IMMUNOFLUORESCENCE DETEC TION OF ANTI-NUCLEAR ANTIBODIES ON Hep2 CELLS

ANA-TESTING

IMMUNOFLUORESCENCE ON HEp2 CELLS

CONNECTIVE TISSUE DISEASES

INFECTIOUS DISEASES

DRUG INDUCED

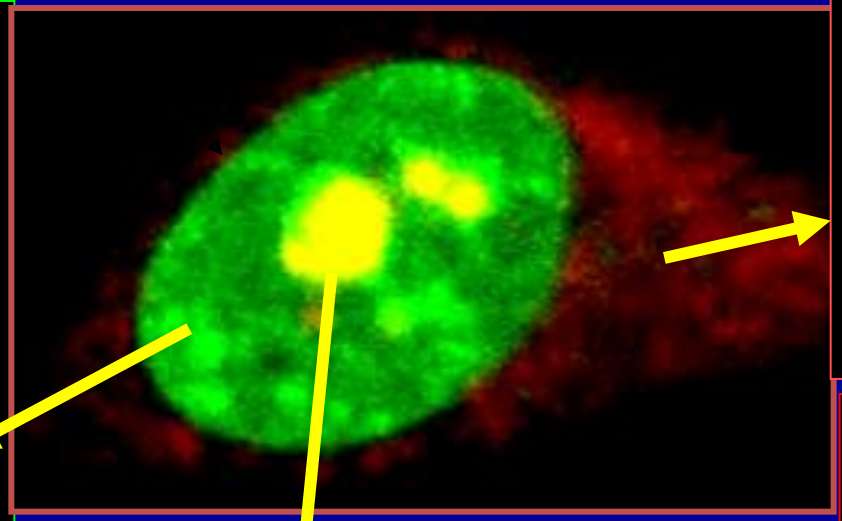
No Disease

CANCER

ANTINUCLEAR ANTIBODIES

NUCLEI

RNP
Sm
SSA
SSB
Scl70
PMScl
Ku
Mi2
PCNA
Centromere A/B
DFS70
RNA POL III
Sp100
gp210



CYTOPLASM

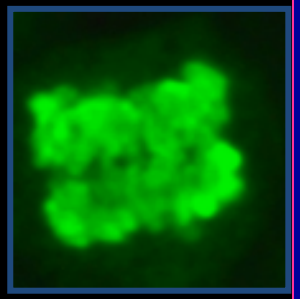
JO1,PL7,PL12,EJ,OJ,
KS,Zo,SRP,
Mitochondria, Golgi
Ribosomes

Actin, Vimentin
Tubulin , Nematine

NUCLEOLI

Th/To
Fibrillarine
NOR90
RNAPol I
PMScl100

MITOTIC CELLS



Nucleosome
DNA, Histones
Spindle Apparatus
Mitotic Chromosomes
CENP E, CENP F, MSA2
Midbody

RL.Humbel

ANA-TESTING

IMMUNOFLUORESCENCE ON HEp2 CELLS

POSITIVE

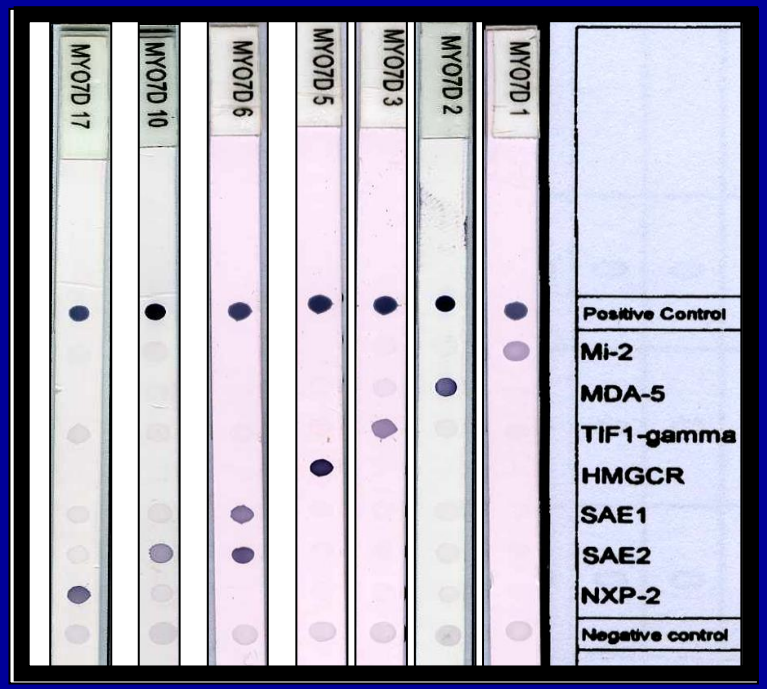
IDENTIFICATION

REFLEX TEST

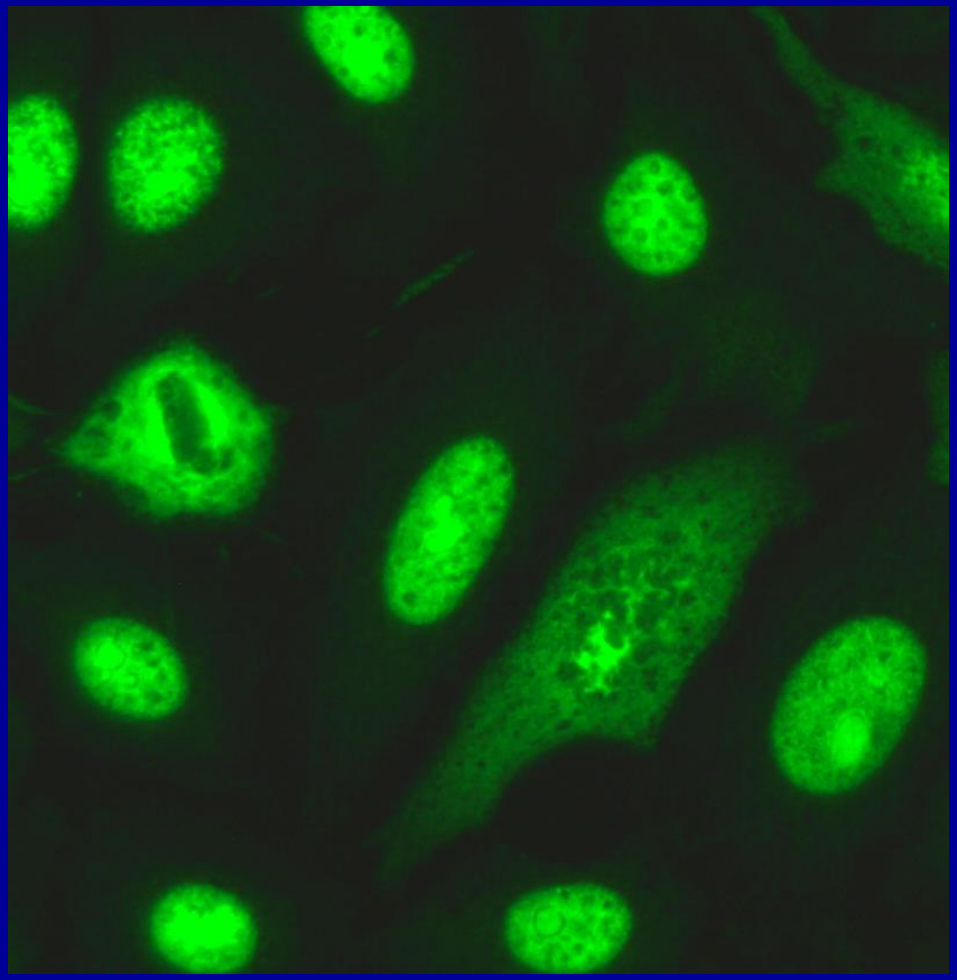
Nucleosome , DNA
ENA PROFILE

NEGATIVE

Ro52/TRIM21
SOME MYOSITIS ANTIBODIES



Hep2 CELLS



JOURNAL OF CLINICAL MICROBIOLOGY, July 1975, p. 42–45
Vol. 2, No. 1

Evaluation of Cellular Substrates for Antinuclear Antibody Determinations

NICHOLAS HAHON,* HERBERT L. ECKERT, AND JOHN STEWART

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Received for publication 18 April 1975

HEp2

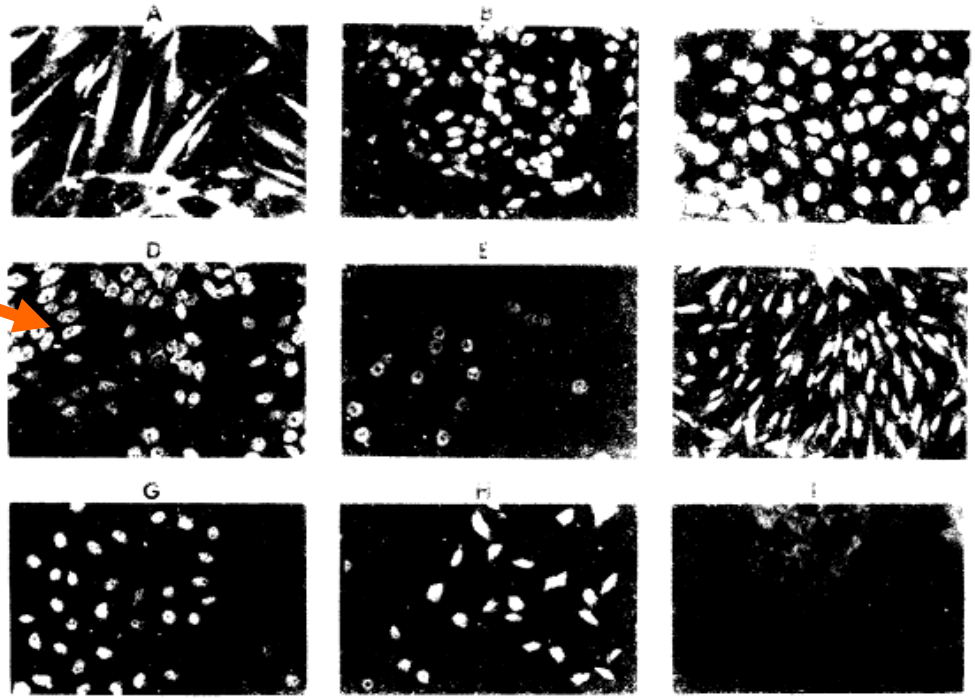


FIG. 1. *Nuclear fluorescence in different cell substrates using an identical dilution of one ANA-positive serum sample. A, WI-38; B, 1-5c-4; C, LLC-MK₂, D, HEp-2; E, E-N; F, BHK 21/C13; G, HeLa; H, L-929; I, RK. ×125.*

Hep-2 CELLS are HeLa CELLS !!

Cells of this line contain HeLa marker chromosomes, and were derived via HeLa contamination. This line was originally thought to be derived from an epidermoid carcinoma of the larynx, but was subsequently found, based on isoenzyme analysis, HeLa marker chromosomes, and DNA fingerprinting, to have been established via HeLa cell contamination. The cells are positive for keratin by immunoperoxidase staining.

Henrietta Lacks (1920-1951)

Adenocarcinoma of the Cervix

IMMUNOFLUORESCENCE

SUBSTRAT

Hep2 CELLS

ANTI-GLOBULIN

IgG
IgM

IgG (H+L)

DILUTION BUFFER

PBS or TRIS

Tween 20
Non Fat Dry Milk

Evans Blue

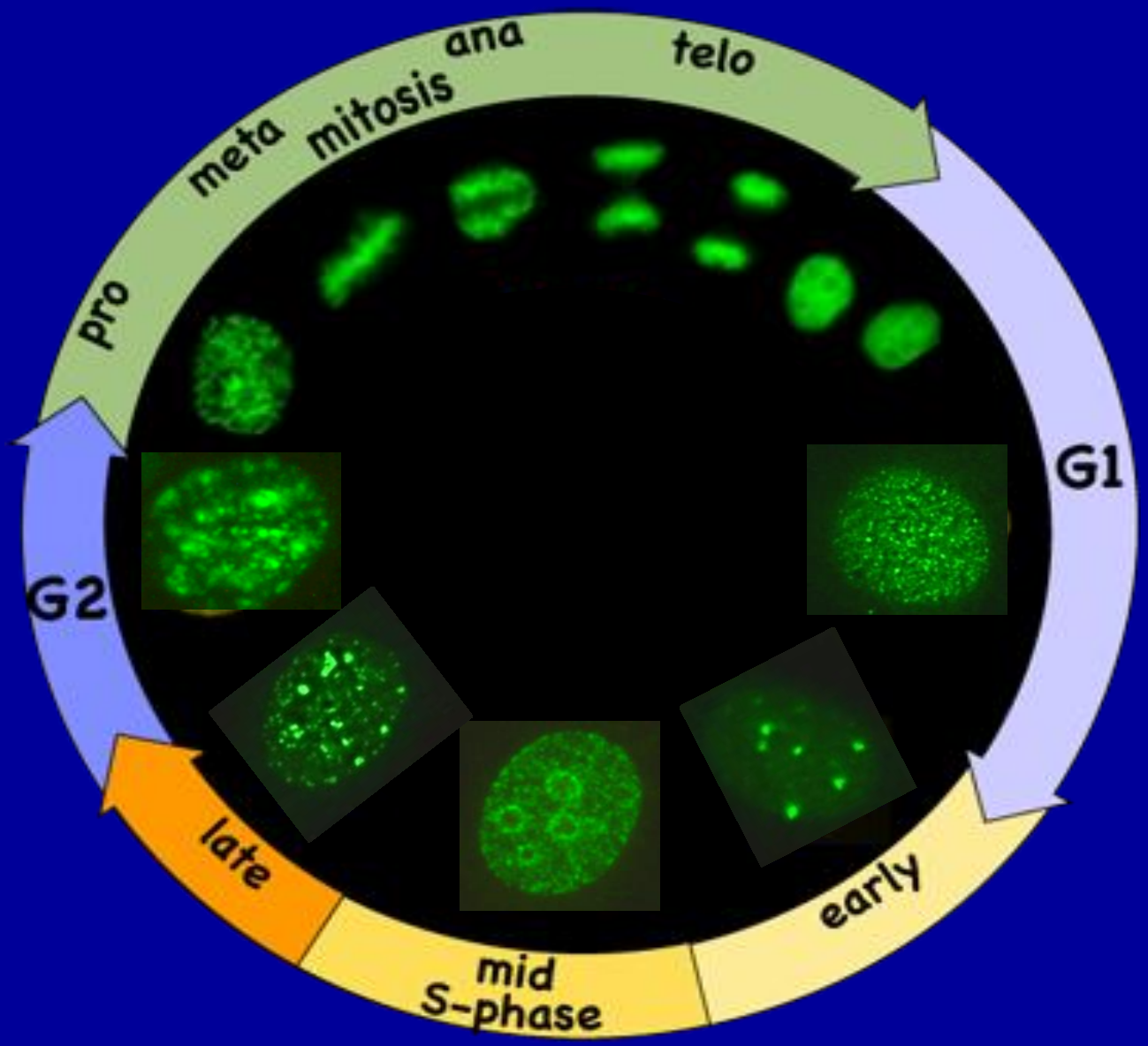
COUNTER STAINING

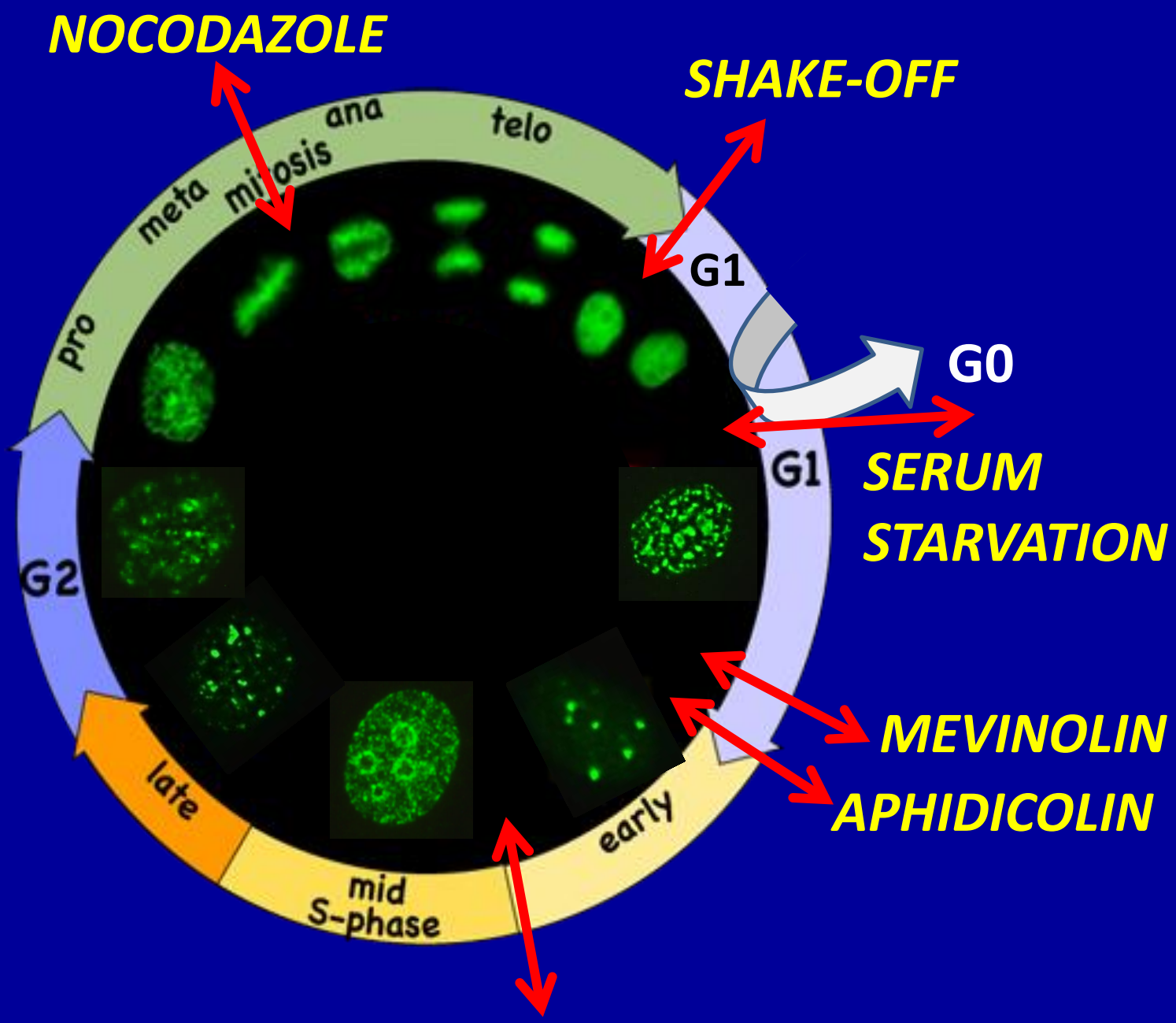
Evans Blue

MOUNTING MEDIUM

Anti-Fading Agents

Hep2 CELL CULTURE




NOCODAZOLE
SHAKE-OFF
ana
telo
meta
mitosis
pro
G1
G0
G1
SERUM
STARVATION
G2
MEVINOLIN
APHIDICOLIN
late
early
mid
S-phase
THYMIDINE

IMMUNOFLUORESCENCE

INTERPRETATION

TITRE

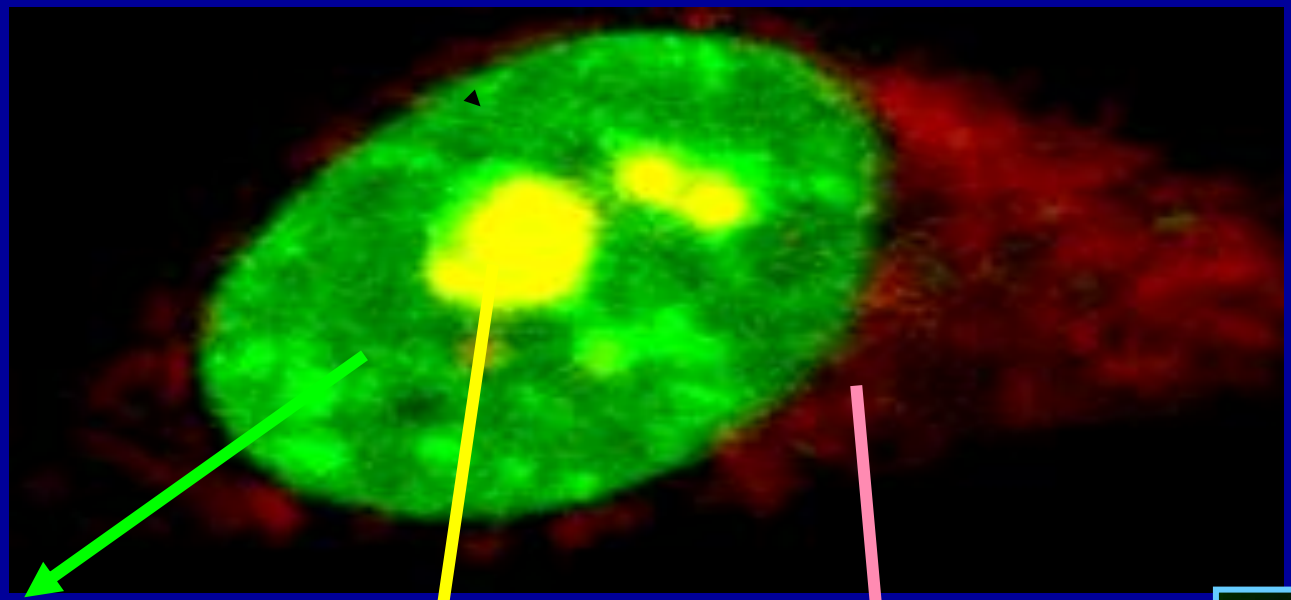
> : 1/320

ASPECT

What is Stained ?

How ?

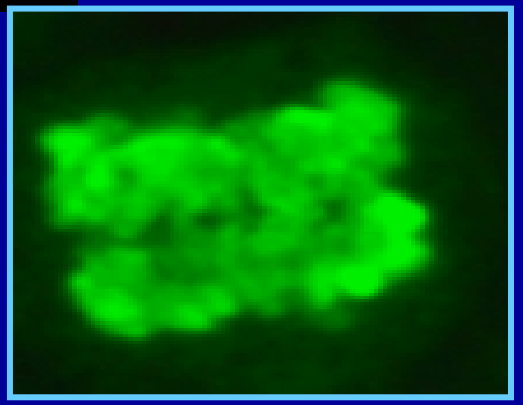
WHAT IS STAINED ?



