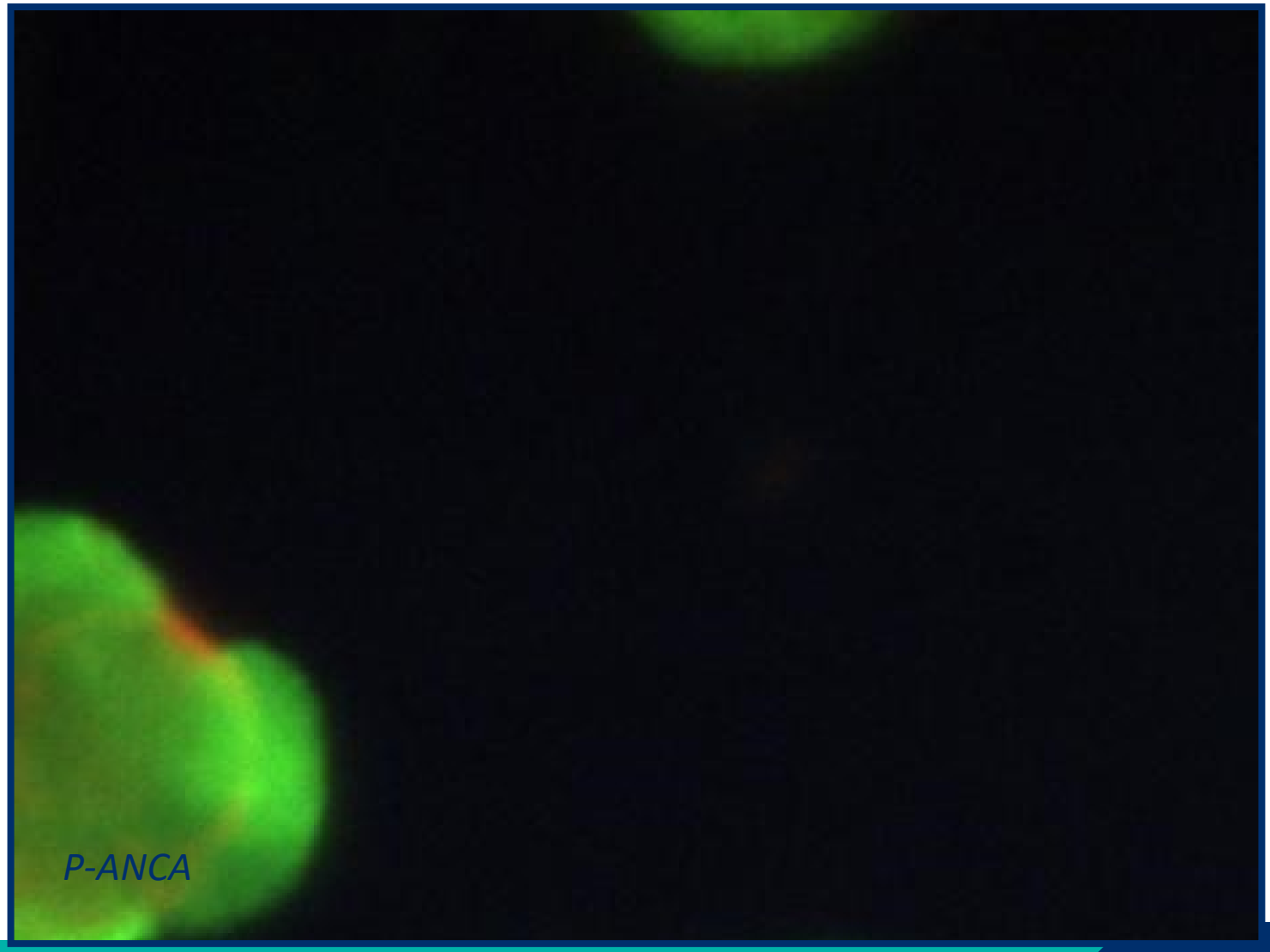
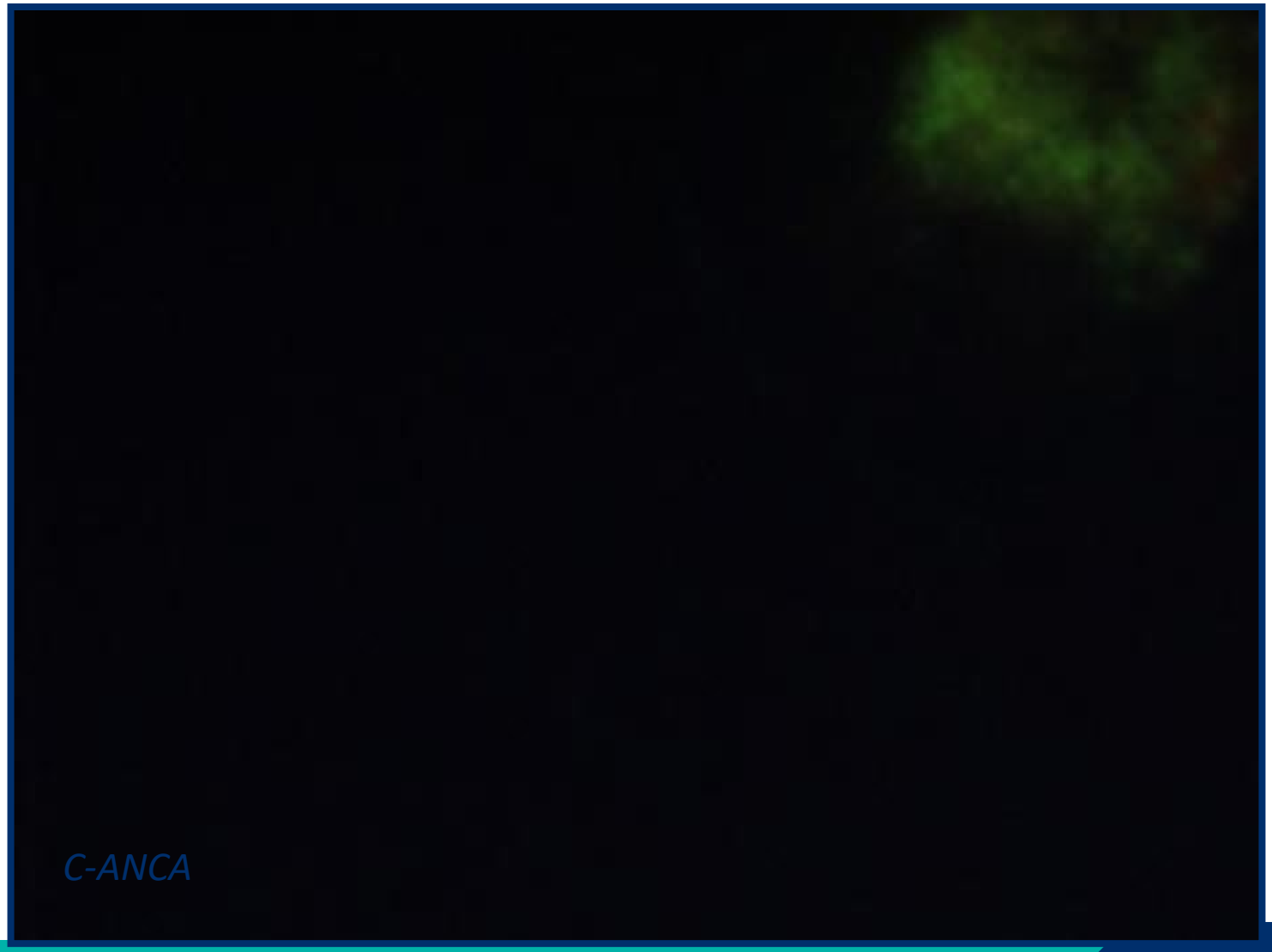
Let us quickly repeat the patterns