NUCLEI

NUCLEOLI

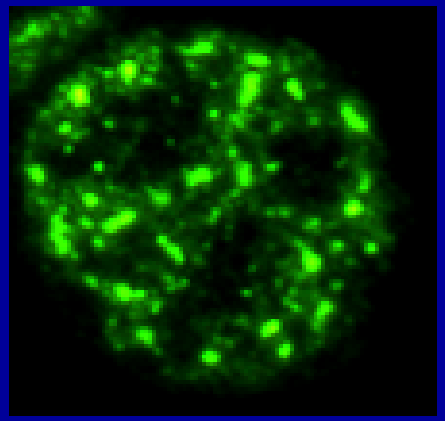
CYTOPLASME



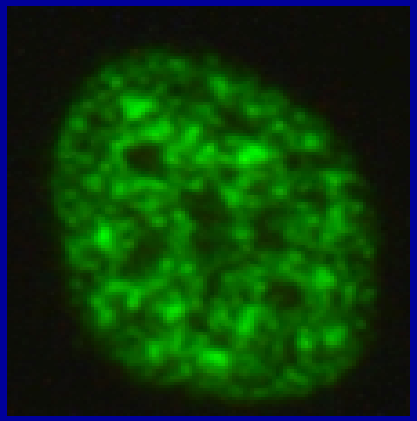
MITOSIS

RL.Humbel

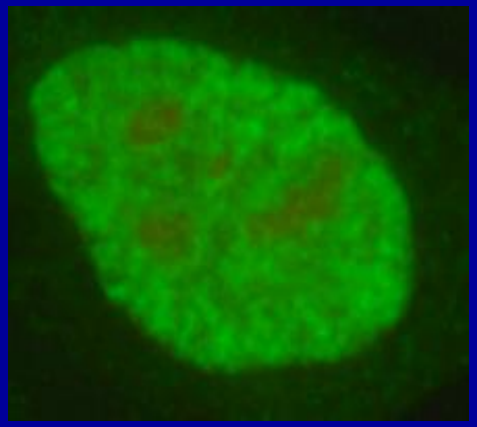
LARGE SPECKLED



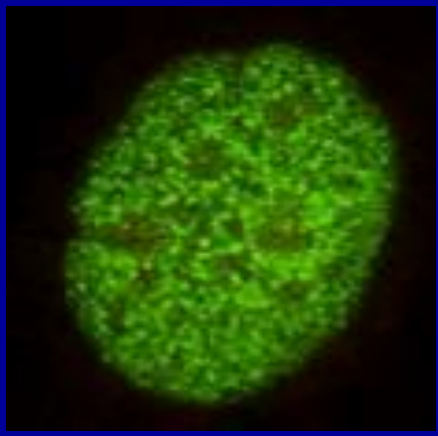
COARSE SPECKLED



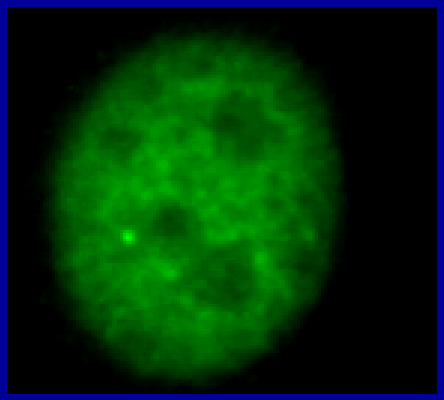
DENSE FINE SPECKLED

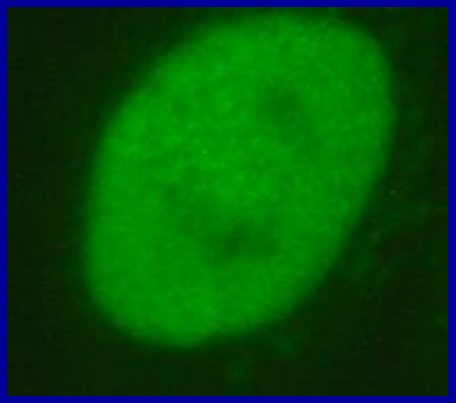


GRANULAR

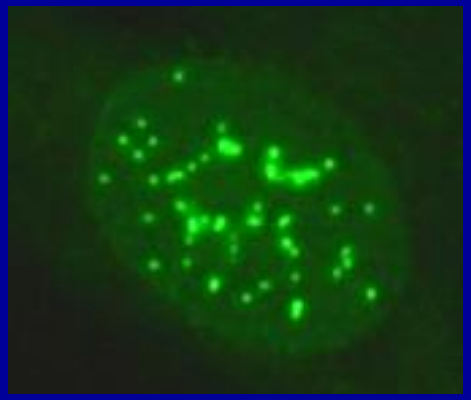


DENSE FINE GRANULAR

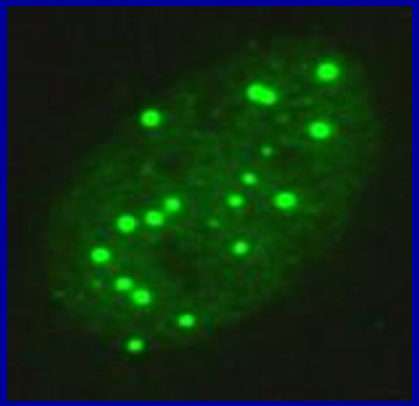




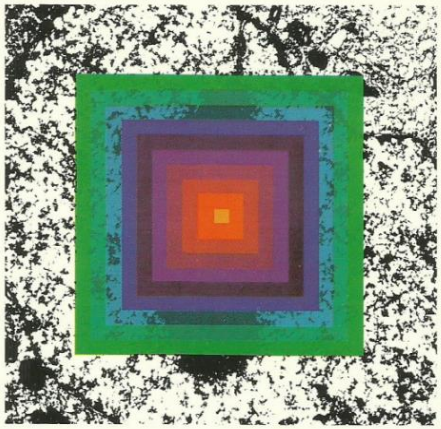
CENTROMERE



DOTS



RL.Humbel



AN ATLAS OF FLUORESCENT PATTERNS ASSOCIATED WITH ANTINUCLEAR AND ANTICYTOPLASMIC ANTIBODIES ON HEp-2 CELLS

R. HUMBEL Chef de laboratoire. Centre Hospitalier du Luxembourg.
G. HULIN Scientific manager. Biolab S.A./N.V.
G. GOESSENS, M. THIRY Laboratoire d'Histologie. Université de Liège - (HEp-2 CELL ULTRASTRUCTURE).

biolab PRODUCTS FOR MEDICAL INVESTIGATION

1985

immuno concepts

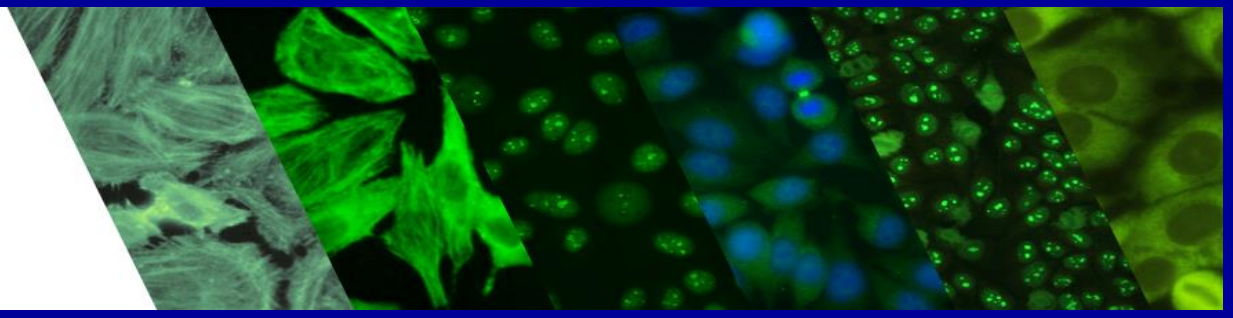
MEMBRANOUS PATTERNS
1. Homogeneous
2. Punctate

NUCLEAR PATTERNS
- Homogeneous
- Speckled 1 - 4
- Pleomorphic
- Centromere
- Nuclear Dots 1-2

NUCLEOLAR PATTERNS
- Homogeneous
- Clumpy
- Speckled
- Dots

SPINDLE APPARATUS 1-4

CYTOPLASMIC PATTERNS
- Fine Speckles
- Fine Dense
- Large Speckles
- Polar Arrow
- Stress Fibers
- Fine Fibers

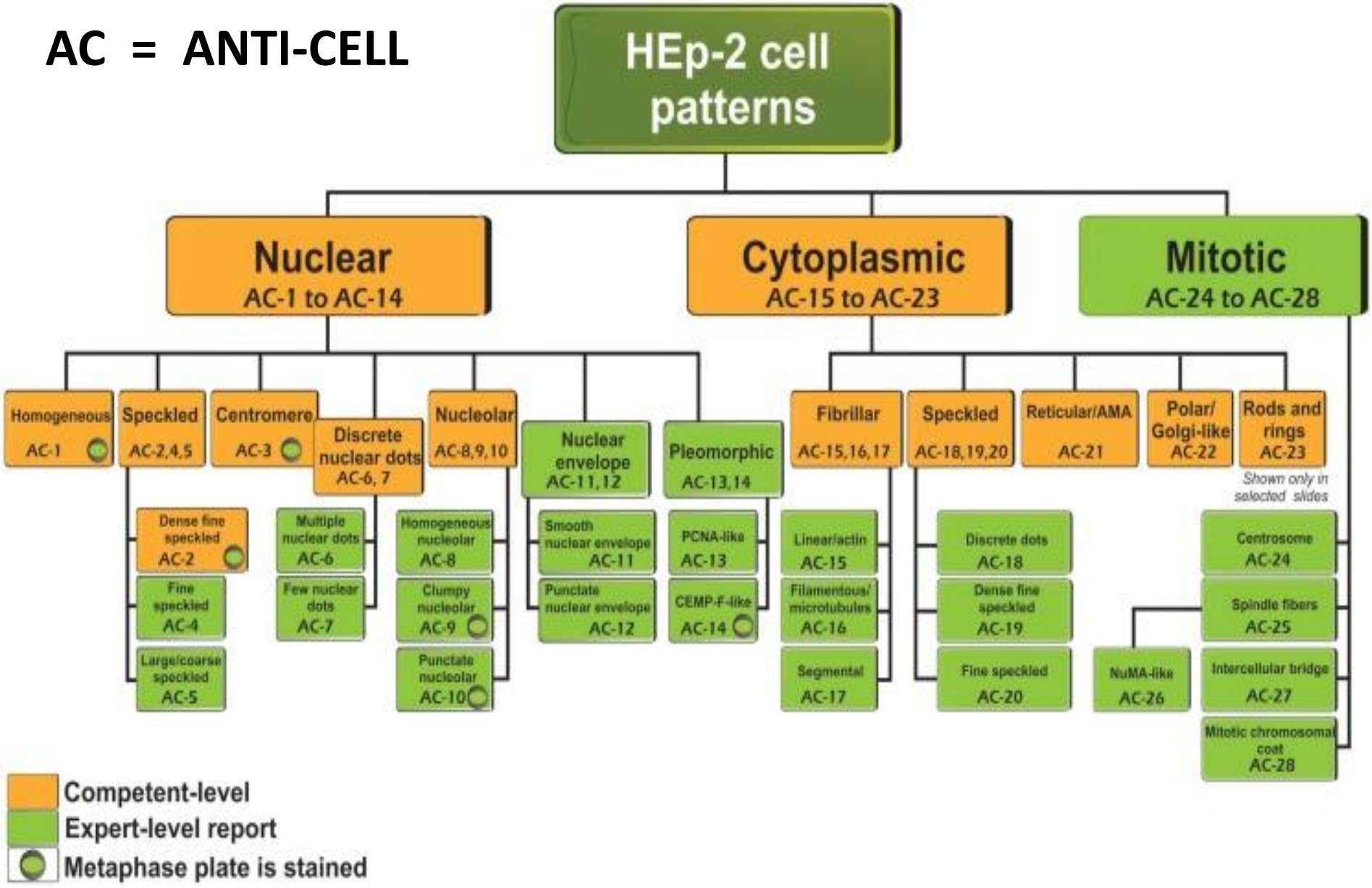


International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies

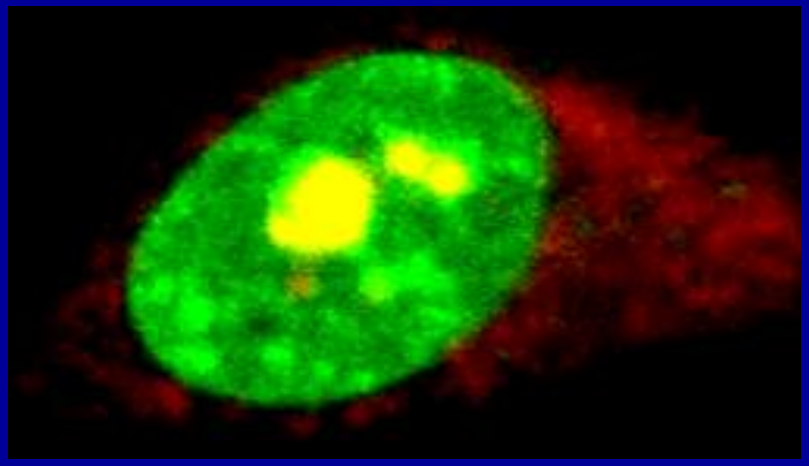
Nancy Agmon-Levin,[1,2] Jan Damoiseaux,[3] Cees Kallenberg,[4] Ulrich Sack,[5] Torsten Witte,[6] Manfred Herold,[7,8] Xavier Bossuyt,[9] Lucille Musset,[10] Ricard Cervera,[11] Aresio Plaza-Lopez,[12] Carlos Dias,[13] Maria José Sousa,[14] Antonella Radice,[15] Catharina Eriksson,[16] Olof Hultgren,[17] Markku Viander,[18] Munther Khamashta,[19] Stephan Regenass,[20] Luis Eduardo Coelho Andrade,[21] Allan Wiik,[22] Angela Tincani,[23] Johan Rönnelid,[24] Donald B Bloch,[25] Marvin J Fritzler,[26] Edward K L Chan,[27] I Garcia-De La Torre,[28] Konstantin N Konstantinov,[29] Robert Lahita,[30] Merlin Wilson,[31] Olli Vainio,[32] Nicole Fabien,[33] Renato Alberto Sinico,[34] Pierluigi Meroni,[35] Yehuda Shoenfeld[1,2,36]

NEW NOMENCLATURE OF ANTINUCLEAR ANTIBODIES

AC = ANTI-CELL


HEp-2 cell patterns
Nuclear
AC-1 to AC-14
Cytoplasmic
AC-15 to AC-23
Mitotic
AC-24 to AC-28
Homogeneous
AC-1
Speckled
AC-2,4,5
Centromere
AC-3
Discrete nuclear dots
AC-6, 7
Nucleolar
AC-8,9,10
Nuclear envelope
AC-11,12
Pleomorphic
AC-13,14
Fibrillar
AC-15,16,17
Speckled
AC-18,19,20
Reticular/AMA
AC-21
Polar/ Golgi-like
AC-22
Rods and rings
AC-23
Shown only in selected slides
Dense fine speckled
AC-2
Fine speckled
AC-4
Large/coarse speckled
AC-5
Multiple nuclear dots
AC-6
Few nuclear dots
AC-7
Homogeneous nucleolar
AC-8
Clumpy nucleolar
AC-9
Punctate nucleolar
AC-10
Smooth nuclear envelope
AC-11
Punctate nuclear envelope
AC-12
PCNA-like
AC-13
CEMP-F-like
AC-14
Linear/actin
AC-15
Filamentous/ microtubules
AC-16
Segmental
AC-17
Discrete dots
AC-18
Dense fine speckled
AC-19
Fine speckled
AC-20
NuMA-like
AC-26
Centrosome
AC-24
Spindle fibers
AC-25
Intercellular bridge
AC-27
Mitotic chromosomal coat
AC-28
Competent-level
Expert-level report
Metaphase plate is stained

NUCLEI



MEMBRANE

Nuclear Enveloppe

Homogeneous **AC-11**

Punctated **AC-12**

NUCLEOPLASM

Homogeneous **AC-1**

Large Speckled **AC-5**

Coarse Speckled

Dense Fine Speckled **AC-2**

Fine Speckled (granular) **AC-4**

Dense Fine Granular

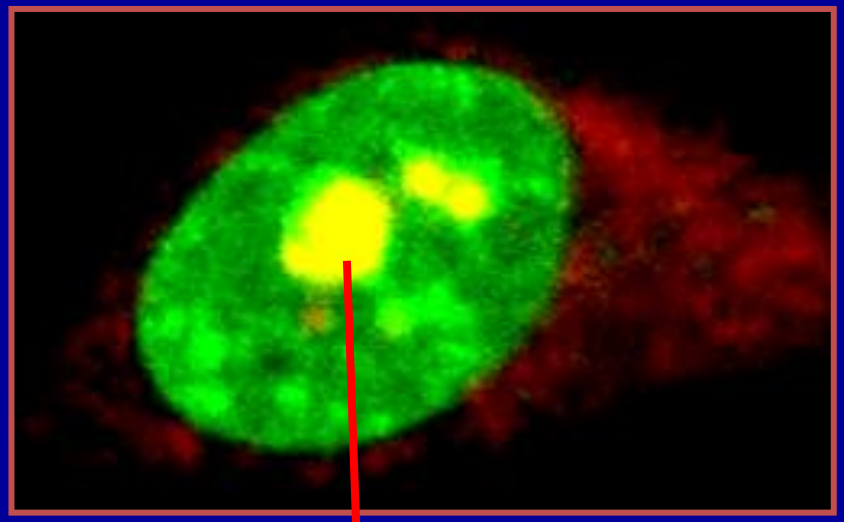
Very Dense Fine Granular

Centromere **AC-3**

Multiple Isolated Dots **AC-6**

Few Isolated Dots **AC-7**

Pleomorphic **AC-13**



NUCLEOLUS

HOMOGENEOUS

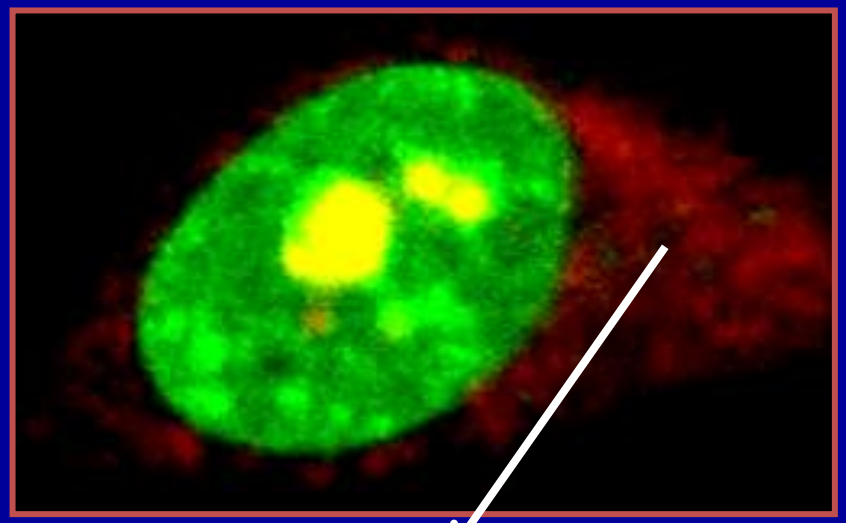
AC-8

CLUMPY

AC-9

GRANULAR
PUNCTATE

AC-10



CYTOPLASM

SPECKLED/GRANULAR

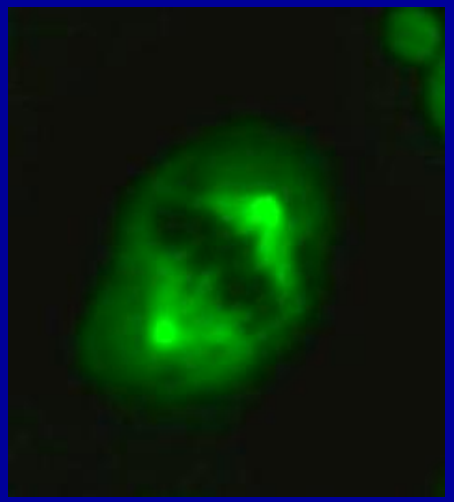
- Large Granules AC-21
- Discrete Dots AC-18
- Fine Granules AC-20
- Dense Fine Granules AC-19

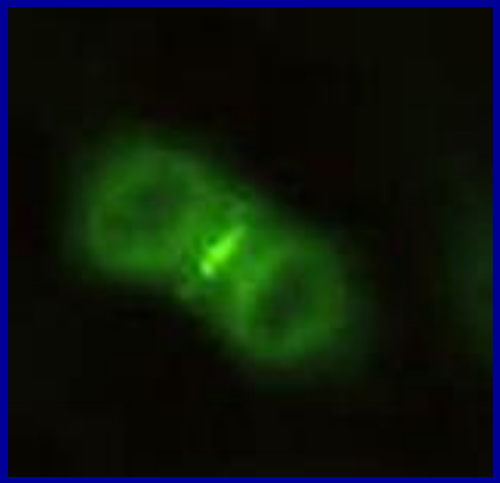
INCLUSIONS

- Golgi AC-22
- Nematin RR AC-23

FILAMENTS

- Linear / Cables AC-15
- Radial Filaments AC-16
- Fine Long Filaments Sequential AC-17