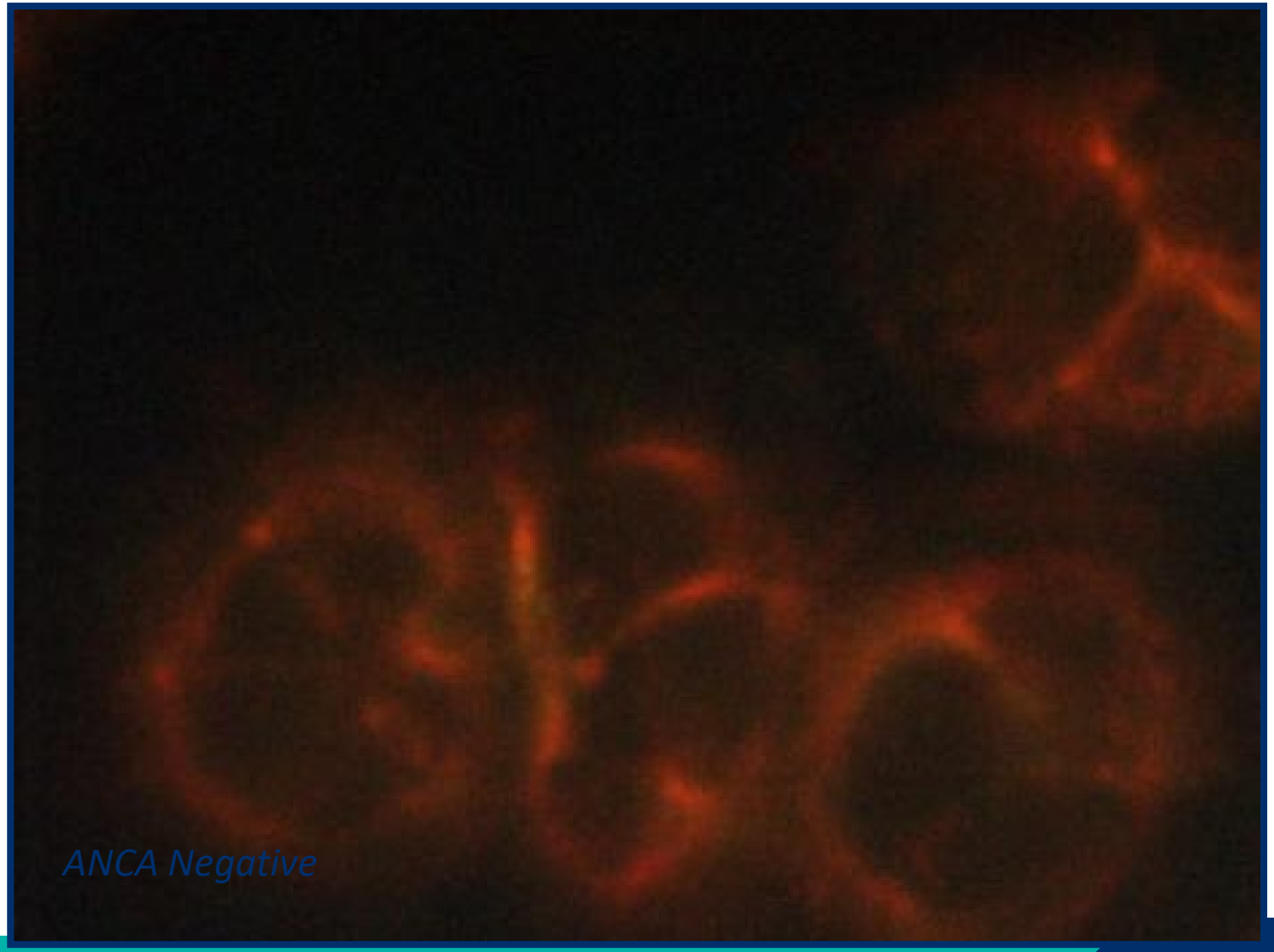
ANCA Patterns



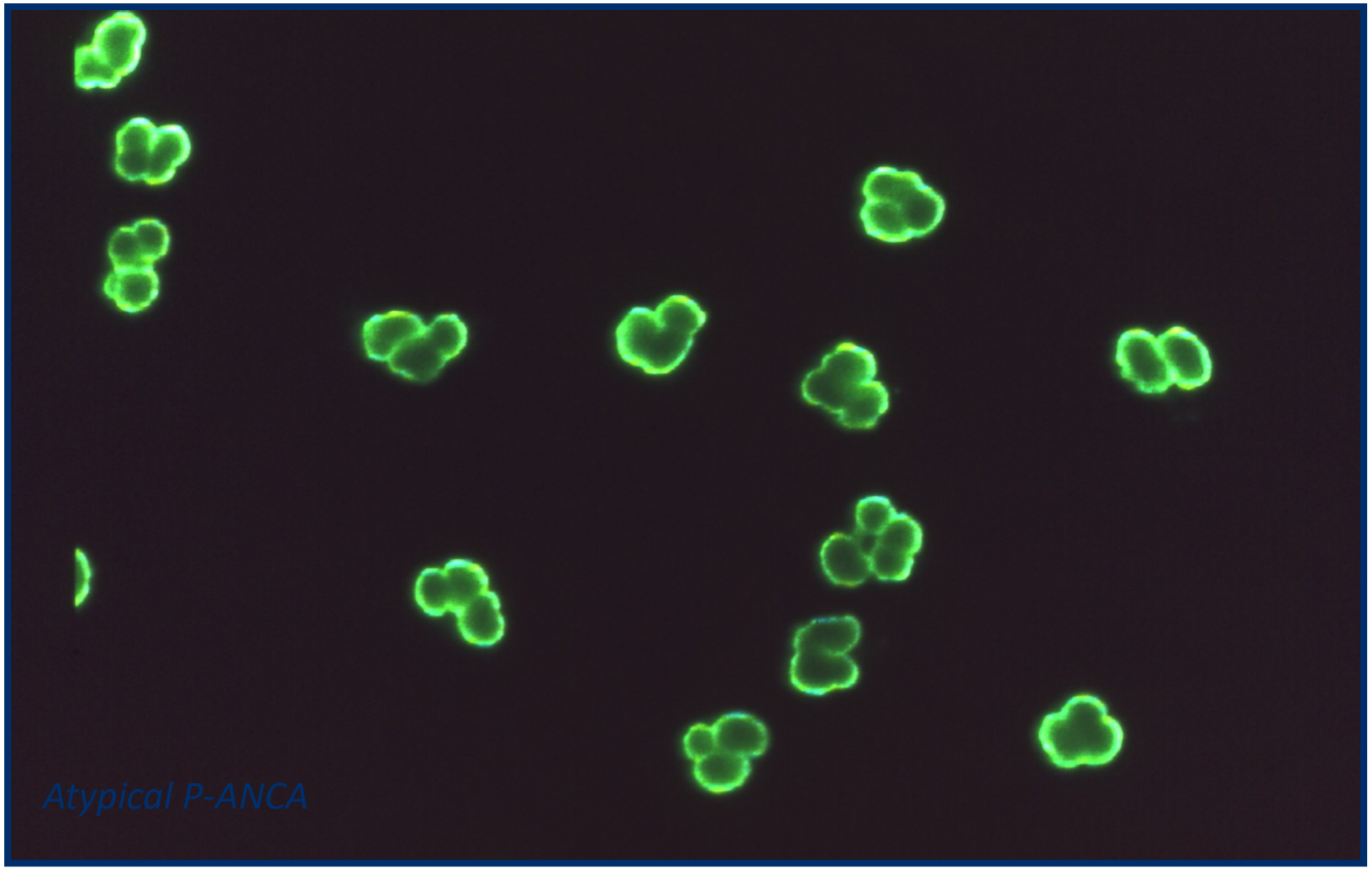
ANCA Patterns