MITOTIC CELLS

CHROMOSOMES

MITOTIC CHROMOSOME

AC-28

MITOTIC CENTROMERE

CHROMOSOMAL PASSENGER ROTEINS

SPINDLE

CENTROSOME

AC-24

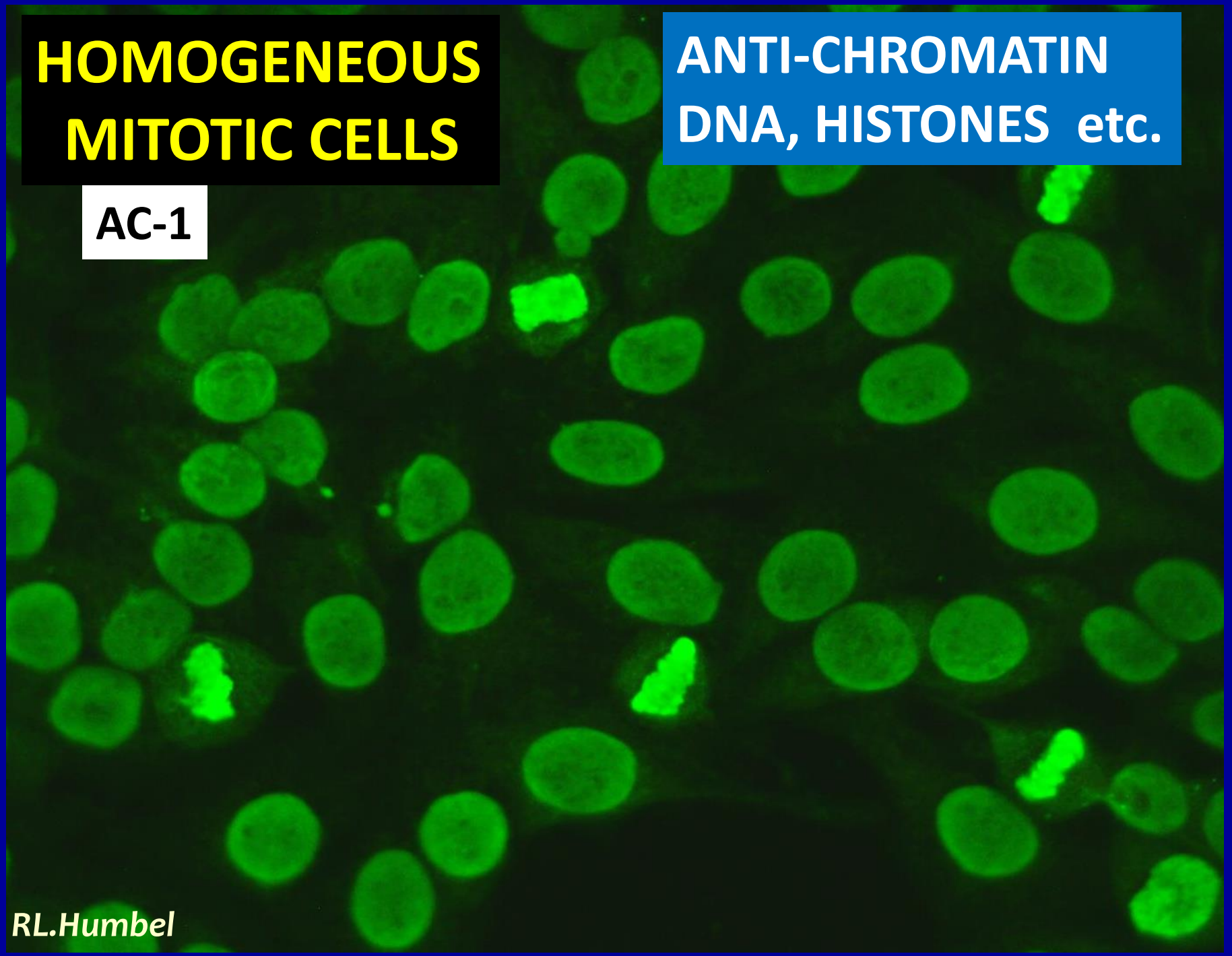
SPINDLE POOL

AC-25 **AC-26**

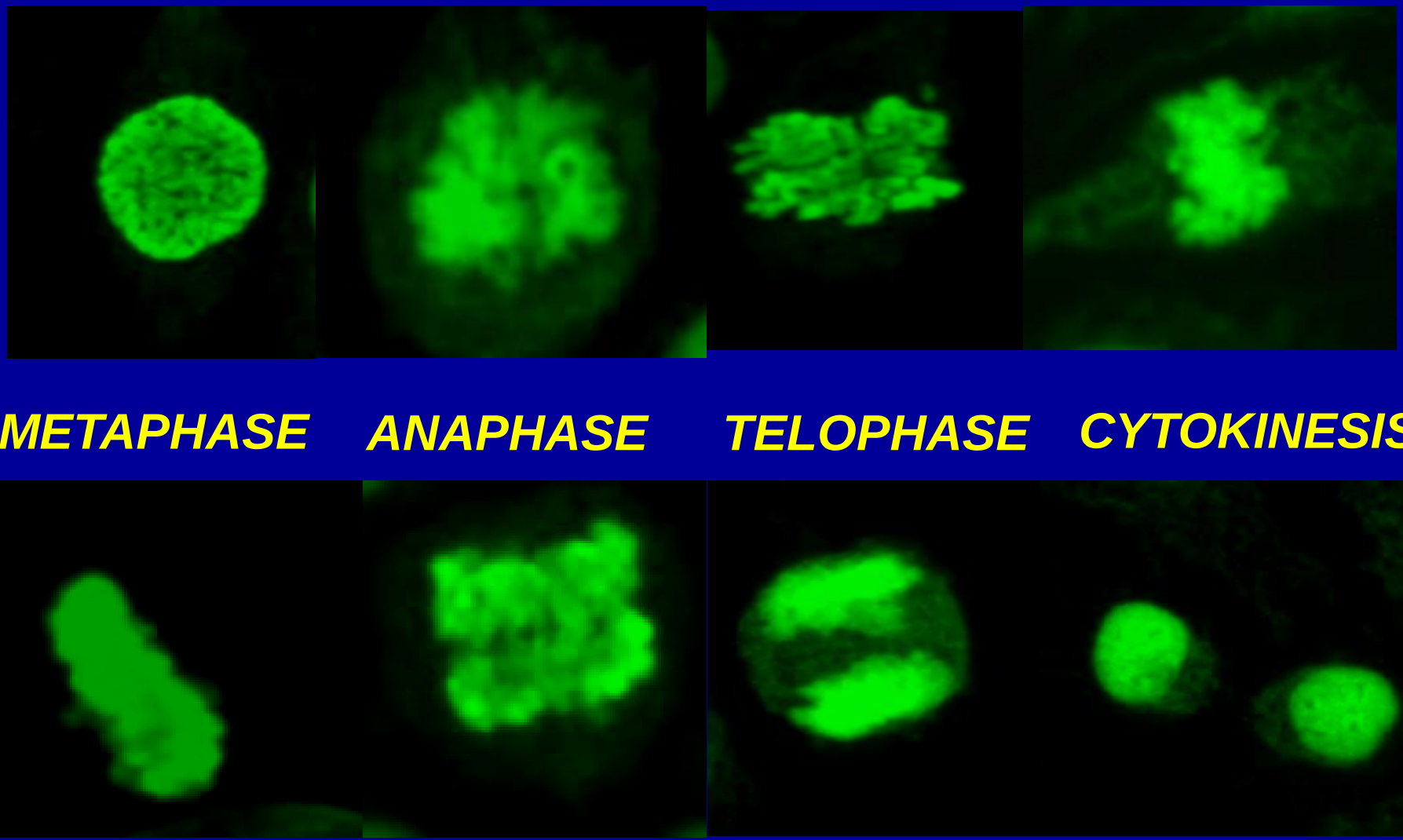
MIDBODY

INTERCELLULAR BRIDGE

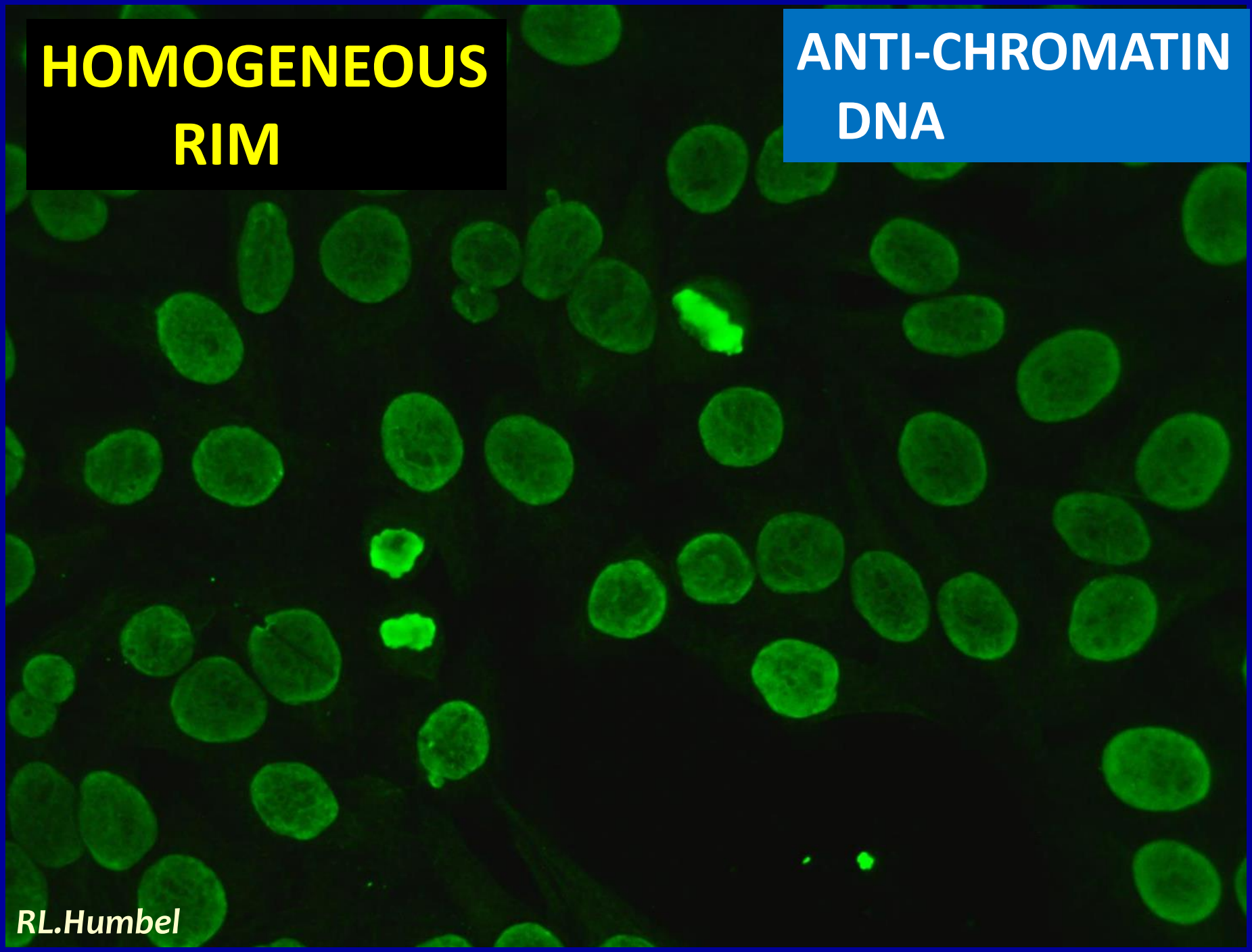
AC-27

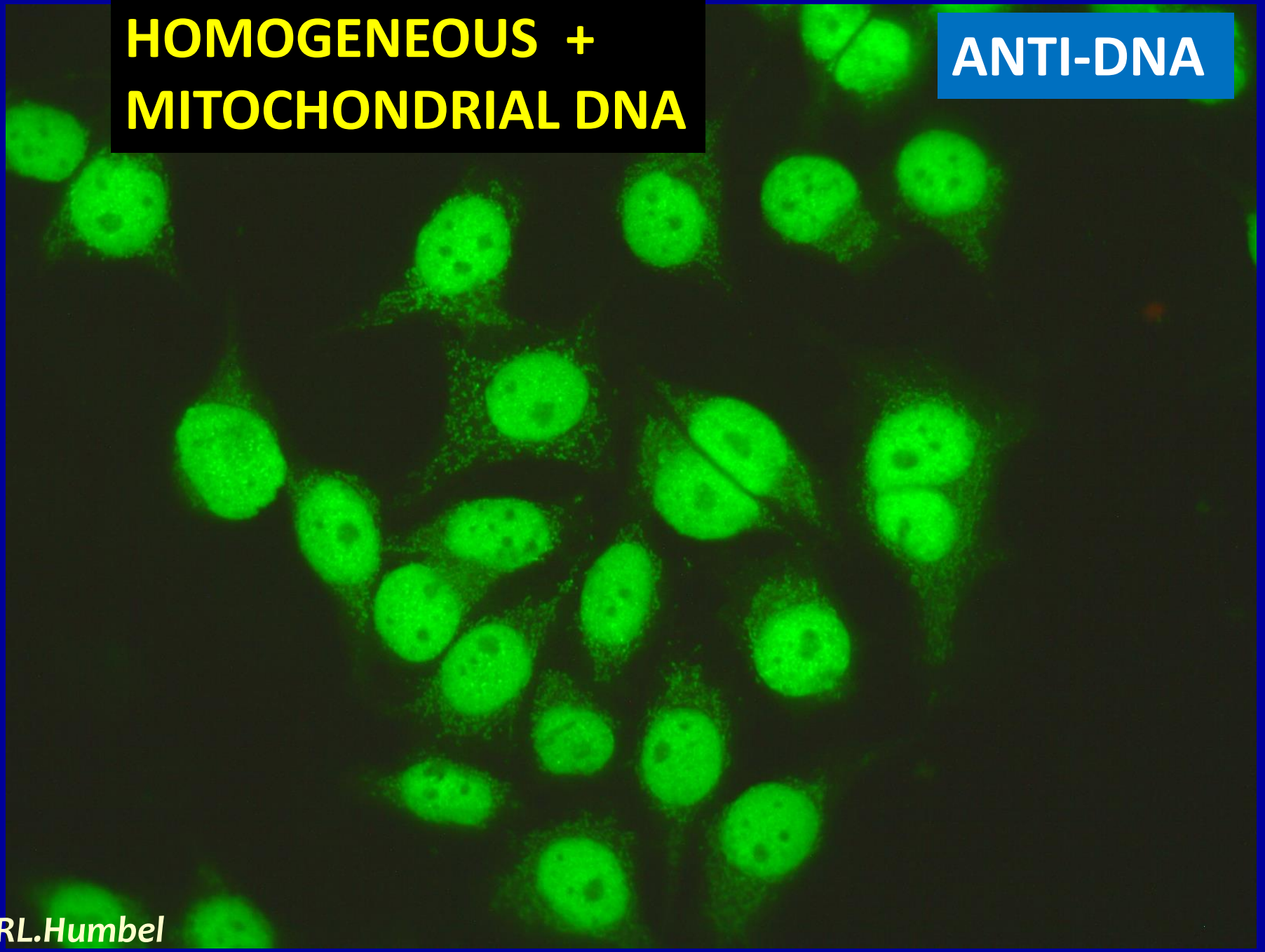

HOMOGENEOUS
MITOTIC CELLS
AC-1
ANTI-CHROMATIN
DNA, HISTONES etc.
RL.Humbel

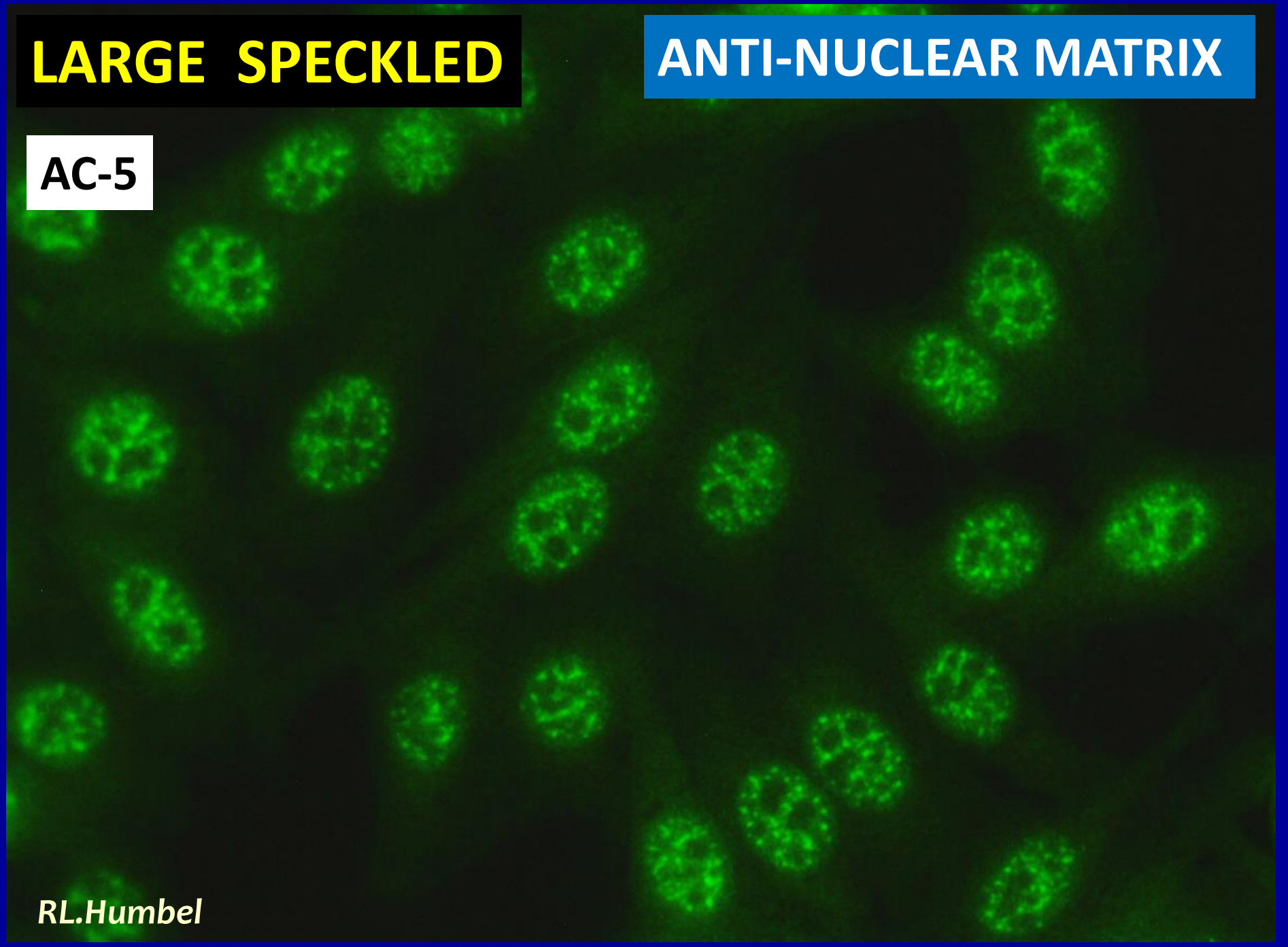
MITOSIS

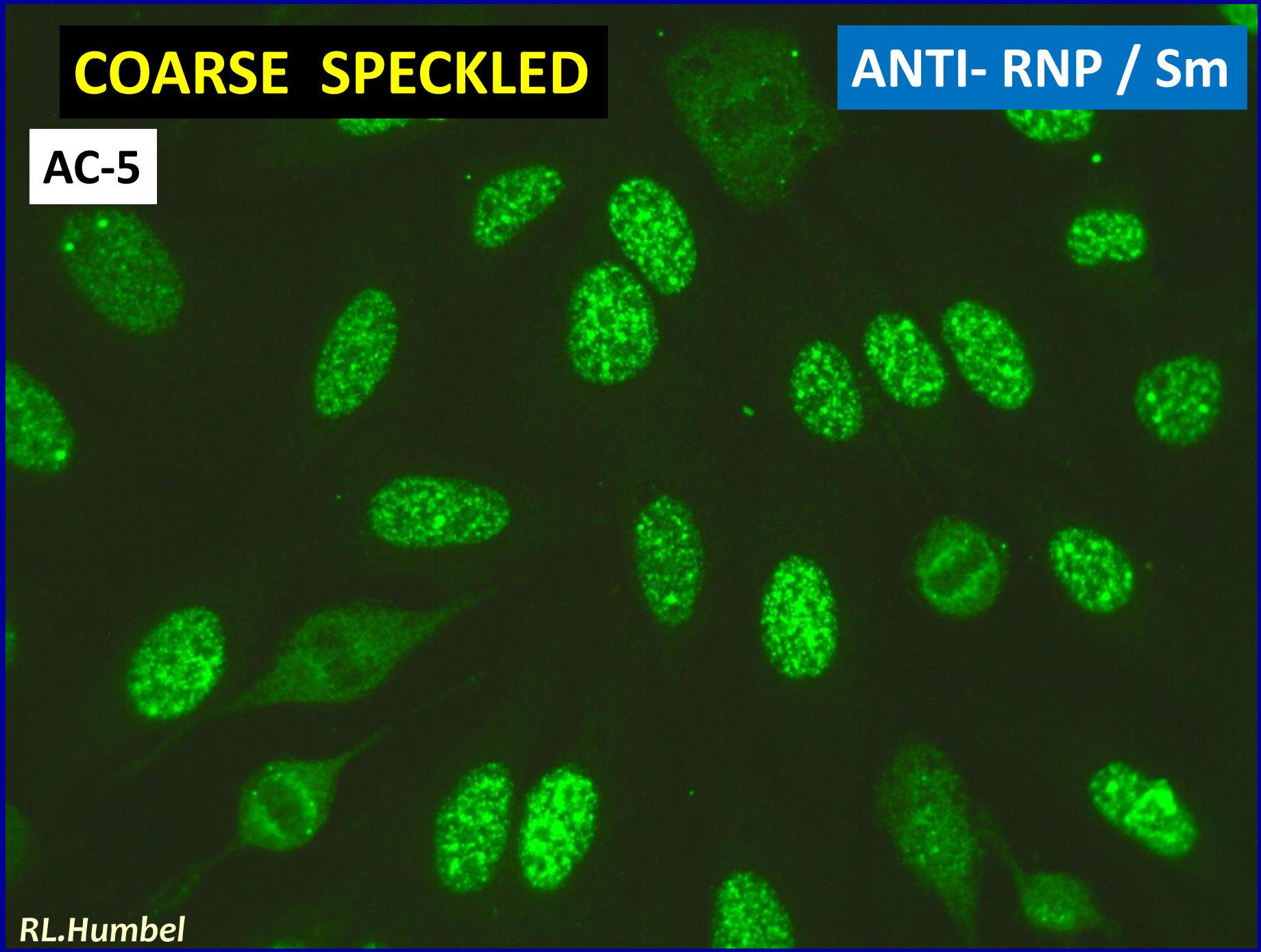