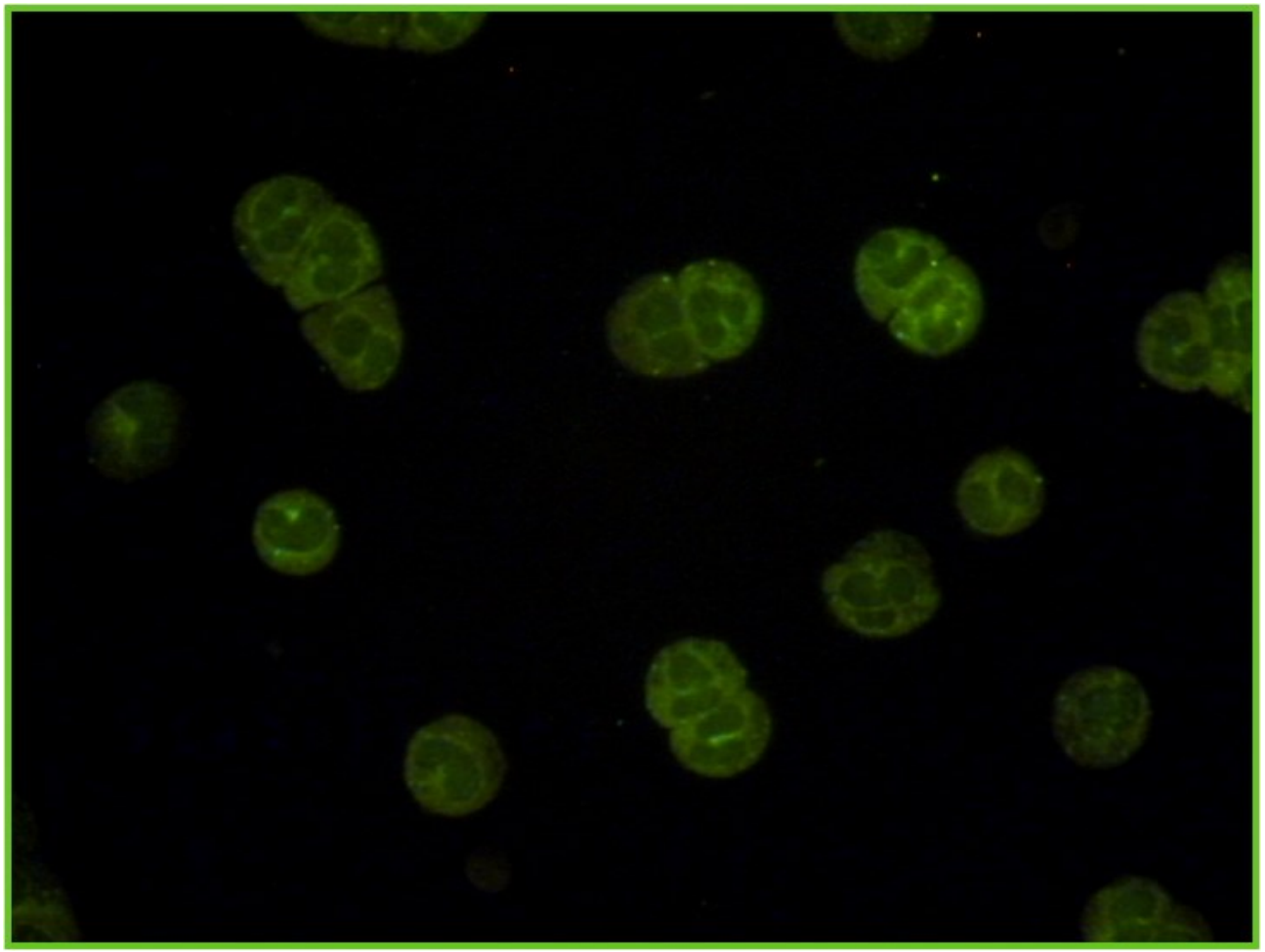
ANCA Patterns



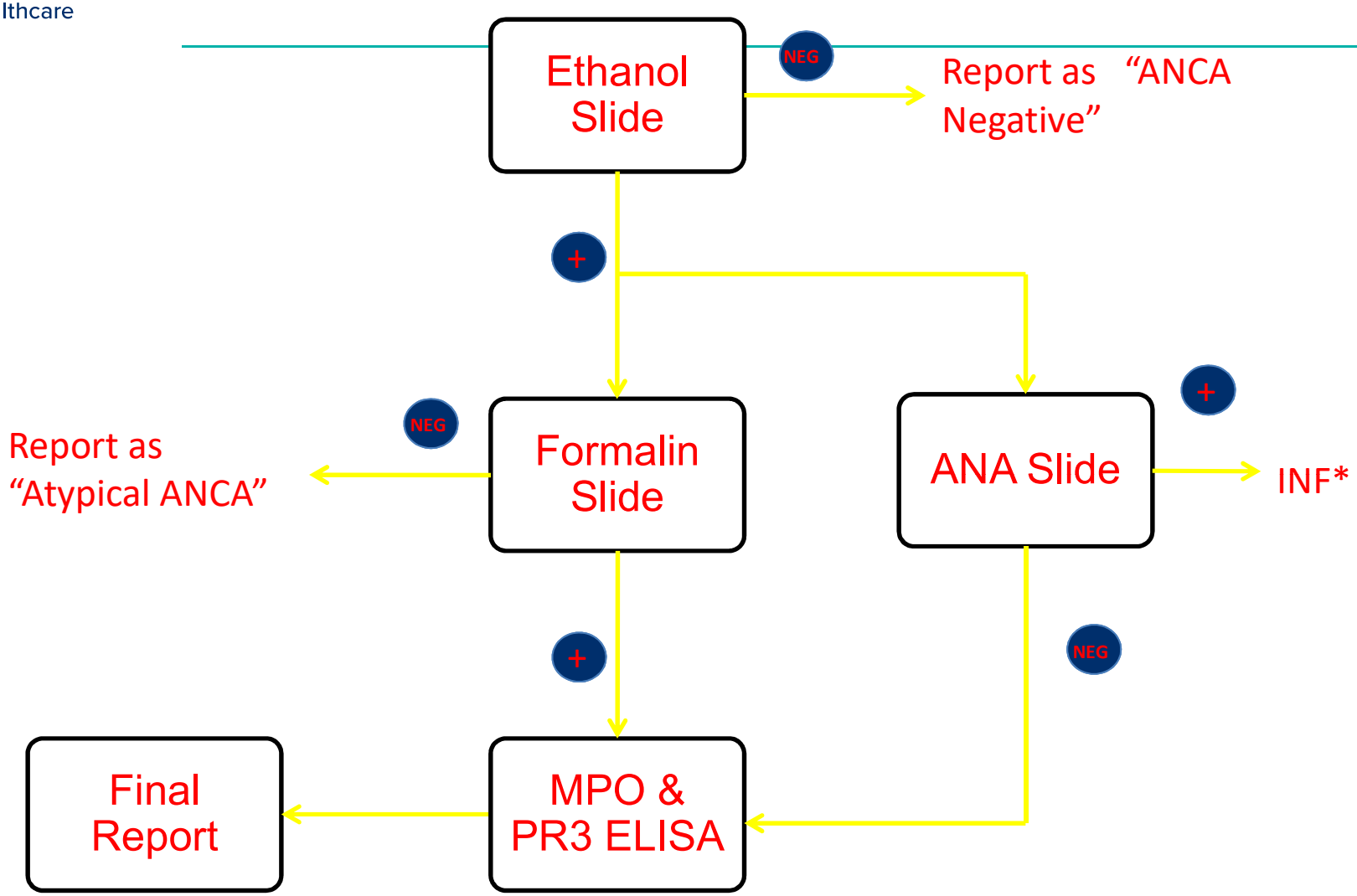
ANCA Patterns



ANCA Patterns



Atypical C-ANCA

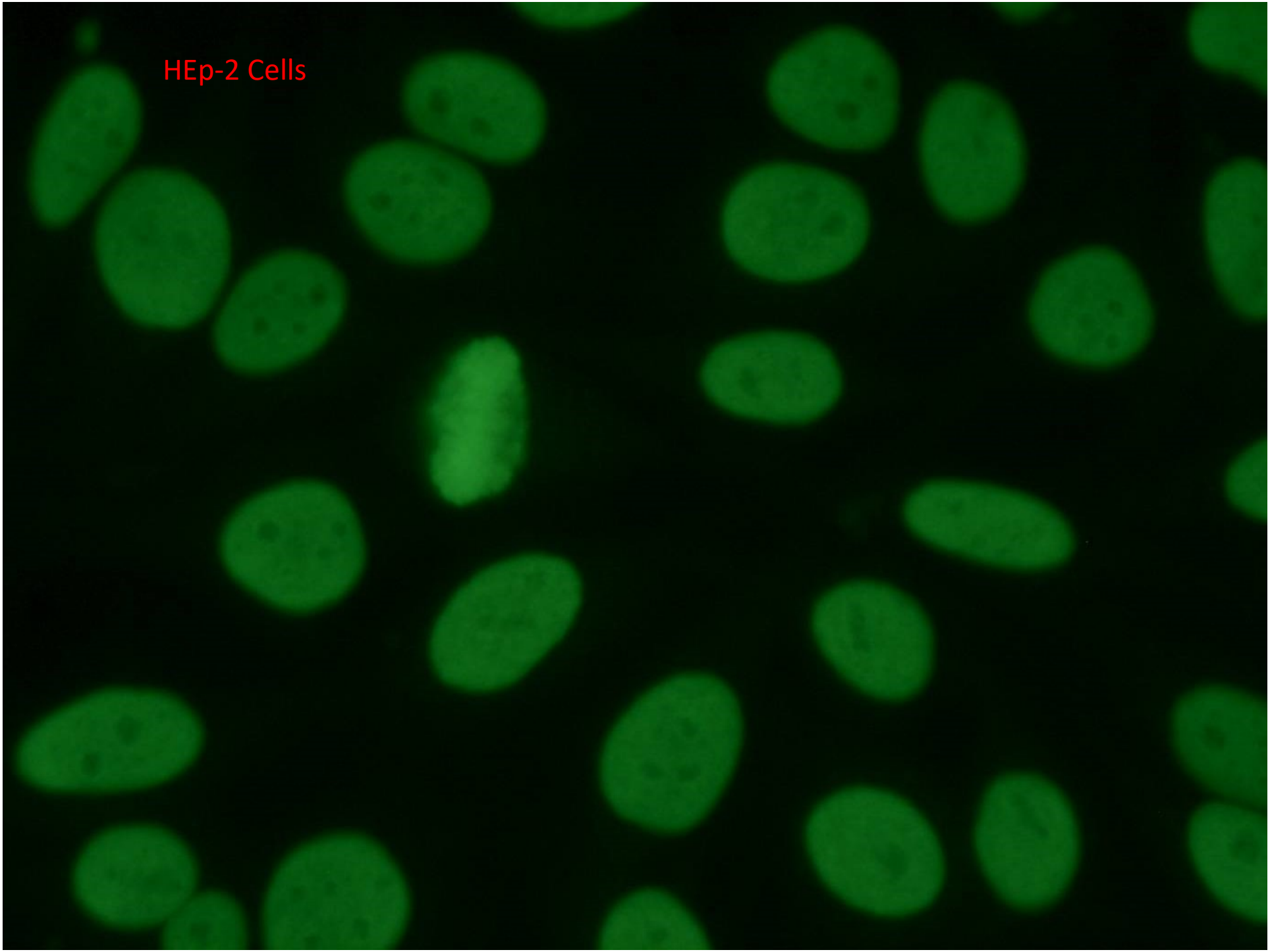


*INF: Report as "ANA