RL.Humbel

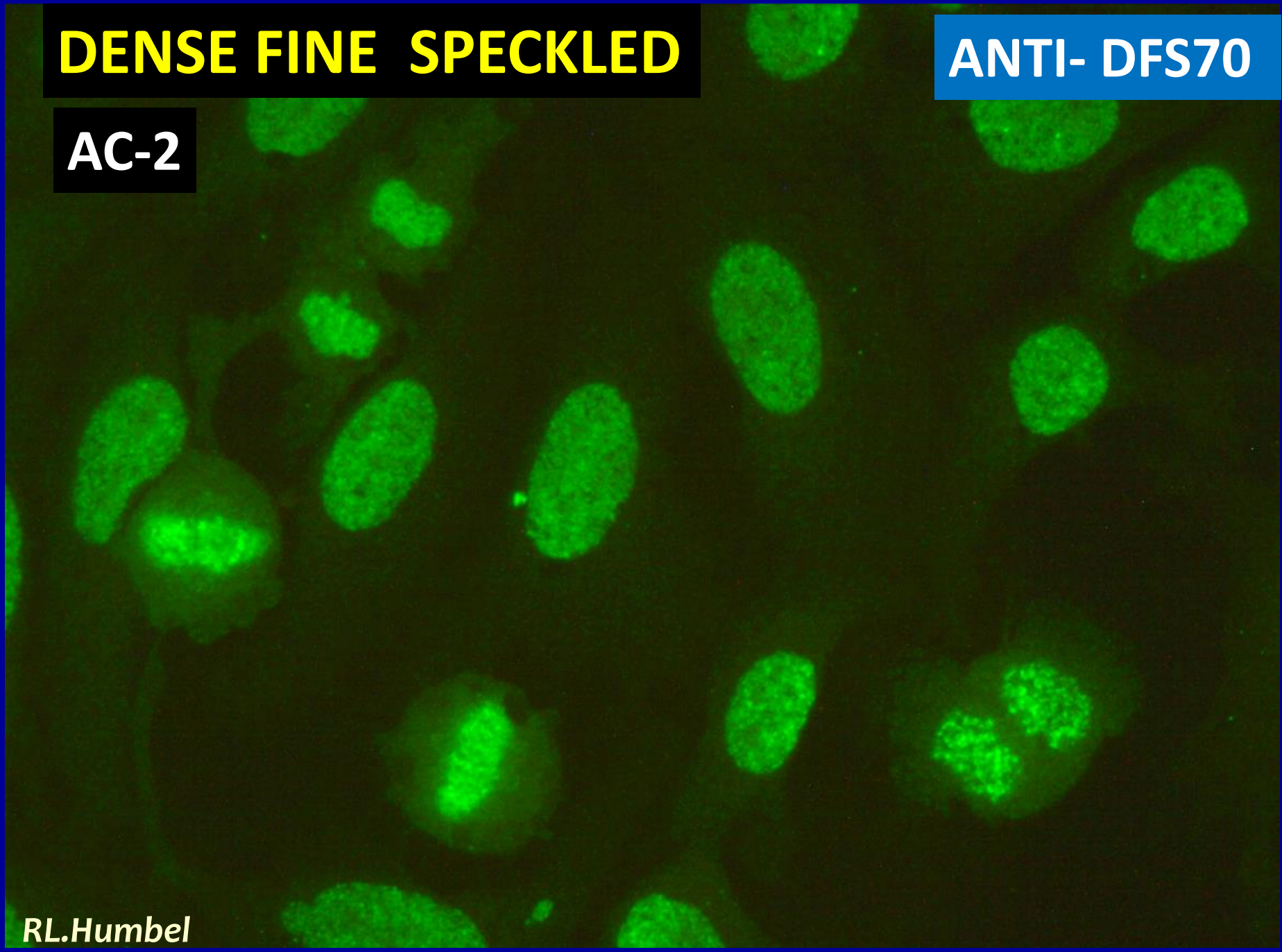

HOMOGENEOUS
RIM
ANTI-CHROMATIN
DNA
RL.Humbel


HOMOGENEOUS +
MITOCHONDRIAL DNA
ANTI-DNA
RL.Humbel


LARGE SPECKLED
ANTI-NUCLEAR MATRIX
AC-5
RL.Humbel


COARSE SPECKLED
ANTI- RNP / Sm
AC-5
RL.Humbel

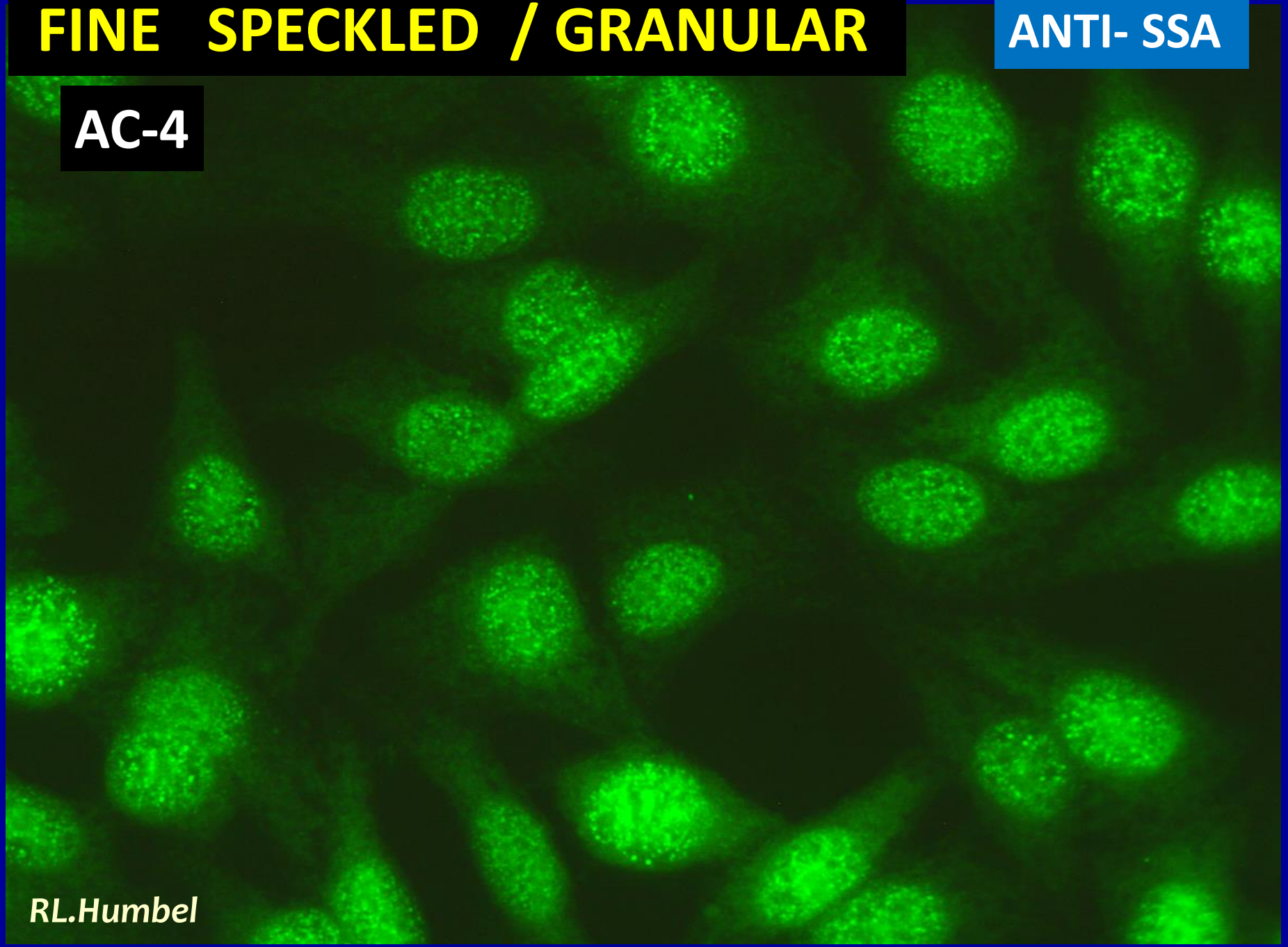

COARSE SPECKLED
+ CAJAL BODIES
ANTI- RNP
RL.Humbel


DENSE FINE SPECKLED
ANTI- DFS70
AC-2
RL.Humbel

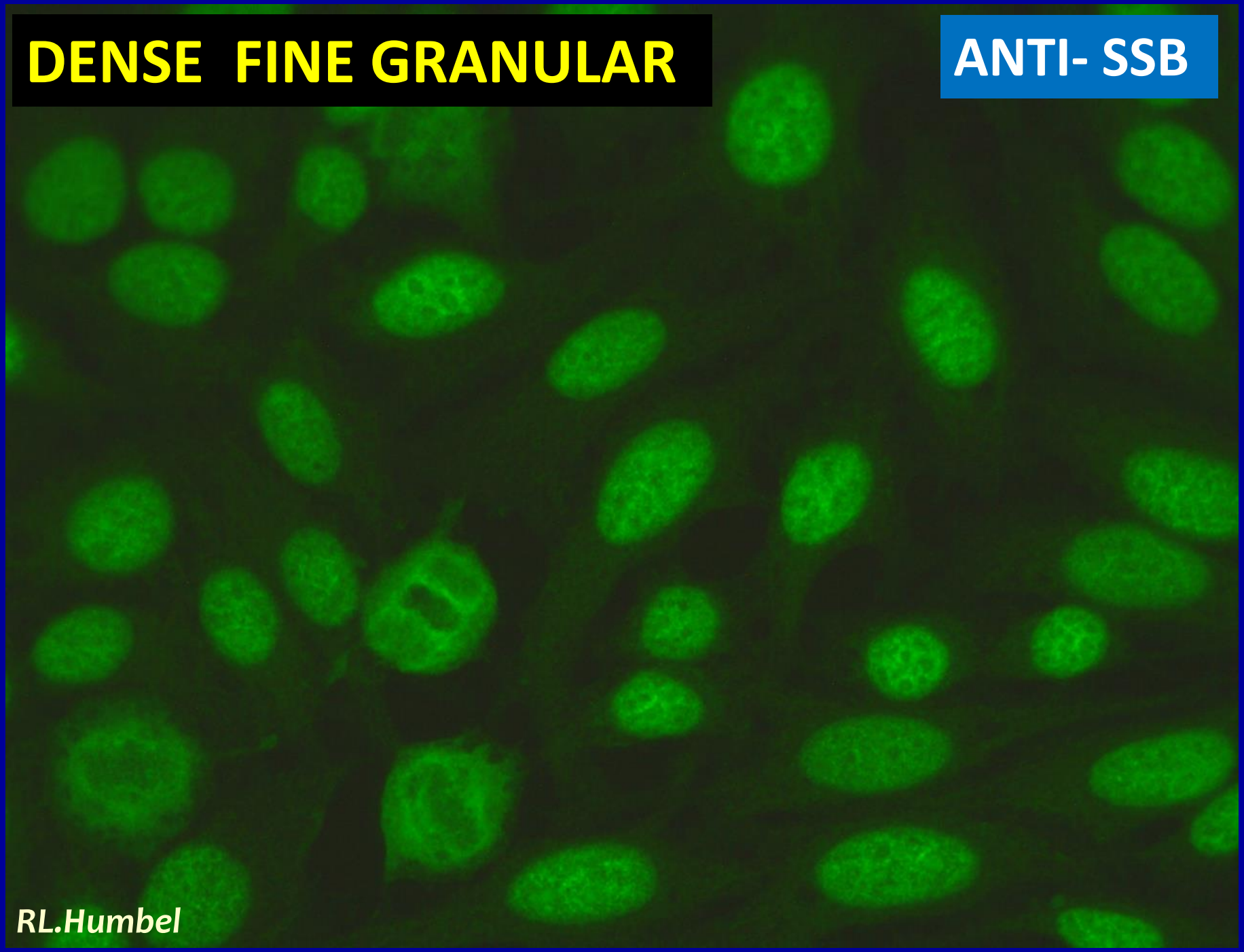
FINE SPECKLED / GRANULAR

ANTI- SSA

AC-4

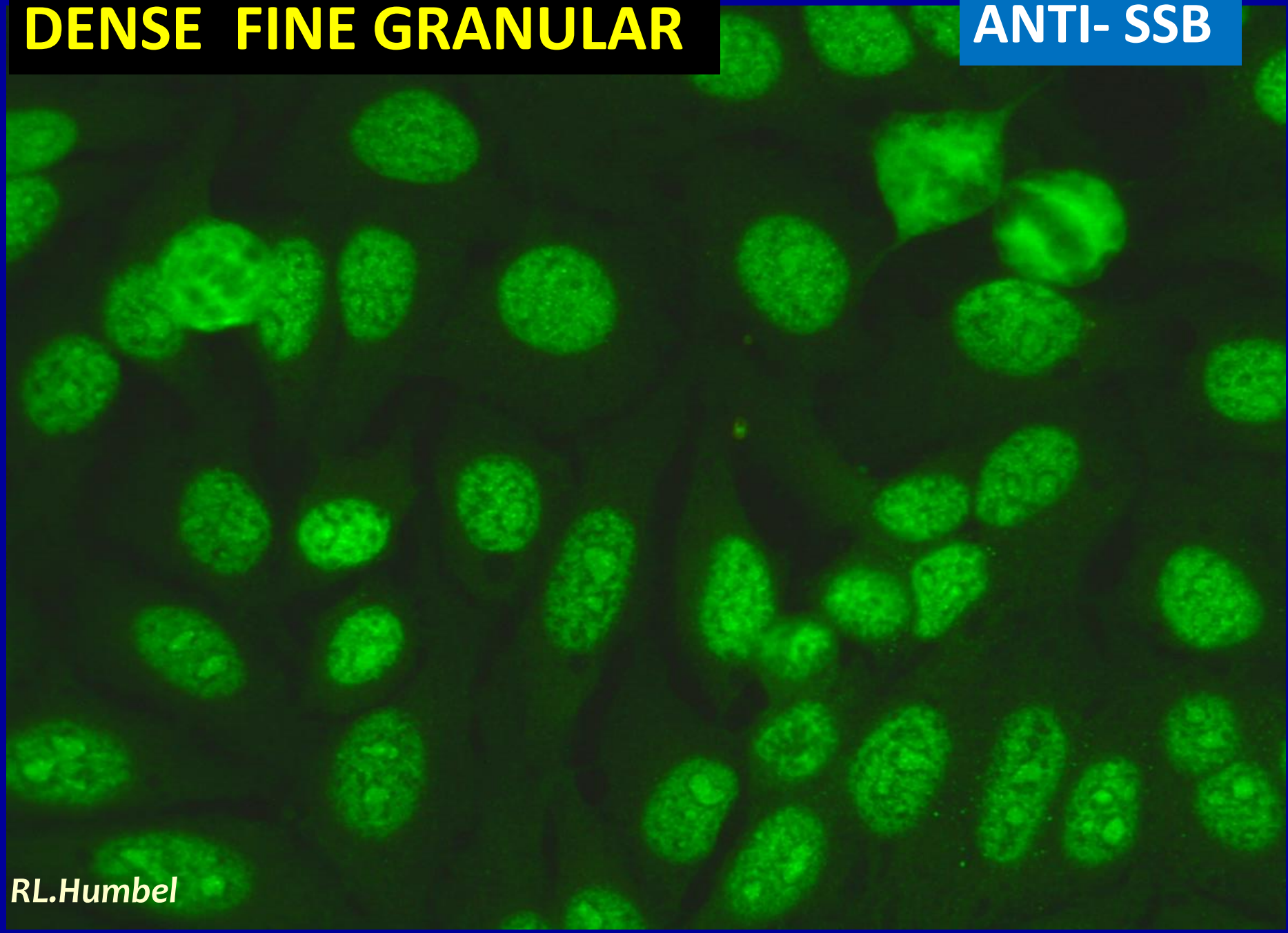