Interference – Cannot Interpret IFA ANCA"

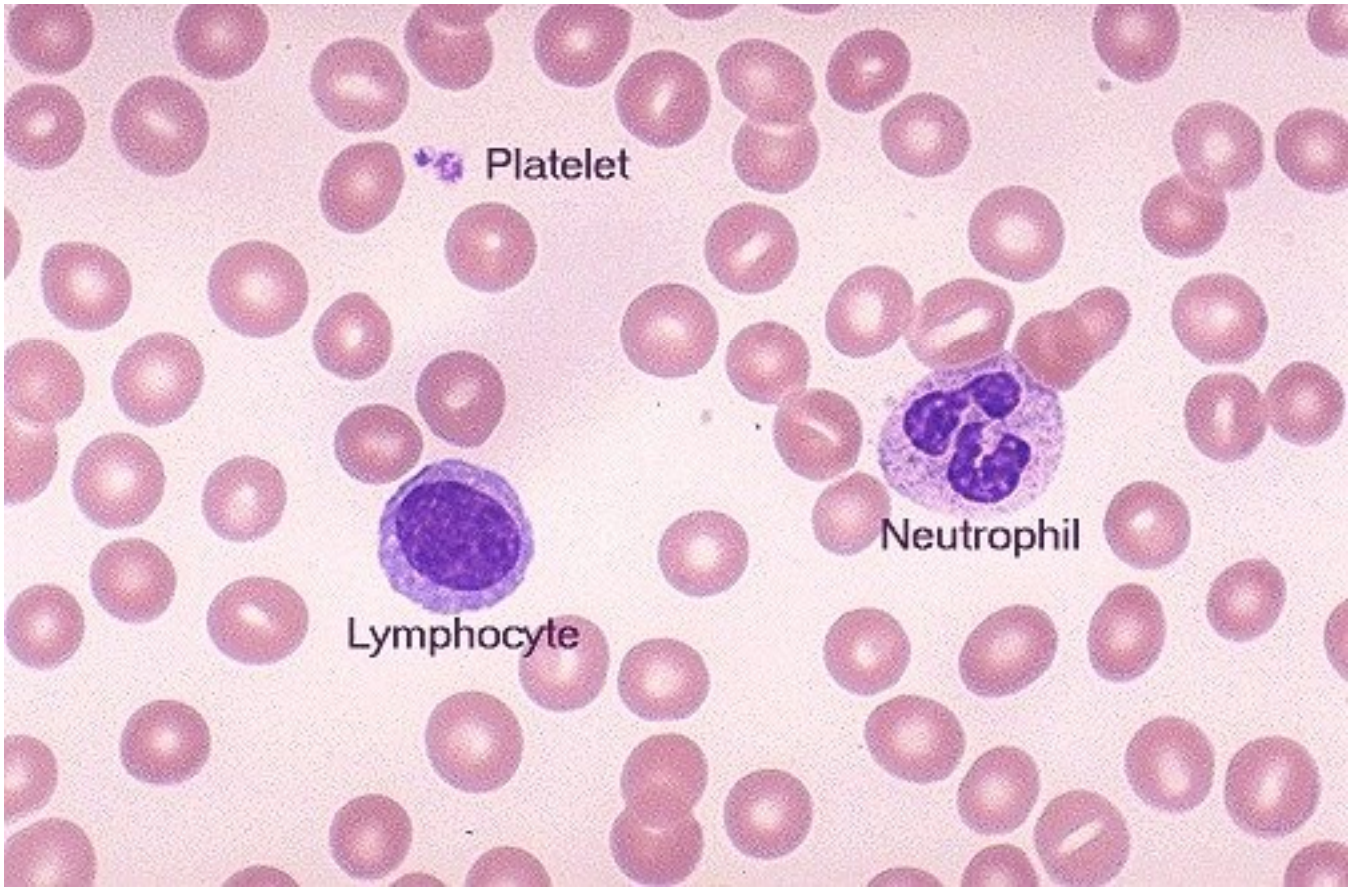
What is a Difficulty with ANCA?

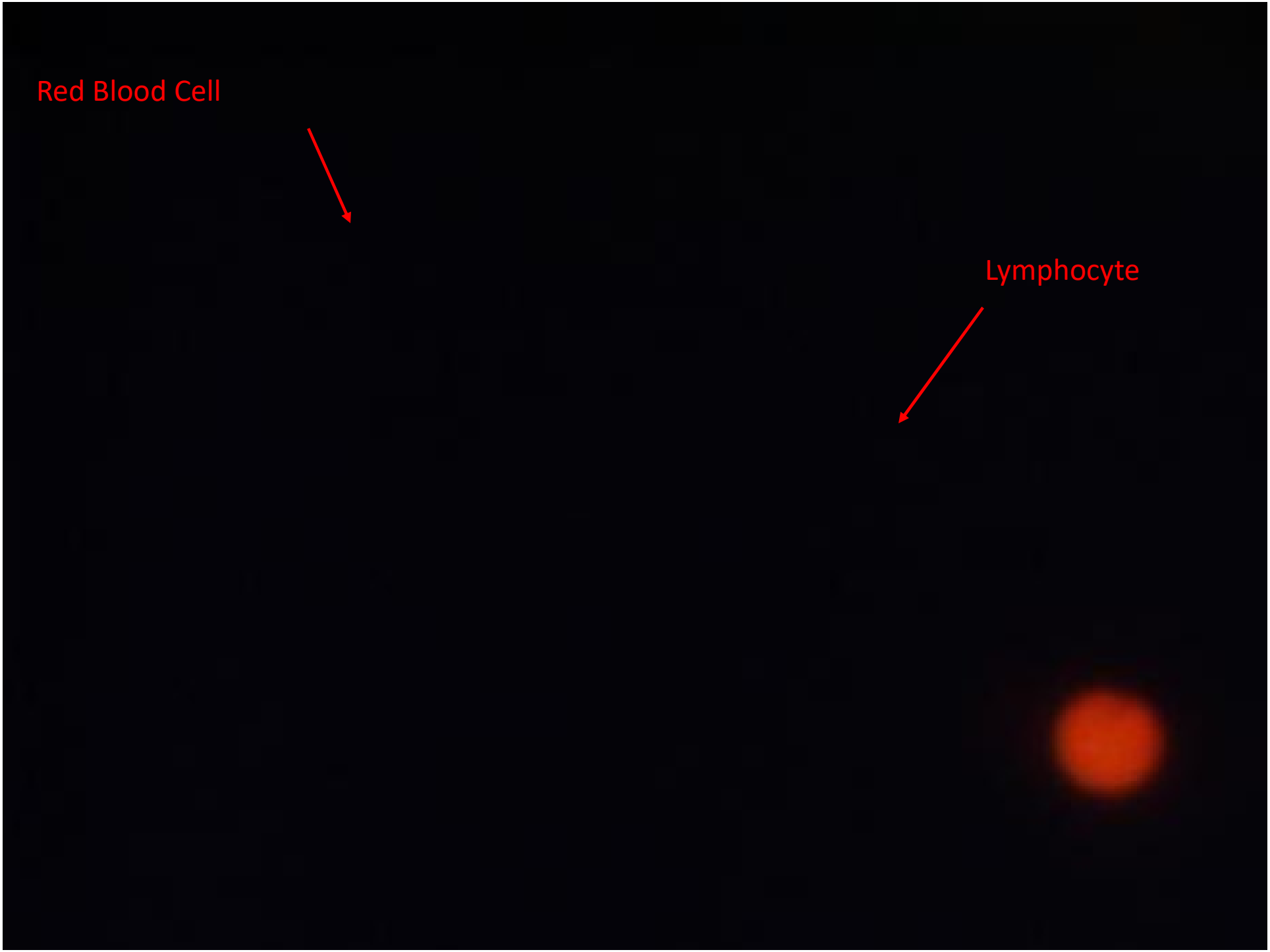
- Antinuclear antibodies can interfere with ANCA interpretation
- A homogeneous ANA pattern can look like a P-ANCA
- The International Consensus Guidelines recommend running a HEp-2 slide for all P-ANCA positive samples


Homogeneous ANA or P-ANCA?