RL.Humbel

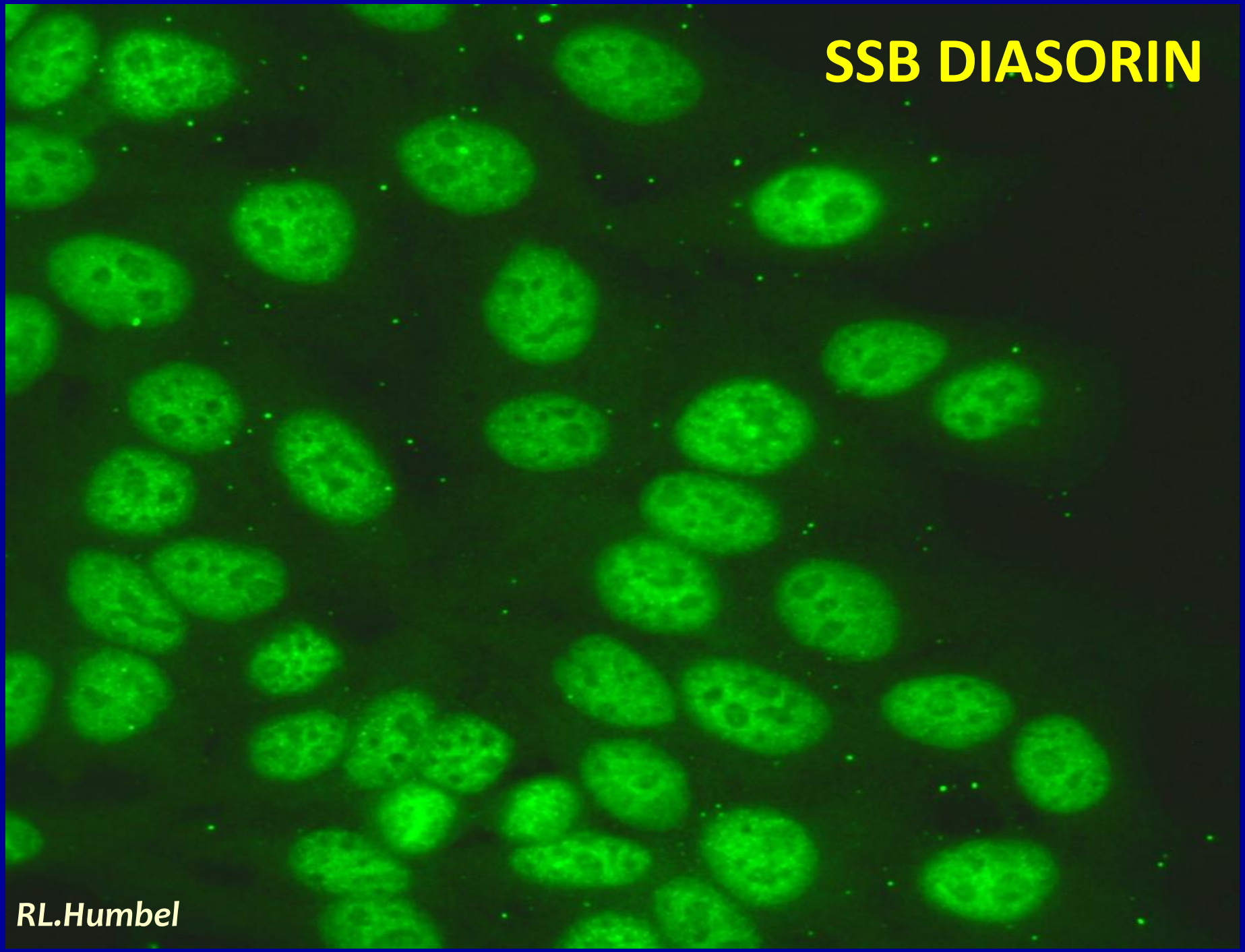

DENSE FINE GRANULAR
ANTI- SSB
RL.Humbel

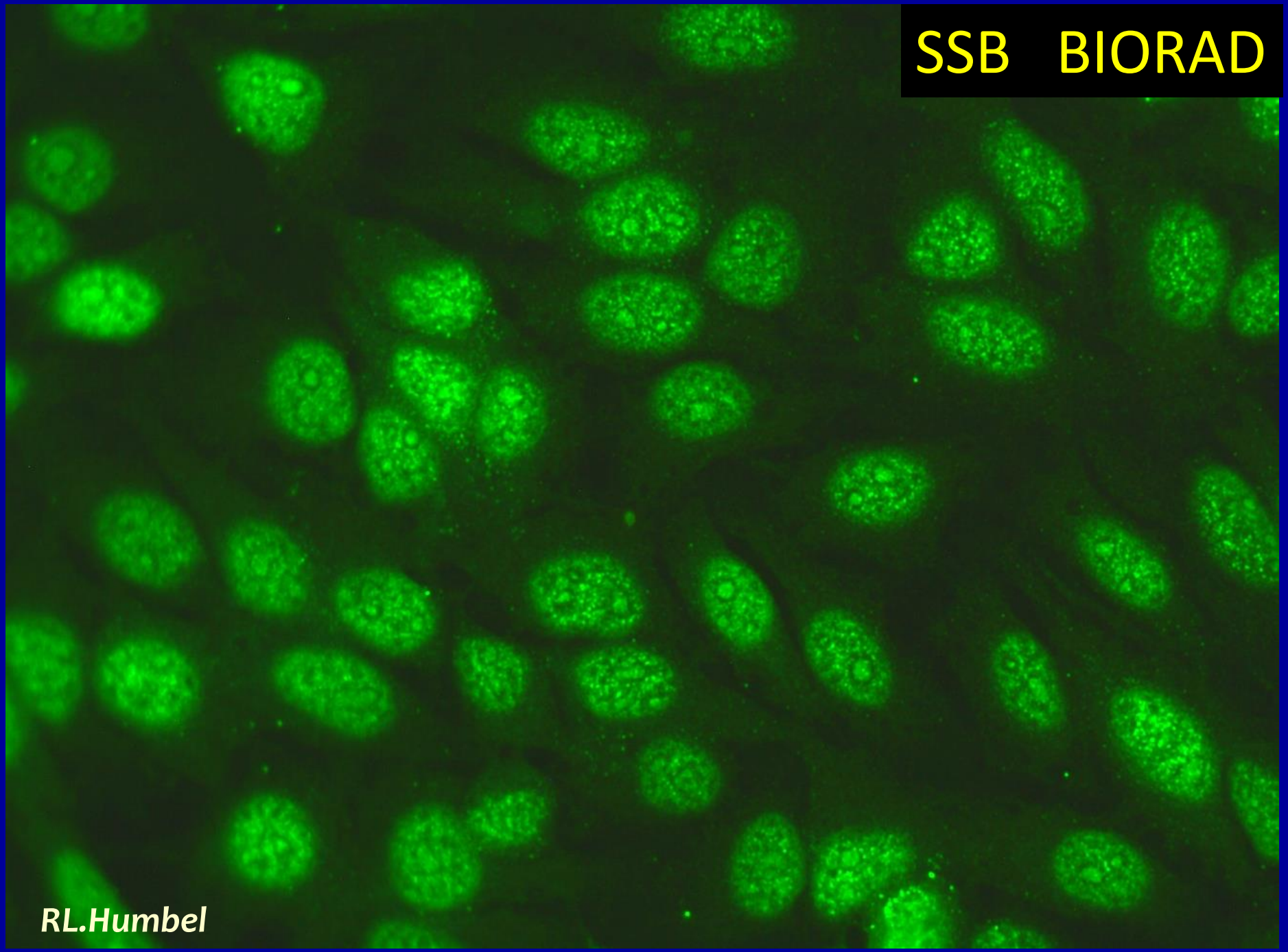
DENSE FINE GRANULAR

ANTI- SSB



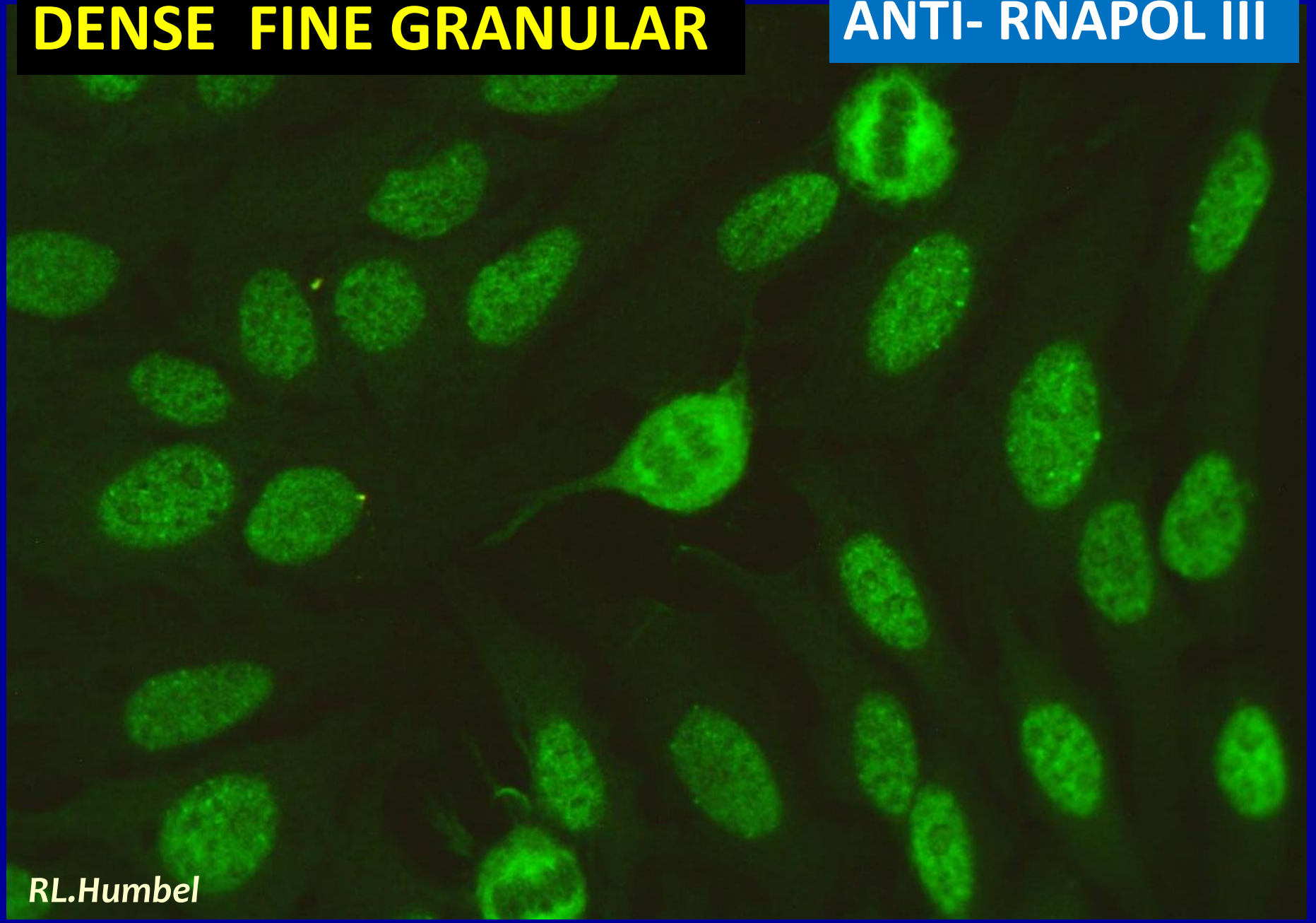
RL.Humbel


SSB DIASORIN
RL.Humbel


SSB BIORAD
RL.Humbel

DENSE FINE GRANULAR

ANTI- RNAPOL III



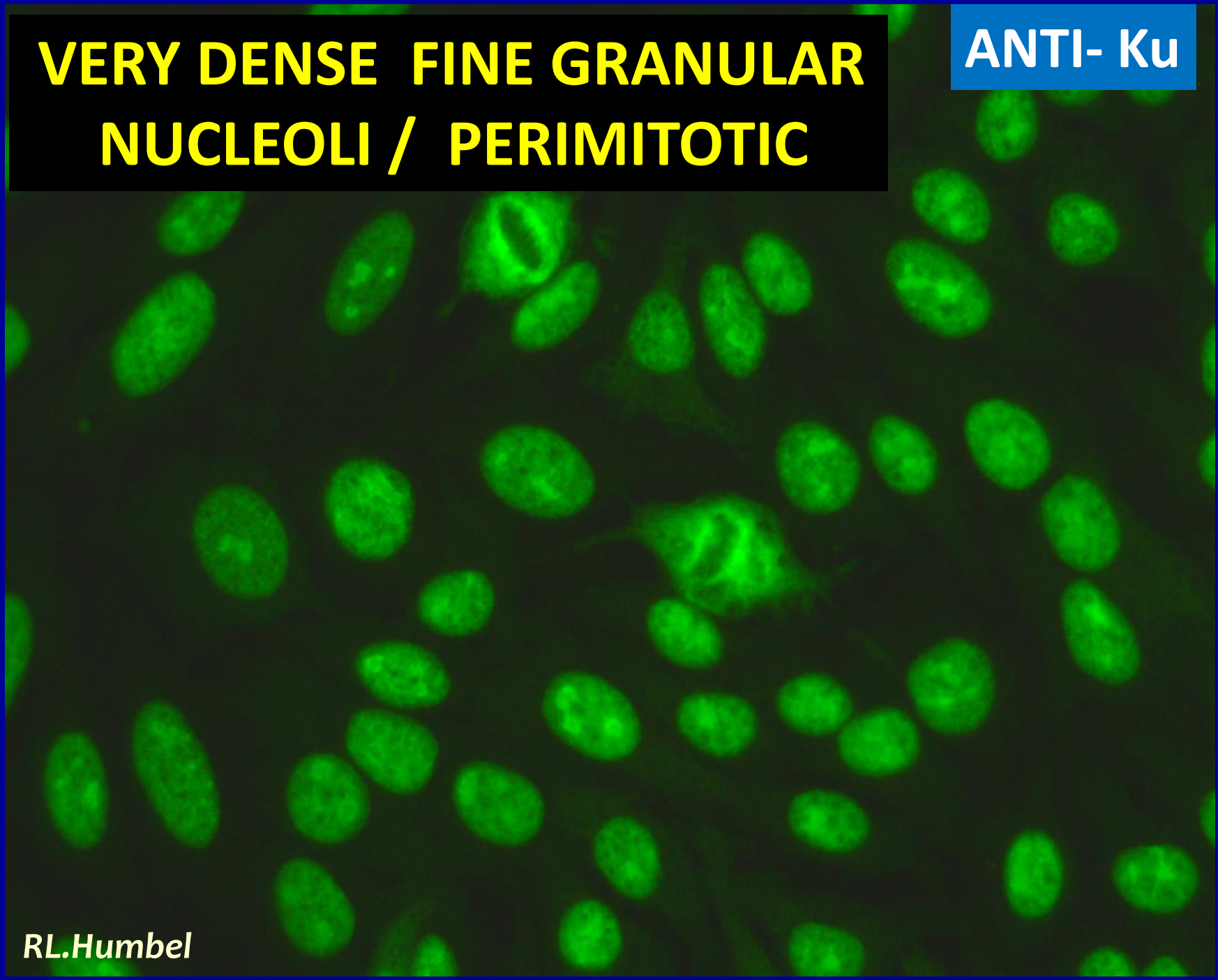
VERY DENSE FINE GRANULAR

ANTI- Mi2

RL.Humbel

VERY DENSE FINE GRANULAR NUCLEOLI / PERIMITOTIC

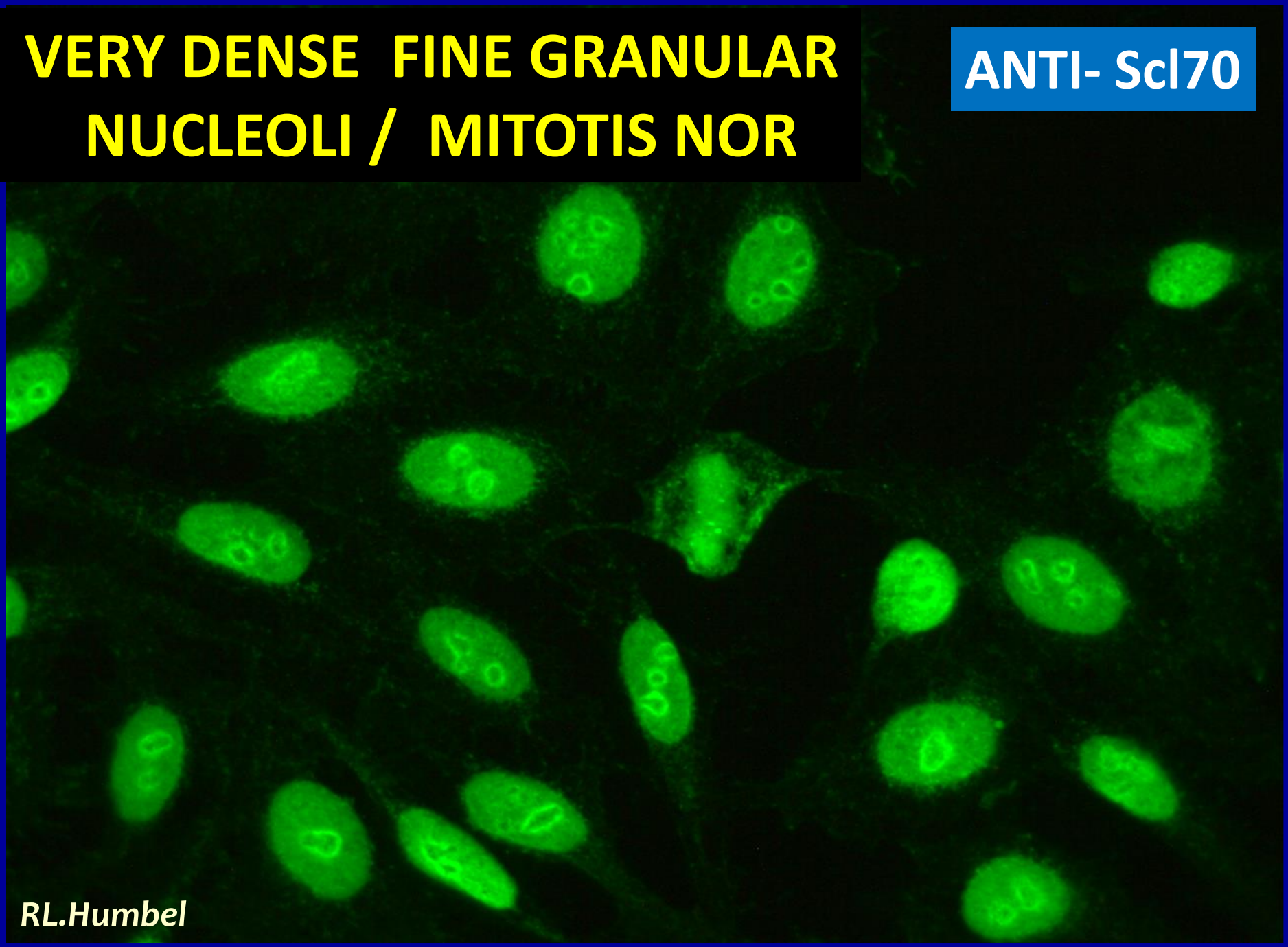
ANTI- Ku



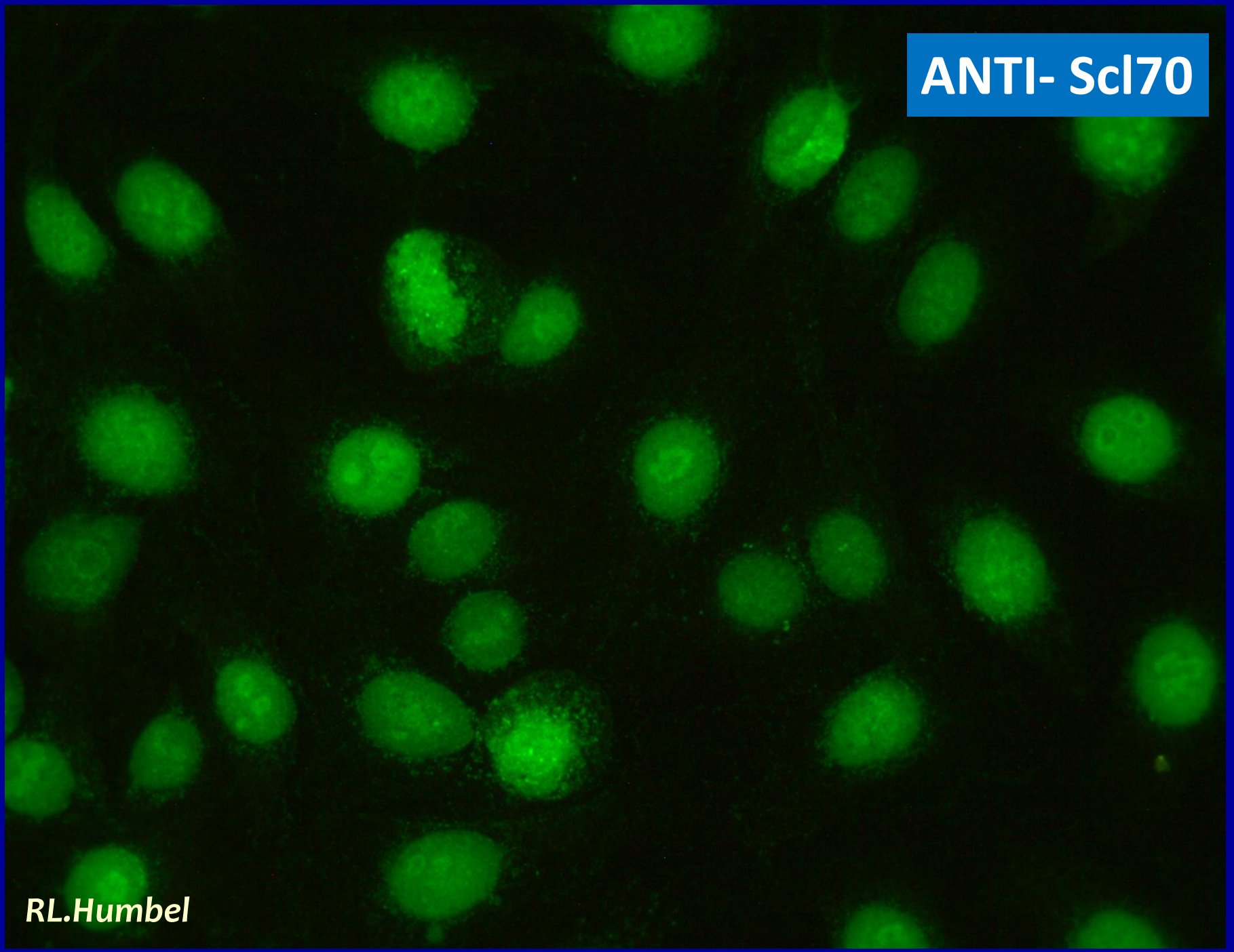