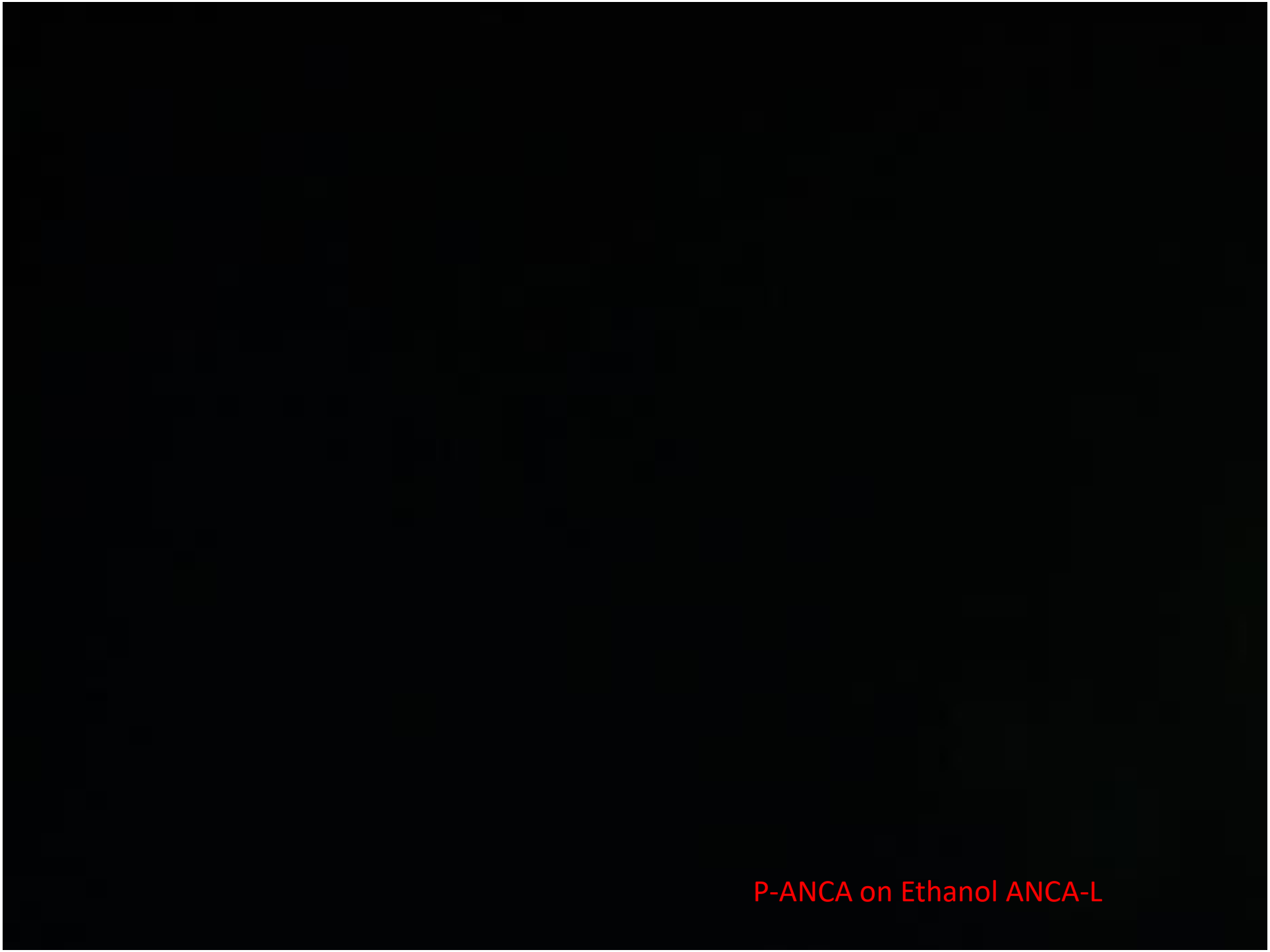

HEp-2 Cells

Blood Film

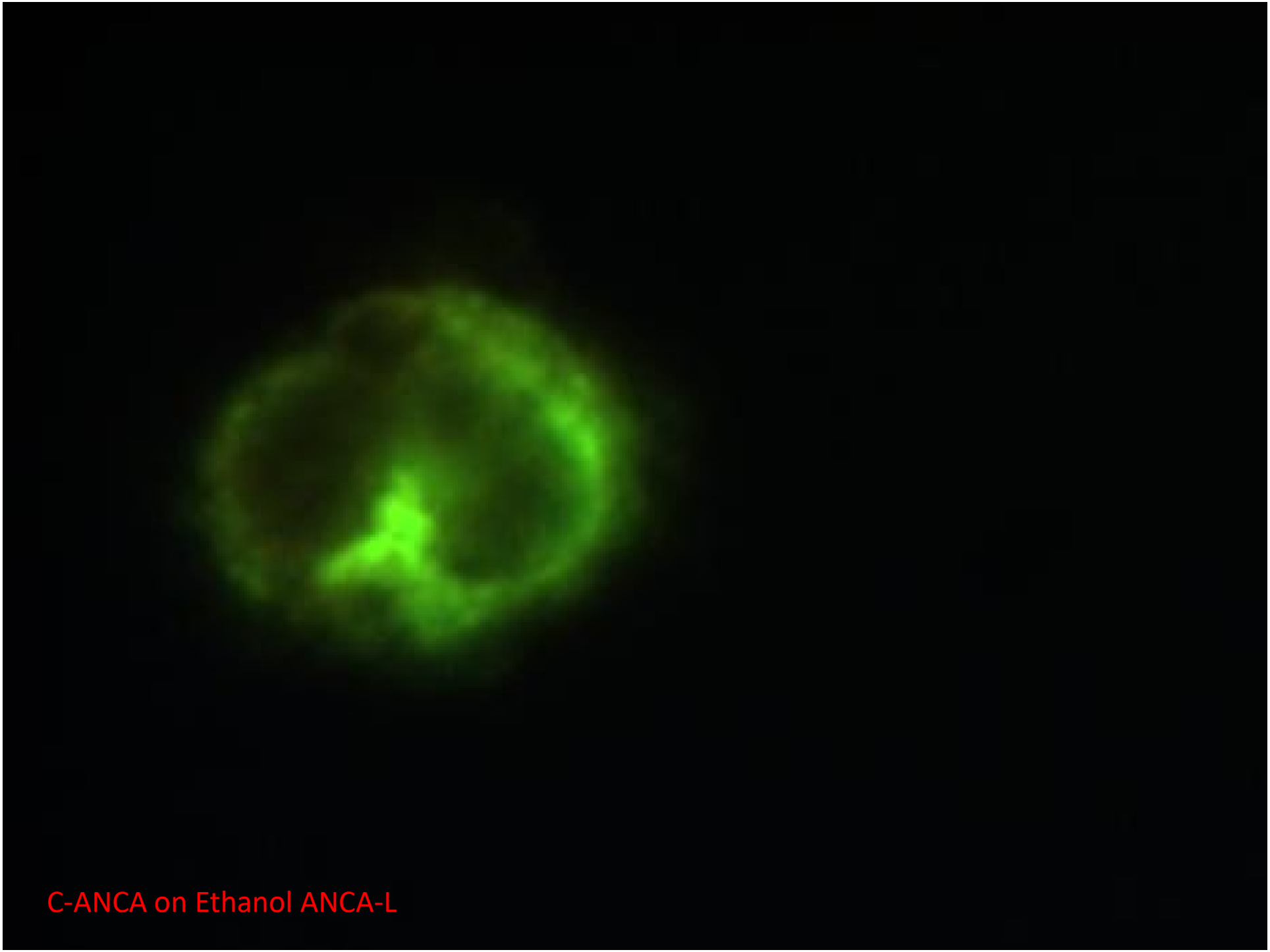



Red Blood Cell
Lymphocyte

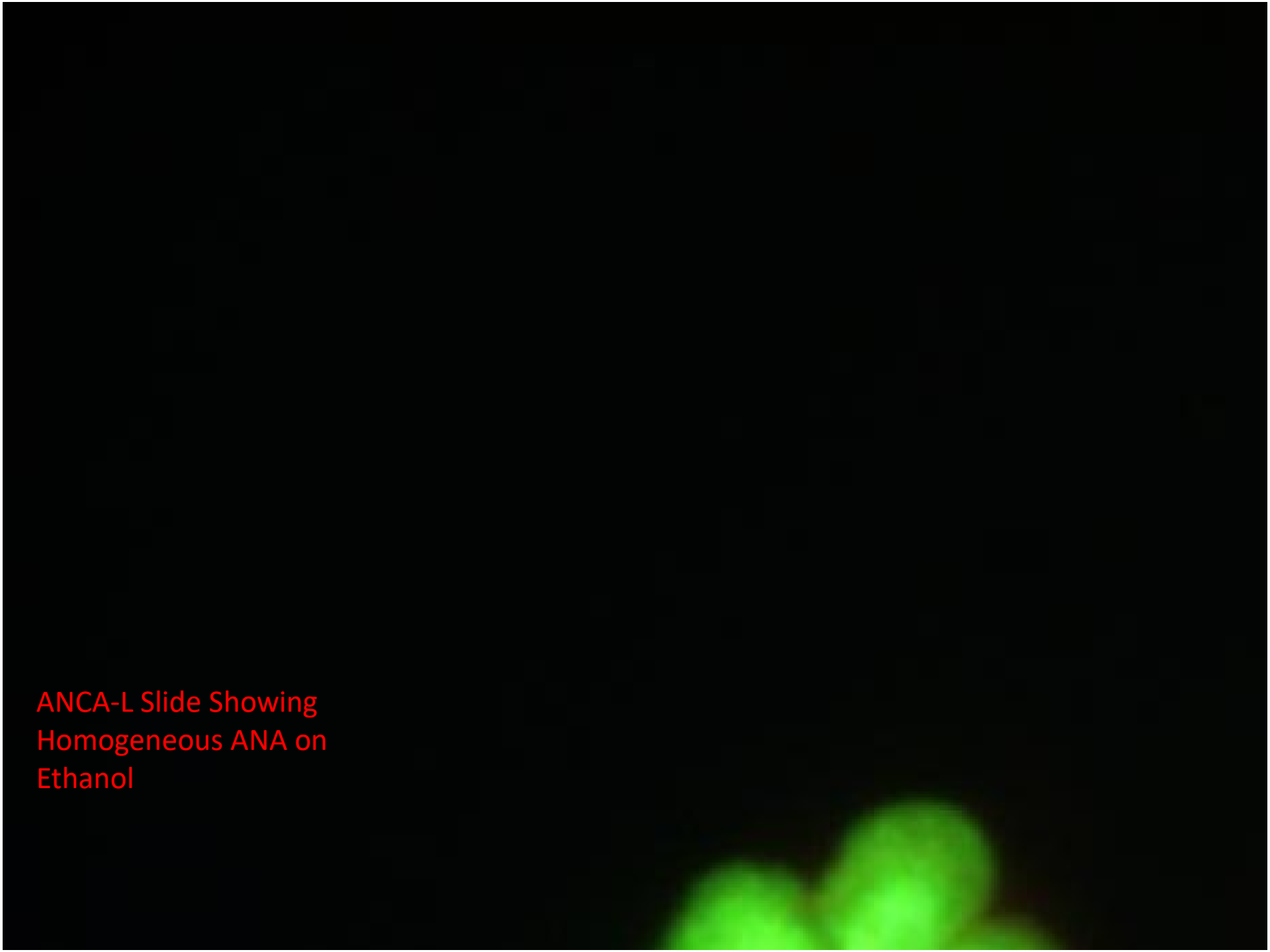

ANCA L
P-ANCA on Ethanol ANCA-L


P-ANCA on Ethanol ANCA-L


P-ANCA on Ethanol ANCA-L


C-ANCA on Ethanol ANCA-L

ANCA Negative on Ethanol ANCA-L


ANCA-L Slide Showing
Homogeneous ANA on
Ethanol


New Slide Showing
Homogeneous ANA on
Ethanol

Current Consensus

- Screen on ethanol fixed slides
- Test ethanol positives on formalin fixed slides
- Test positives on ELISA for anti-MPO and anti-PR3
- Test P-ANCA positives for ANA

Confirmatory Testing for ANCA

- All fluorescent ANCA positive samples should be confirmed using ELISA tests
- P-ANCA should confirm as MPO +
- C-ANCA should confirm as PR3 +
- There are exceptions

Confirmatory Testing for ANCA

- There are IFA positive samples that are ELISA negative.
- Most labs run both MPO and PR3 on positives.

ANCA Summary

	Ethanol	Formalin	MPO ELISA	PR3 ELISA	Disease
C-ANCA	Granular Cytoplasmic	Granular Cytoplasmic	Neg	Pos	Wegener's
P-ANCA	Perinuclear	Granular Cytoplasmic	Pos	Neg	MPA
Atypical C-ANCA	Fine, flat speckled	Neg	Neg	Neg	?
Atypical P-ANCA	Very rim	Neg	Neg	Neg	Ulcerative Colitis
ANA	Nuclear; Lymphocyte	Neg	Neg	Neg	Multiple