RL.Humbel

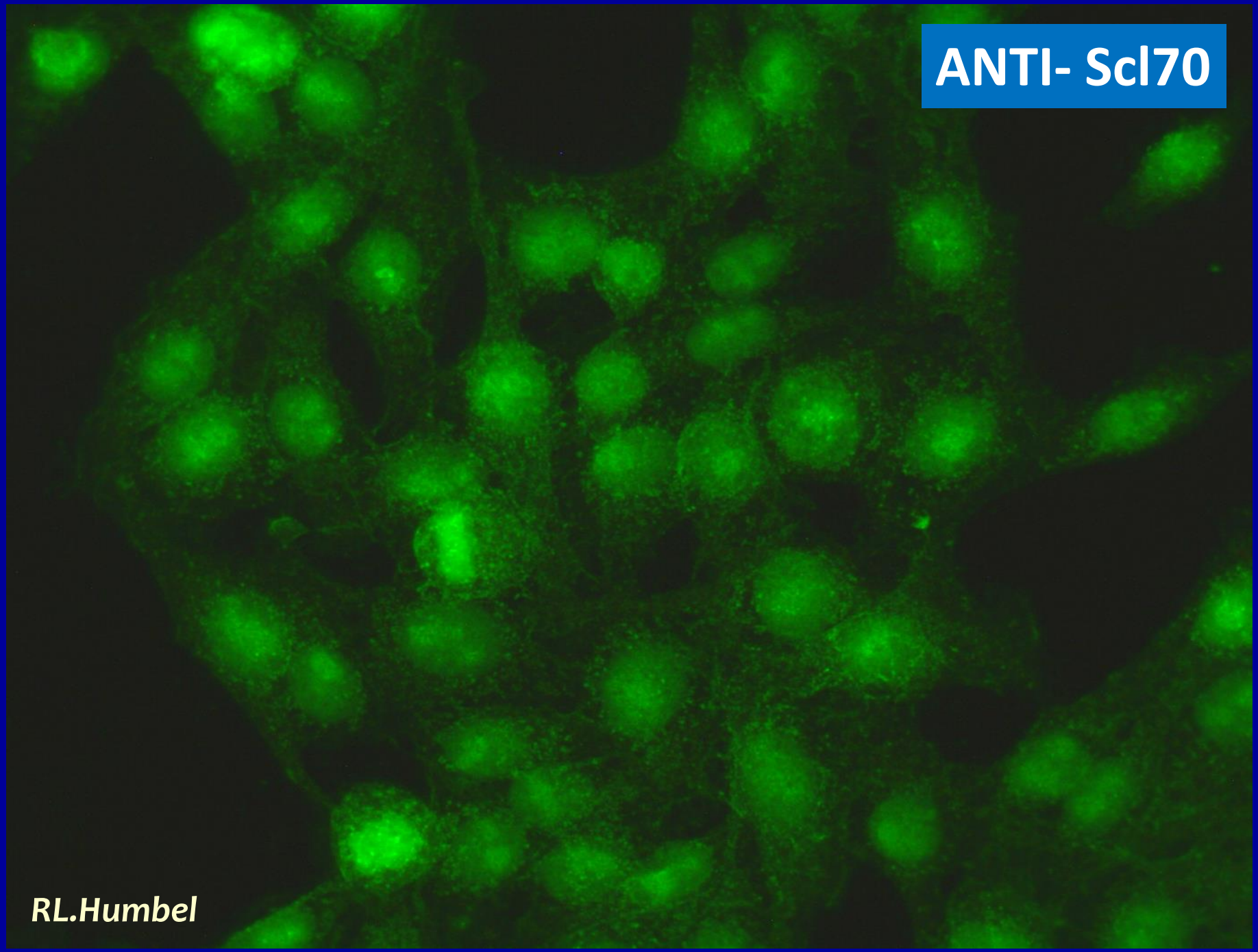
VERY DENSE FINE GRANULAR NUCLEOLI / MITOTIS NOR

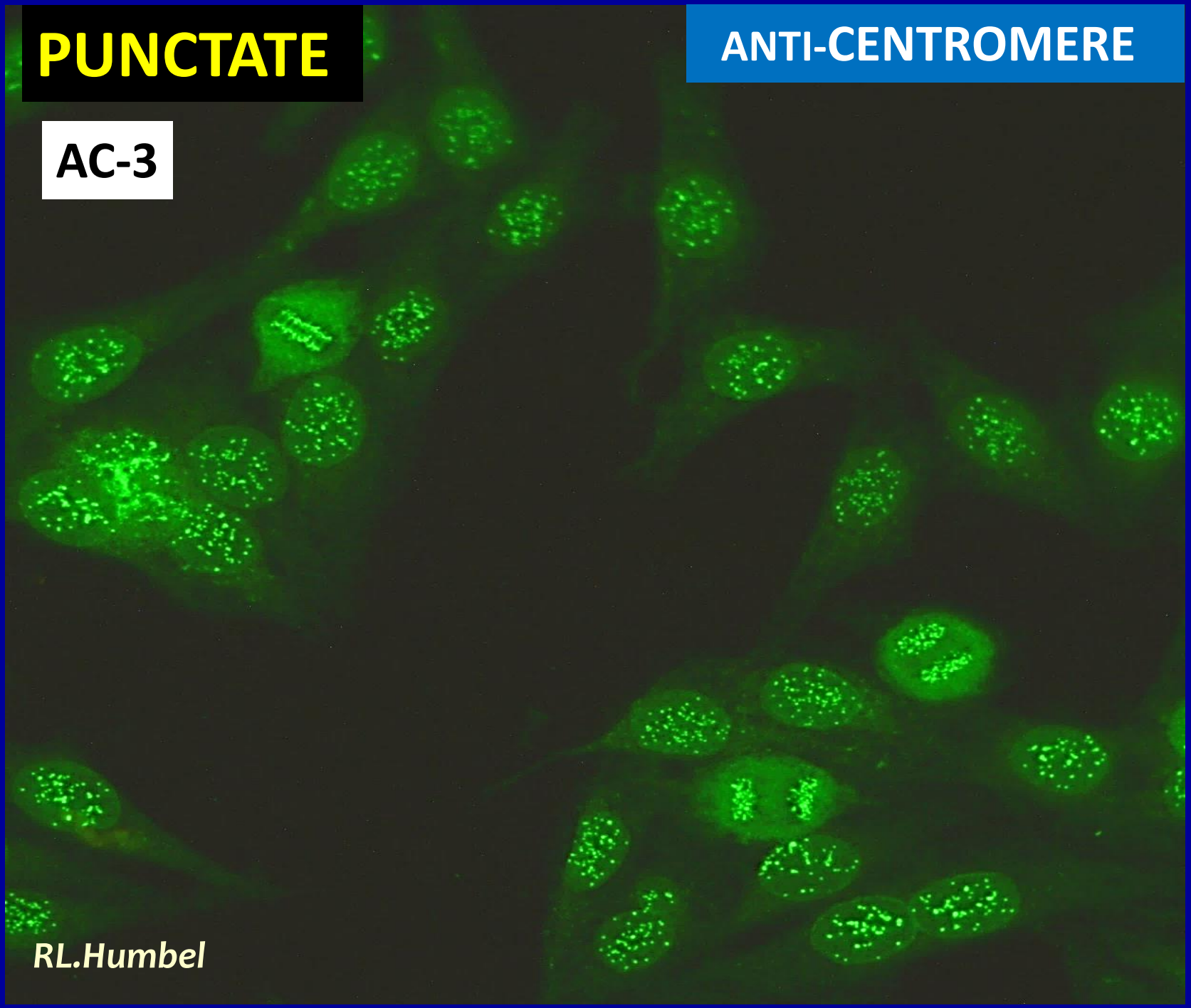
ANTI- Scl70



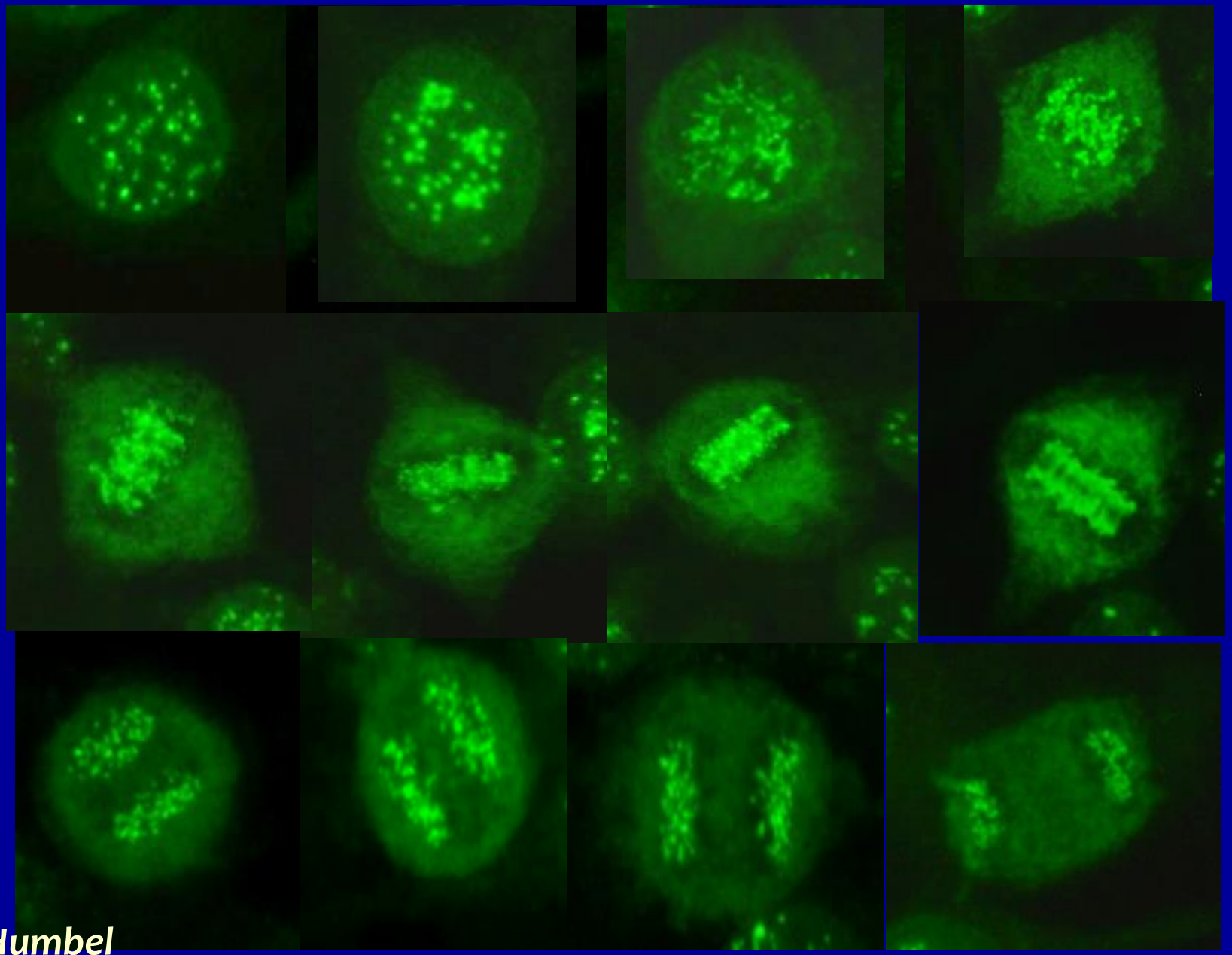
RL.Humbel


ANTI- Scl70
RL.Humbel


ANTI- Scl70
RL.Humbel

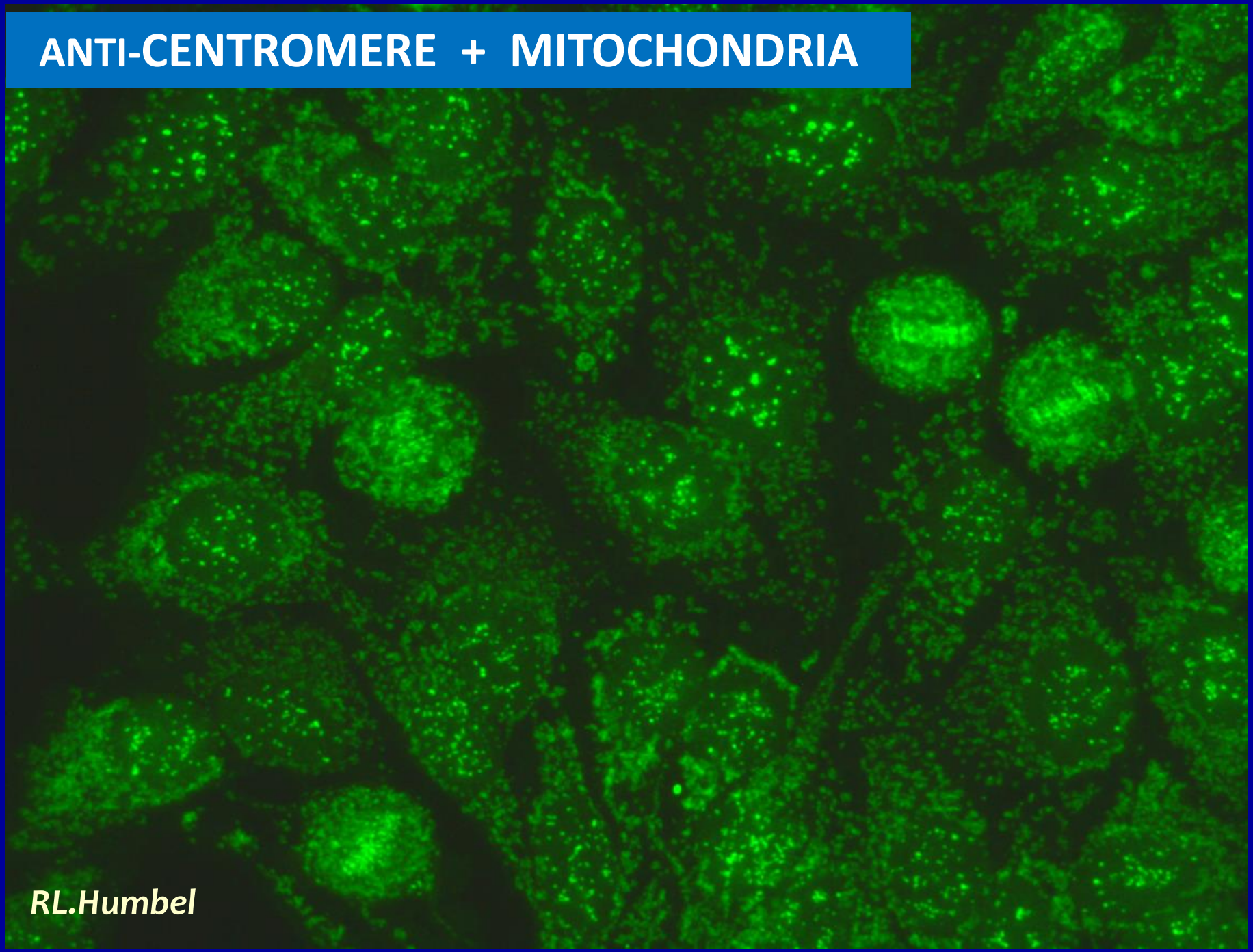

PUNCTATE
ANTI-CENTROMERE
AC-3
RL.Humbel

ANTI - CENTROMERE CENP B



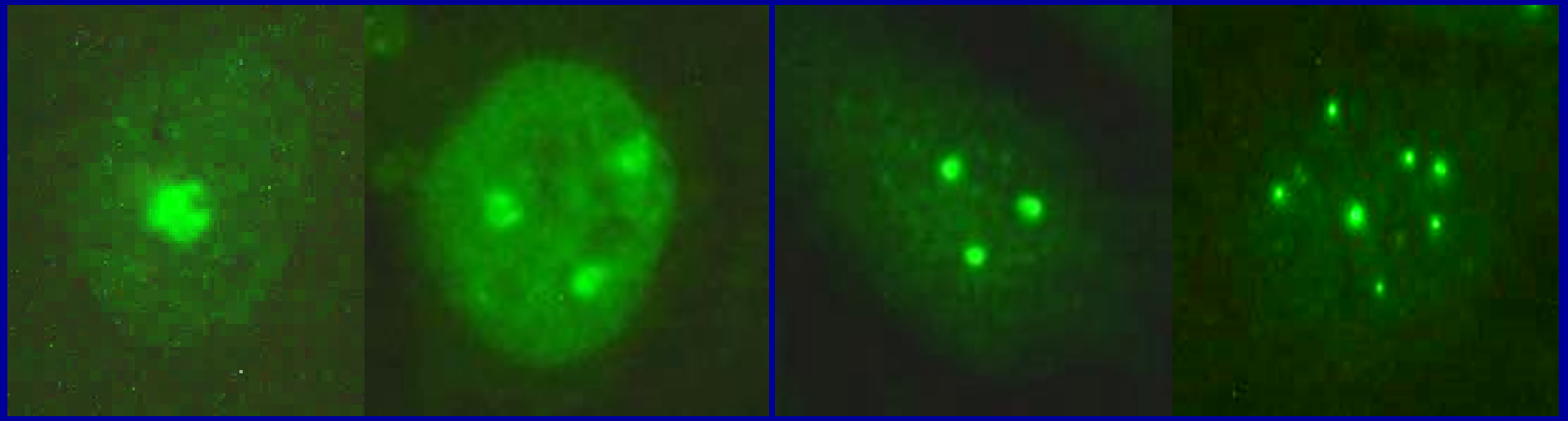
RL.Humbel

ANTI-CENTROMERE + MITOCHONDRIA

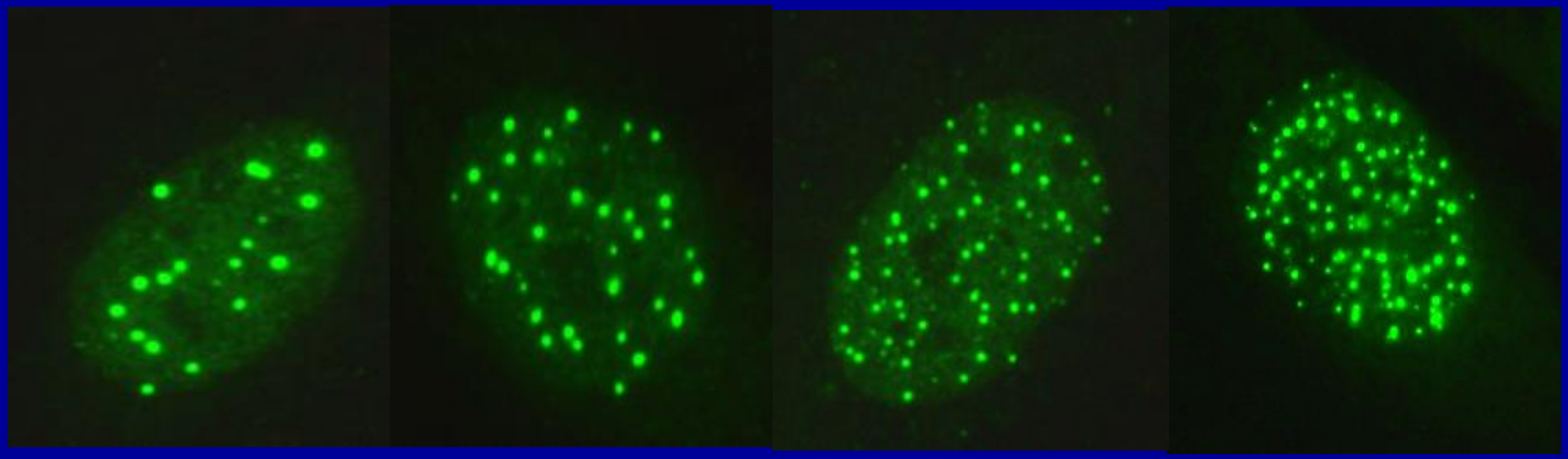


RL.Humbel

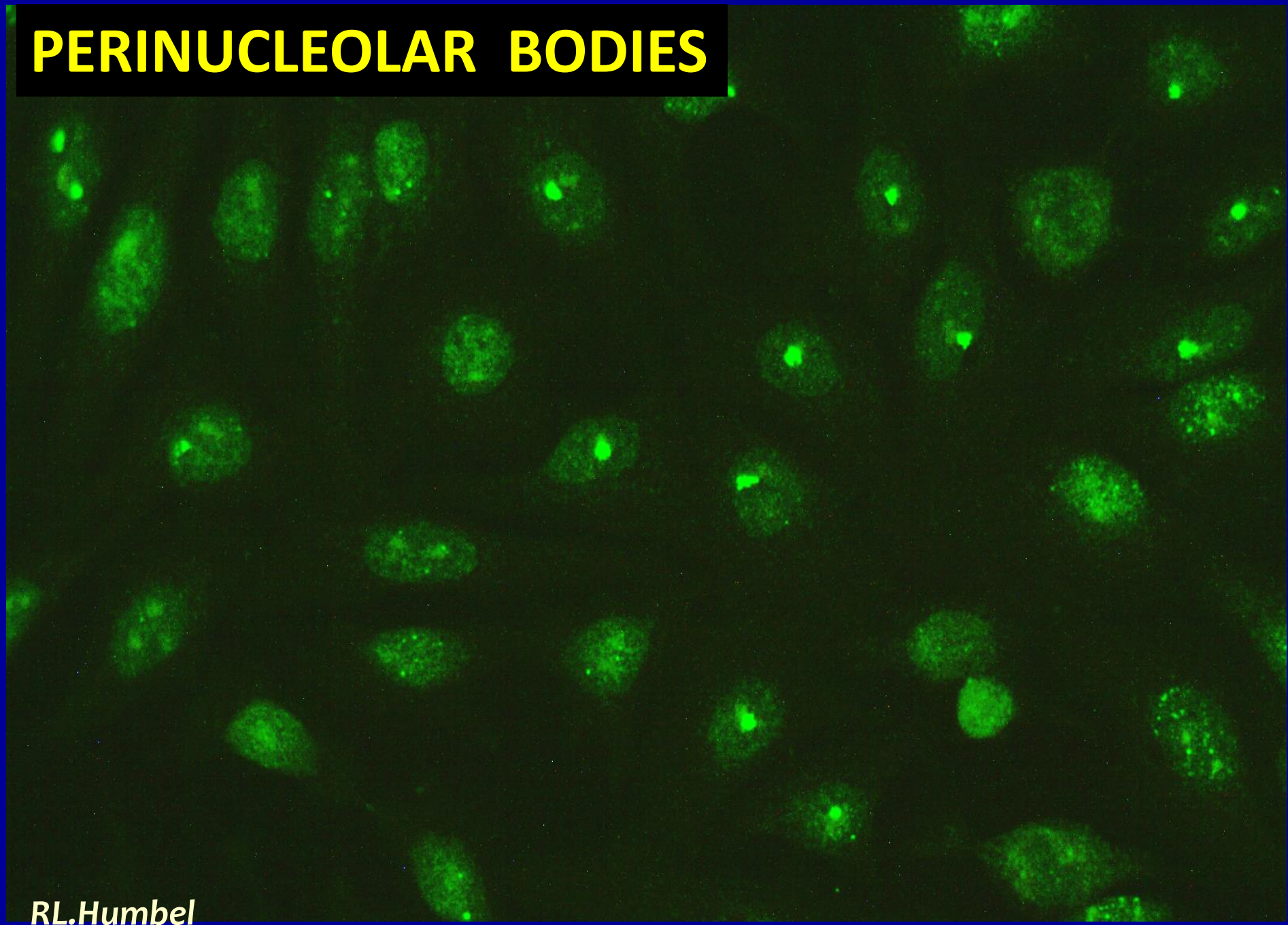
NUCLEAR DOTS



MULTIPLE NUCLEAR DOTS



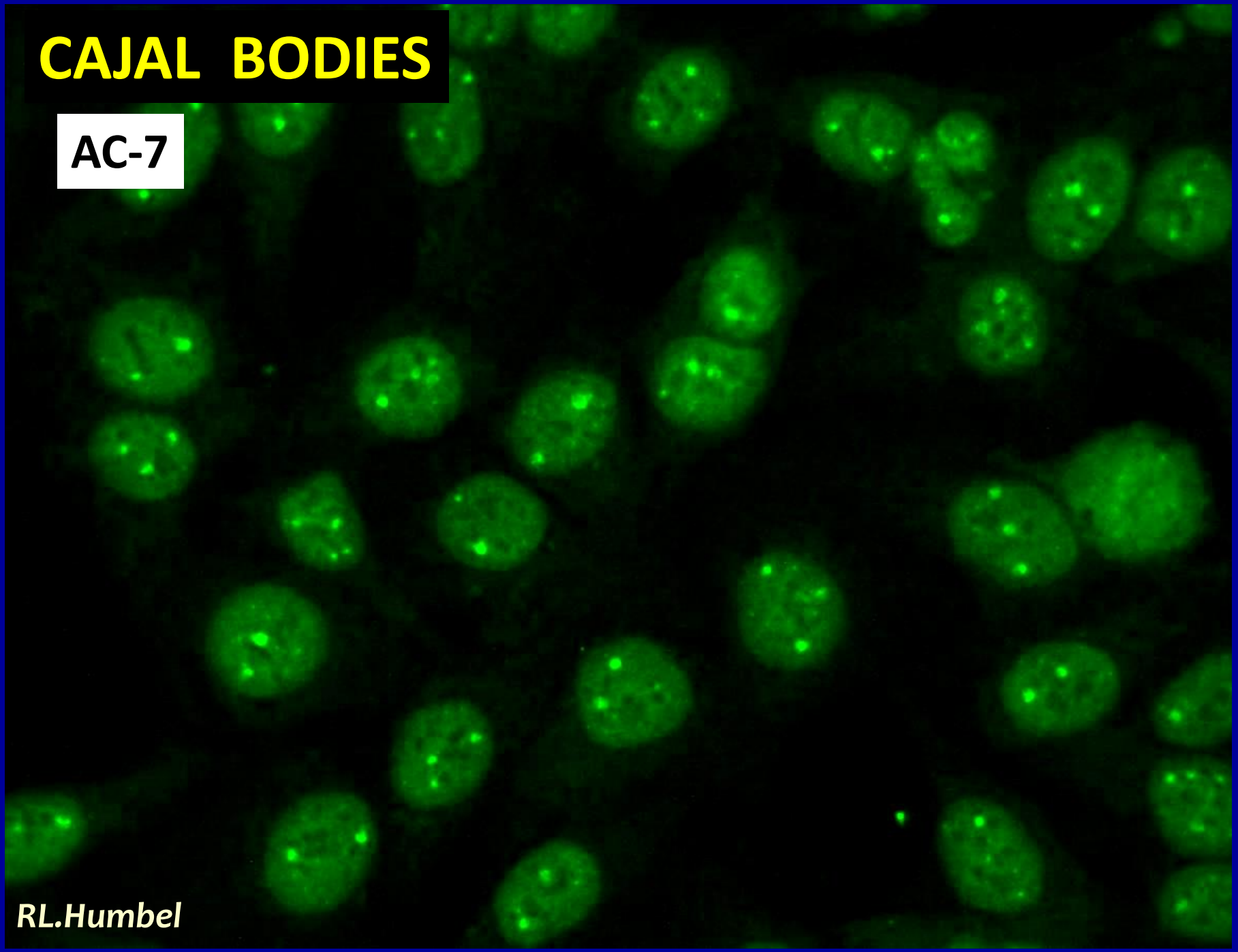
PERINUCLEOLAR BODIES



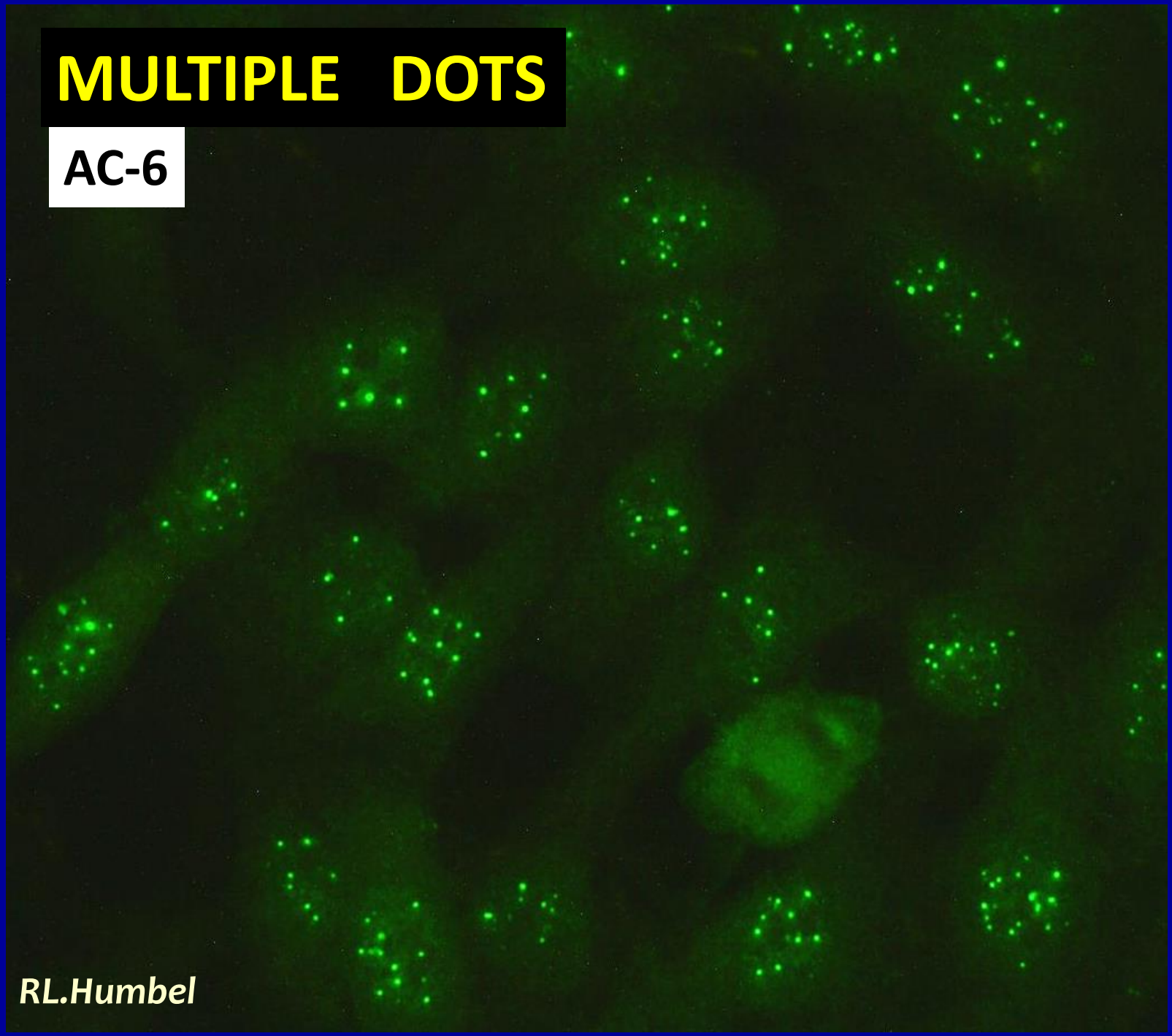
RL.Humbel

CAJAL BODIES

AC-7

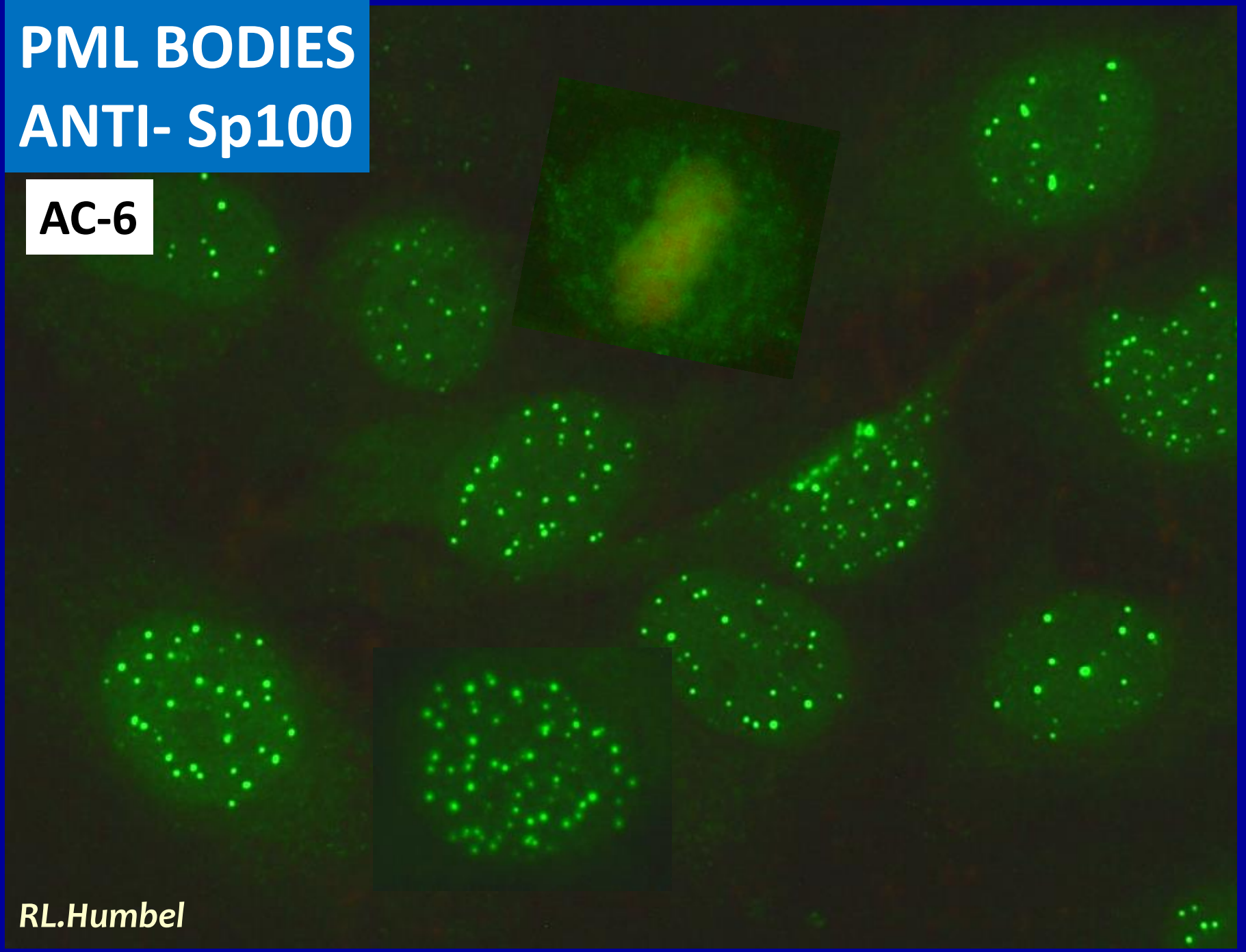


RL.Humbel


MULTIPLE DOTS
AC-6
RL.Humbel

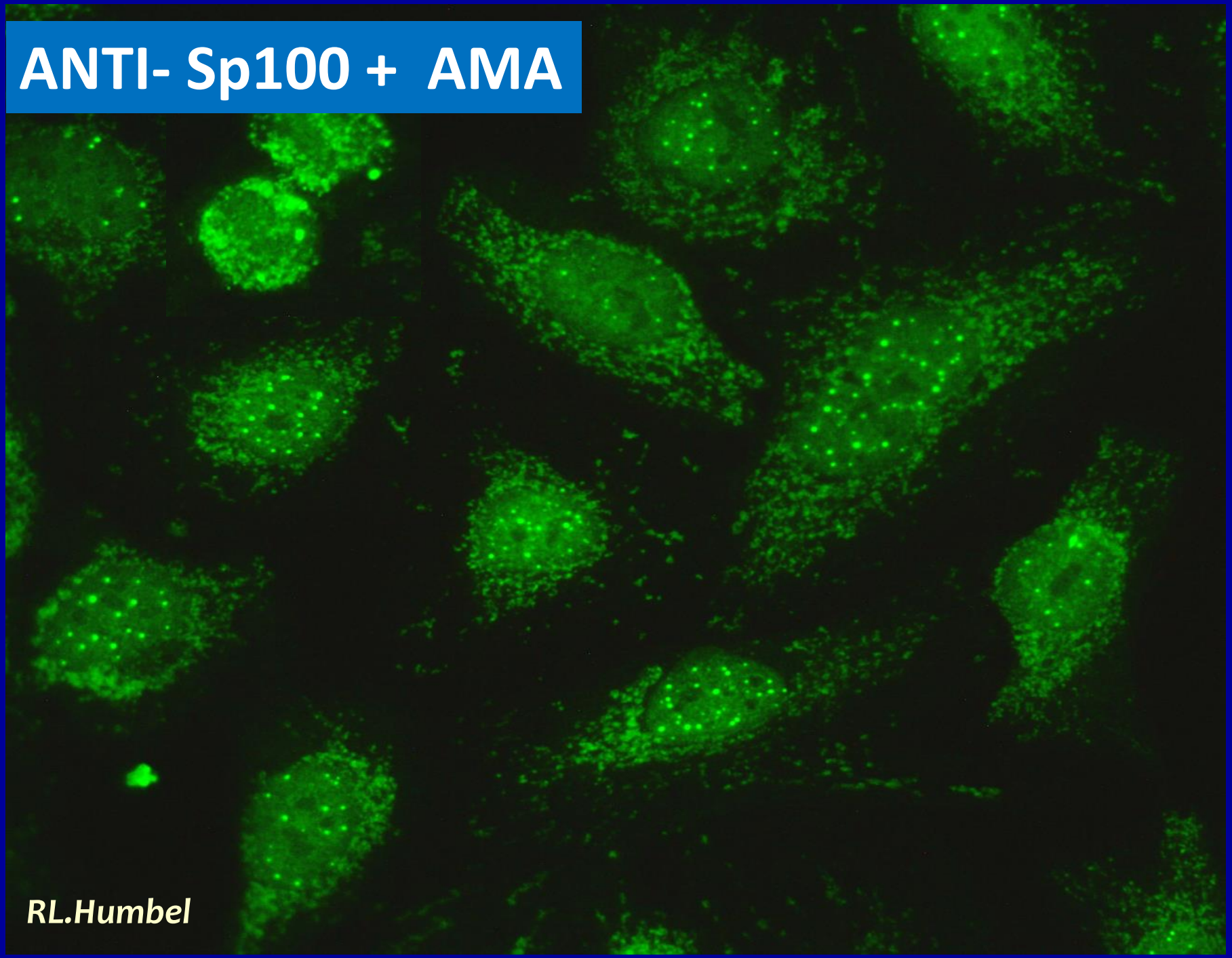
PML BODIES
ANTI- Sp100

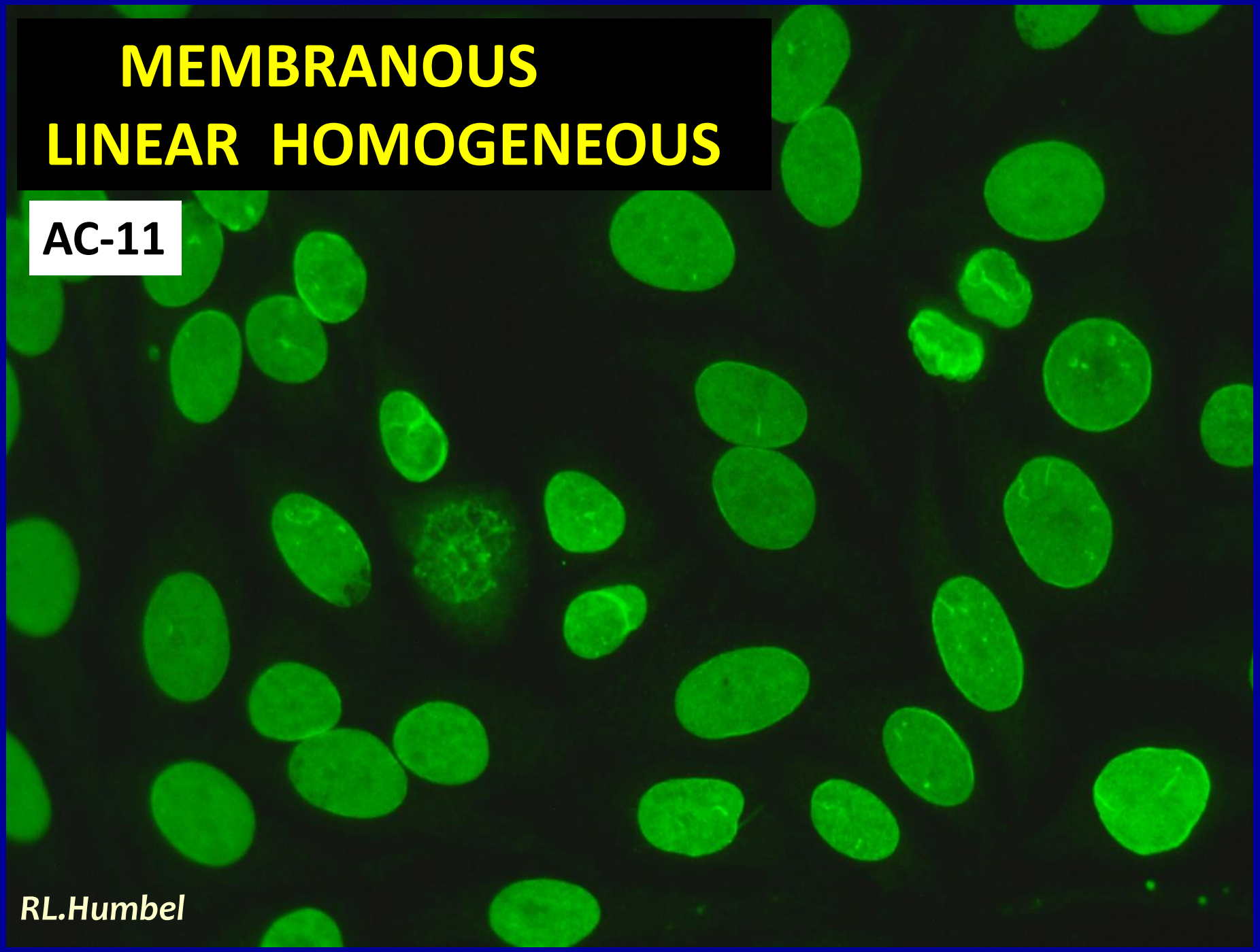
AC-6



RL.Humbel

ANTI- Sp100 + AMA

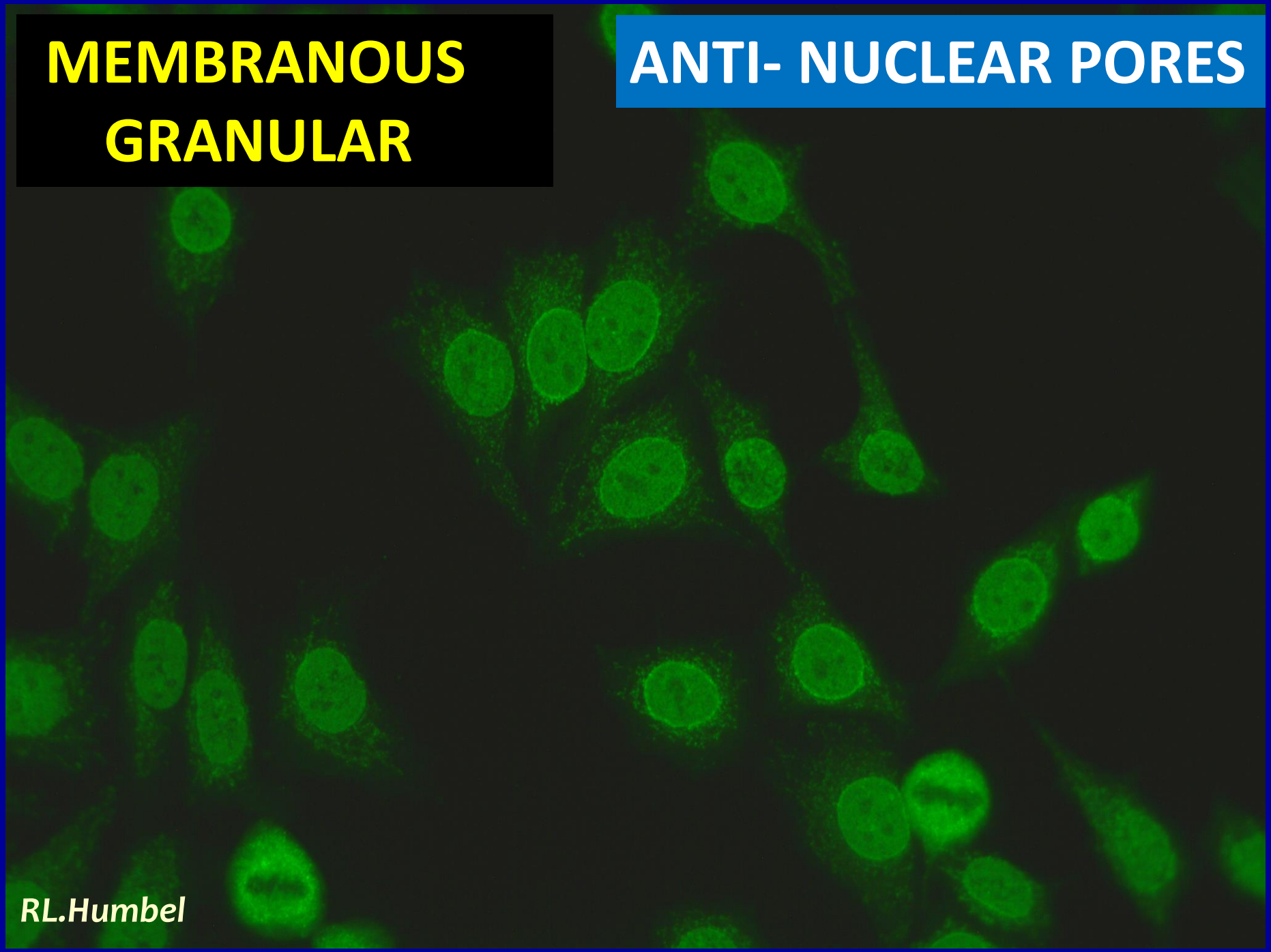



MEMBRANOUS
LINEAR HOMOGENEOUS
AC-11
RL.Humbel


MEMBRANOUS
LINEAR HOMOGENEOUS
RL.Humbel

MEMBRANOUS GRANULAR

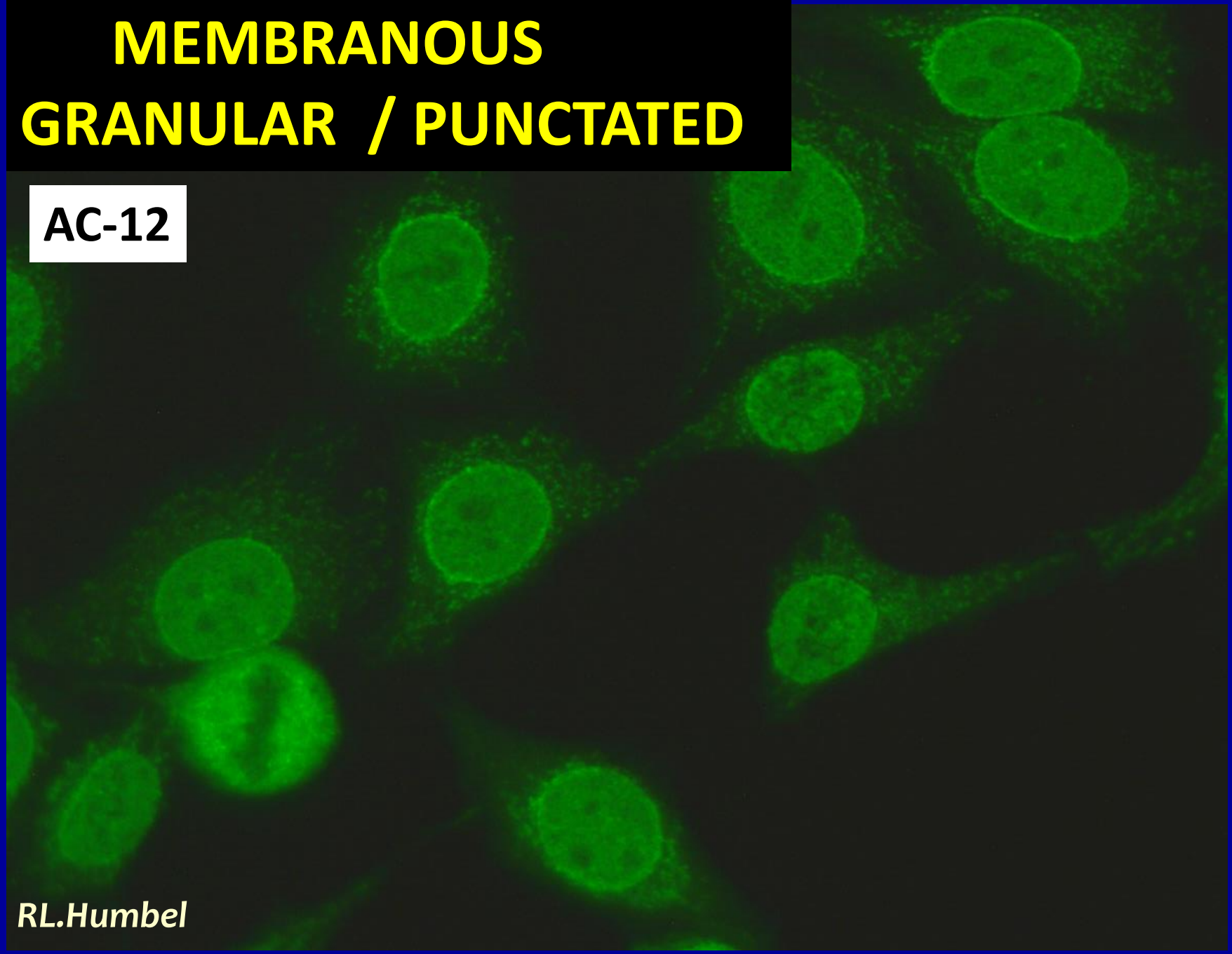
ANTI- NUCLEAR PORES

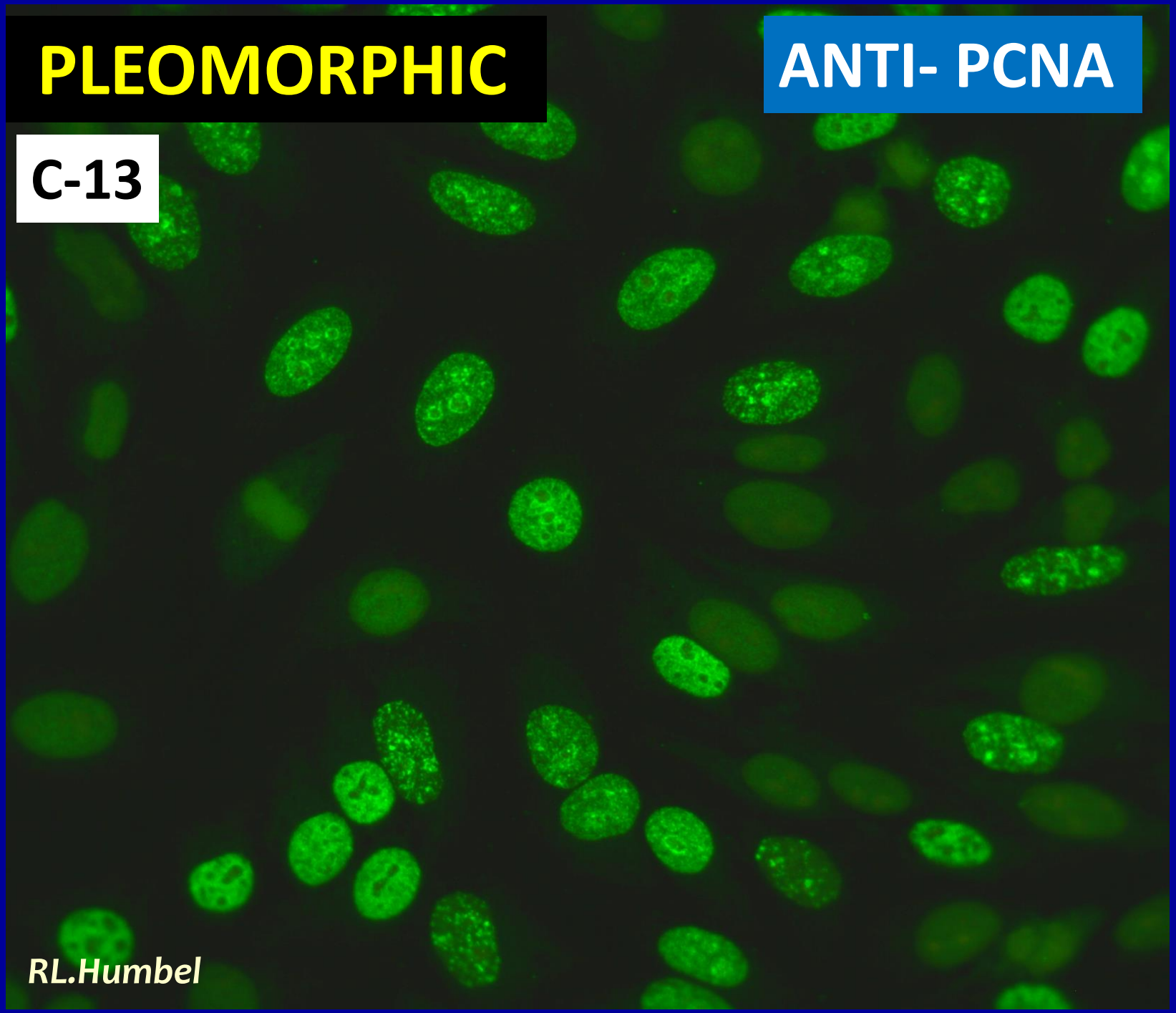


RL.Humbel

MEMBRANOUS
GRANULAR / PUNCTATED

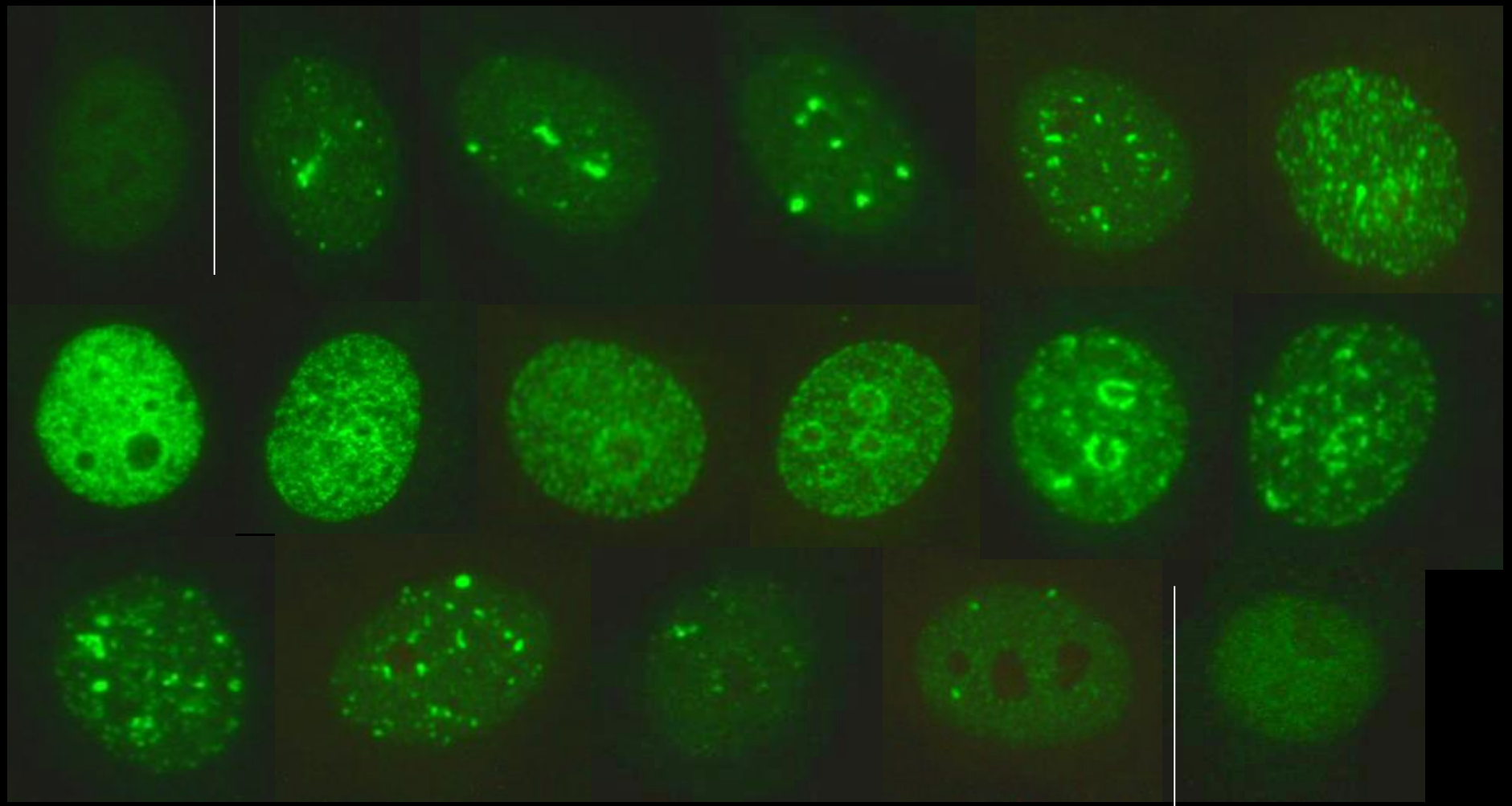
AC-12




PLEOMORPHIC
ANTI- PCNA
C-13
RL.Humbel

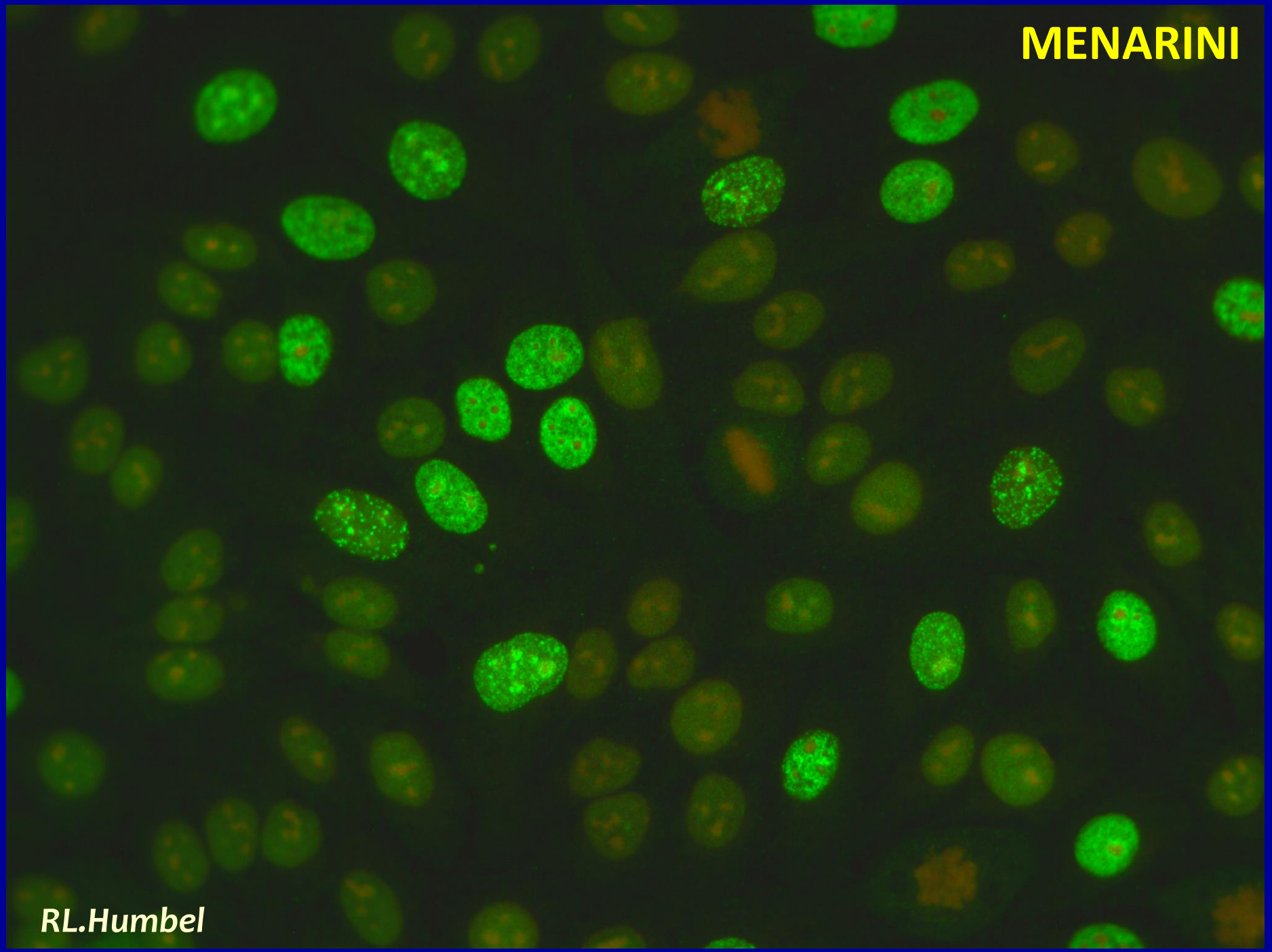
ANTI-PCNA

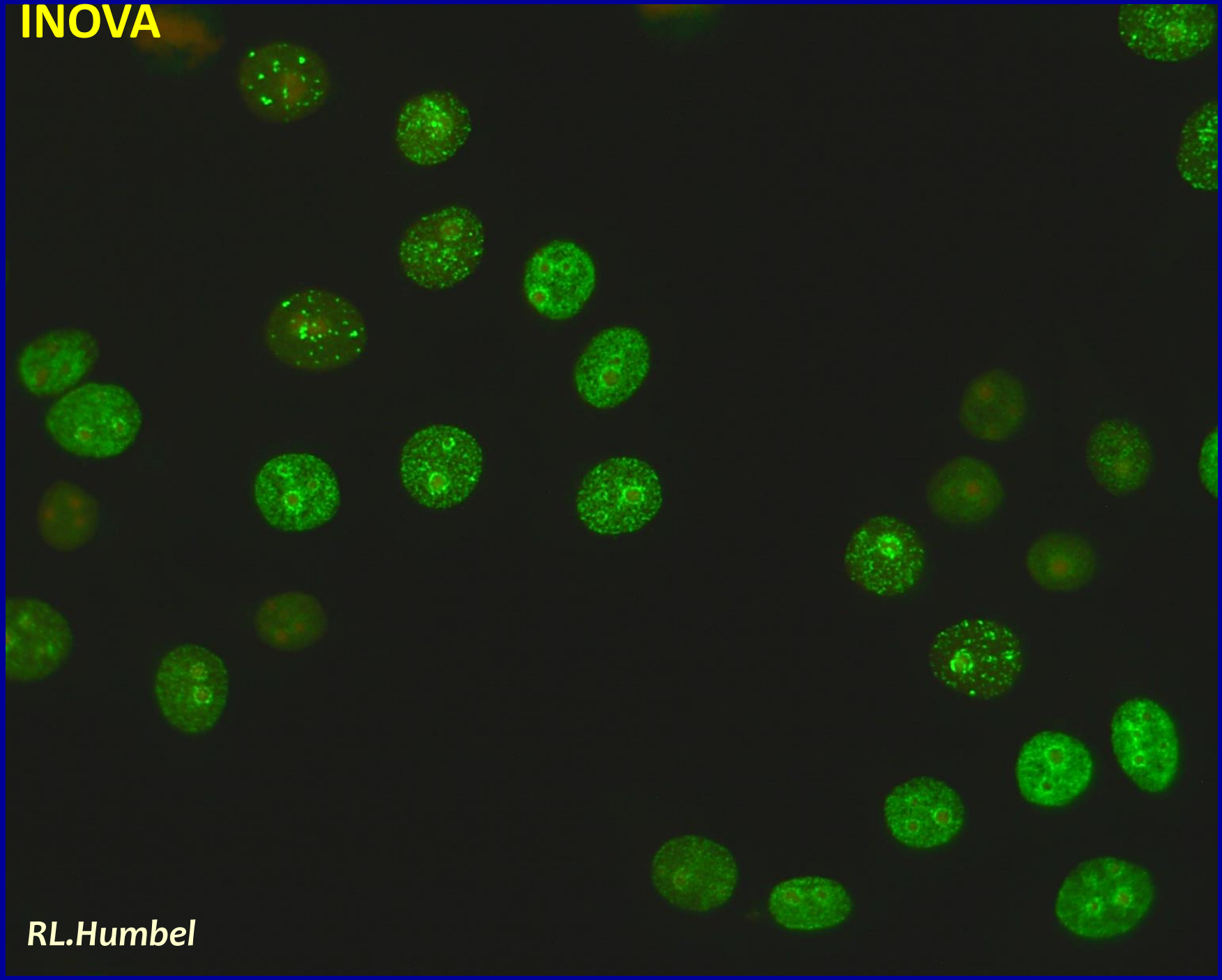
G1 S

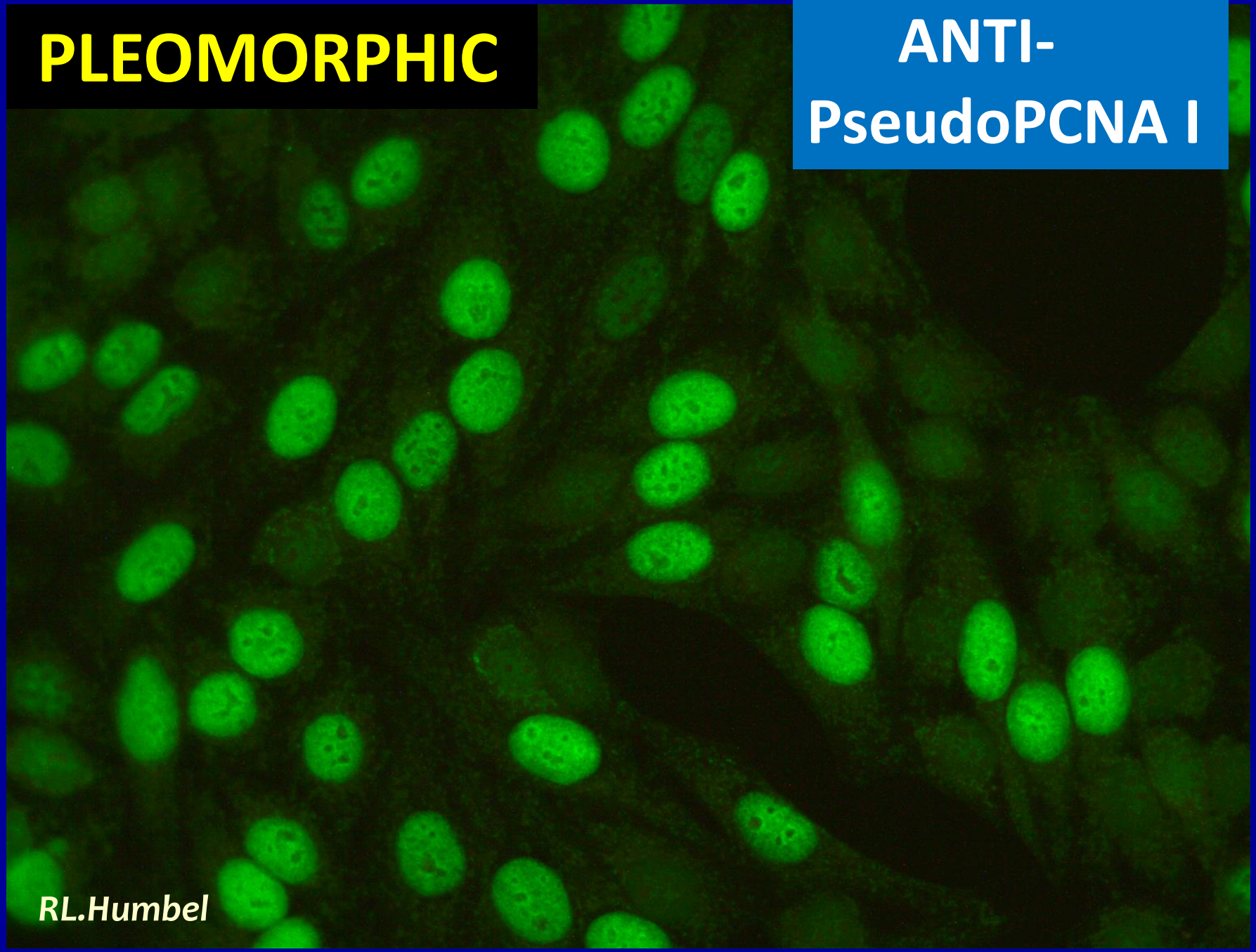


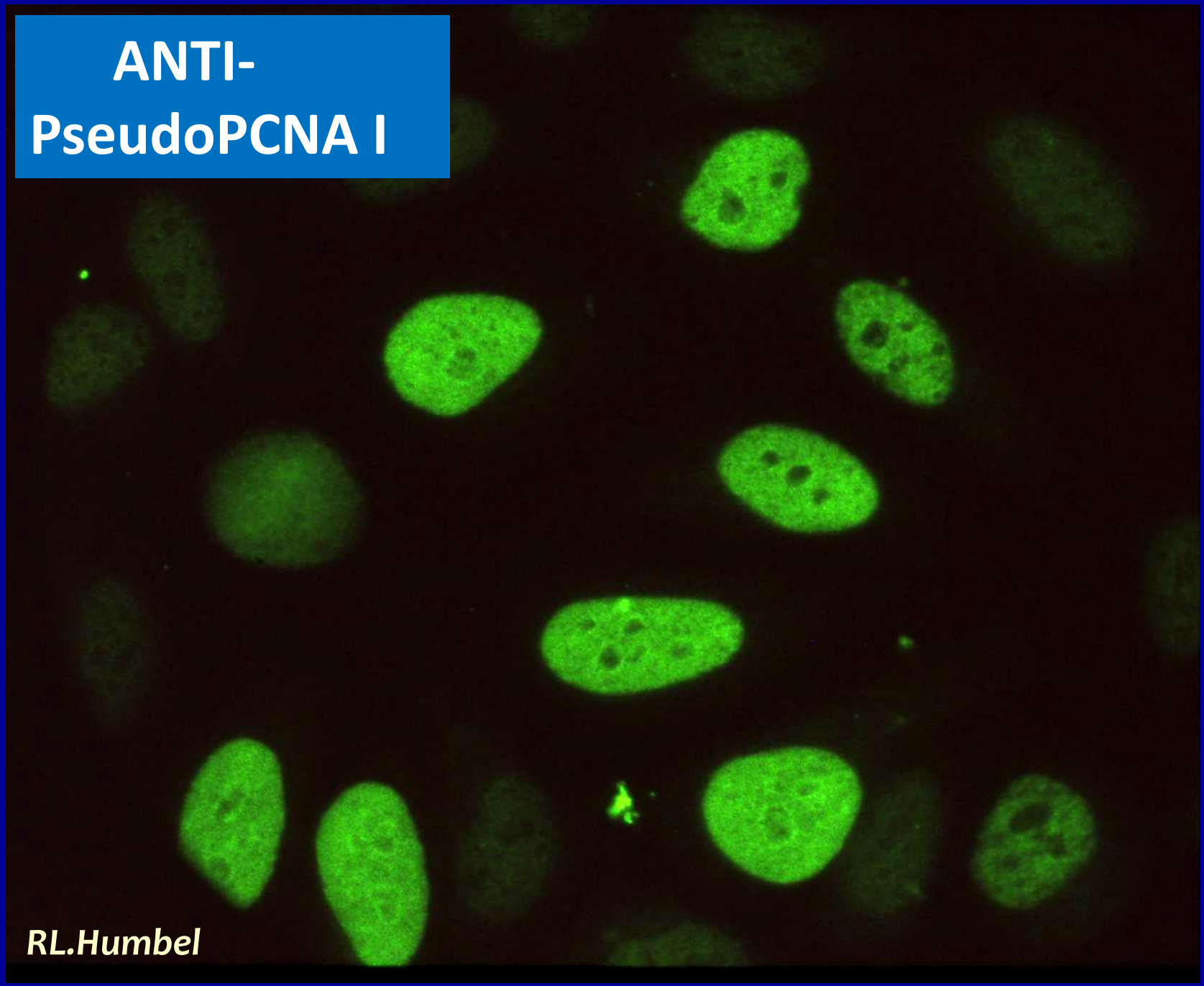
RL.Humbel

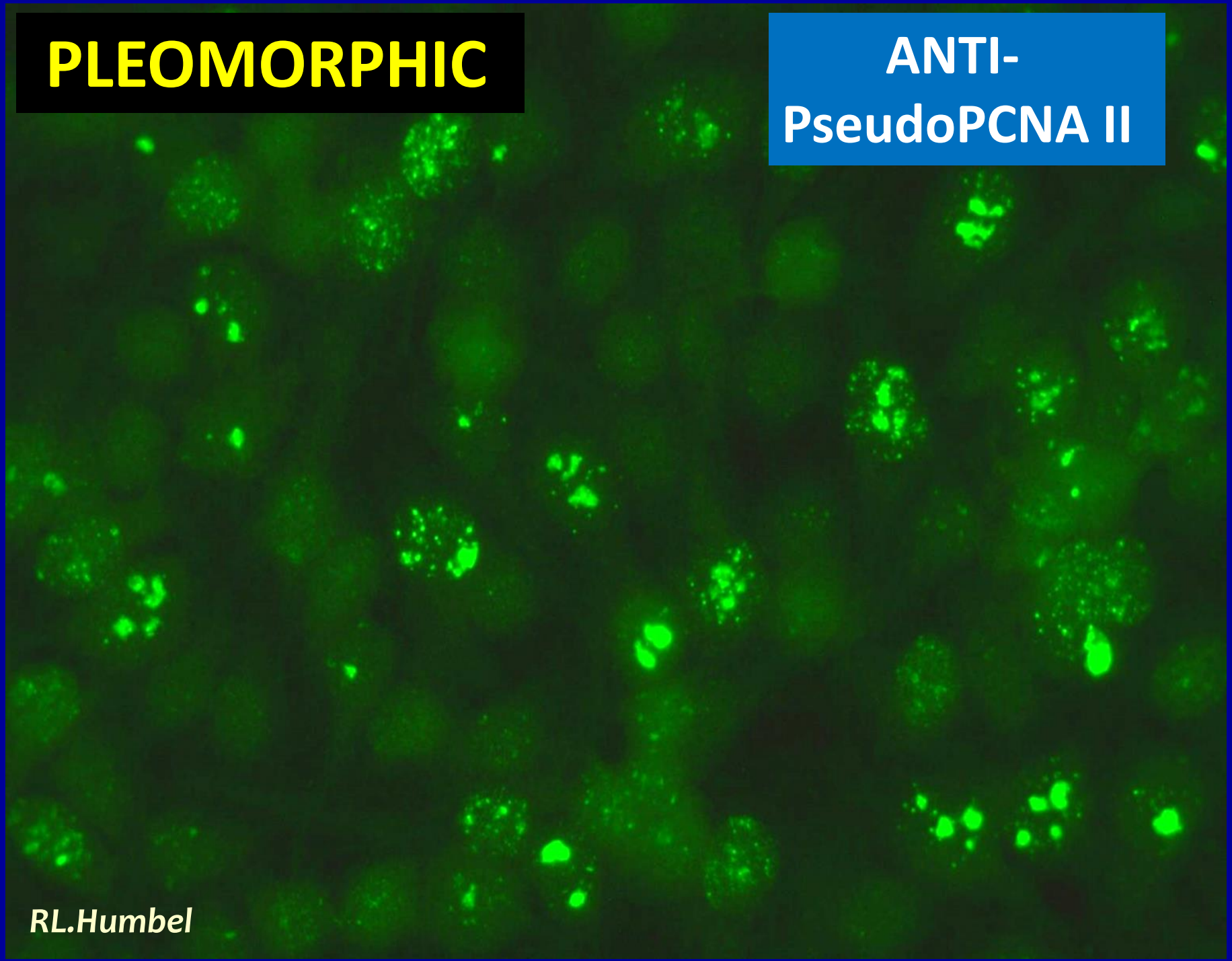
G2

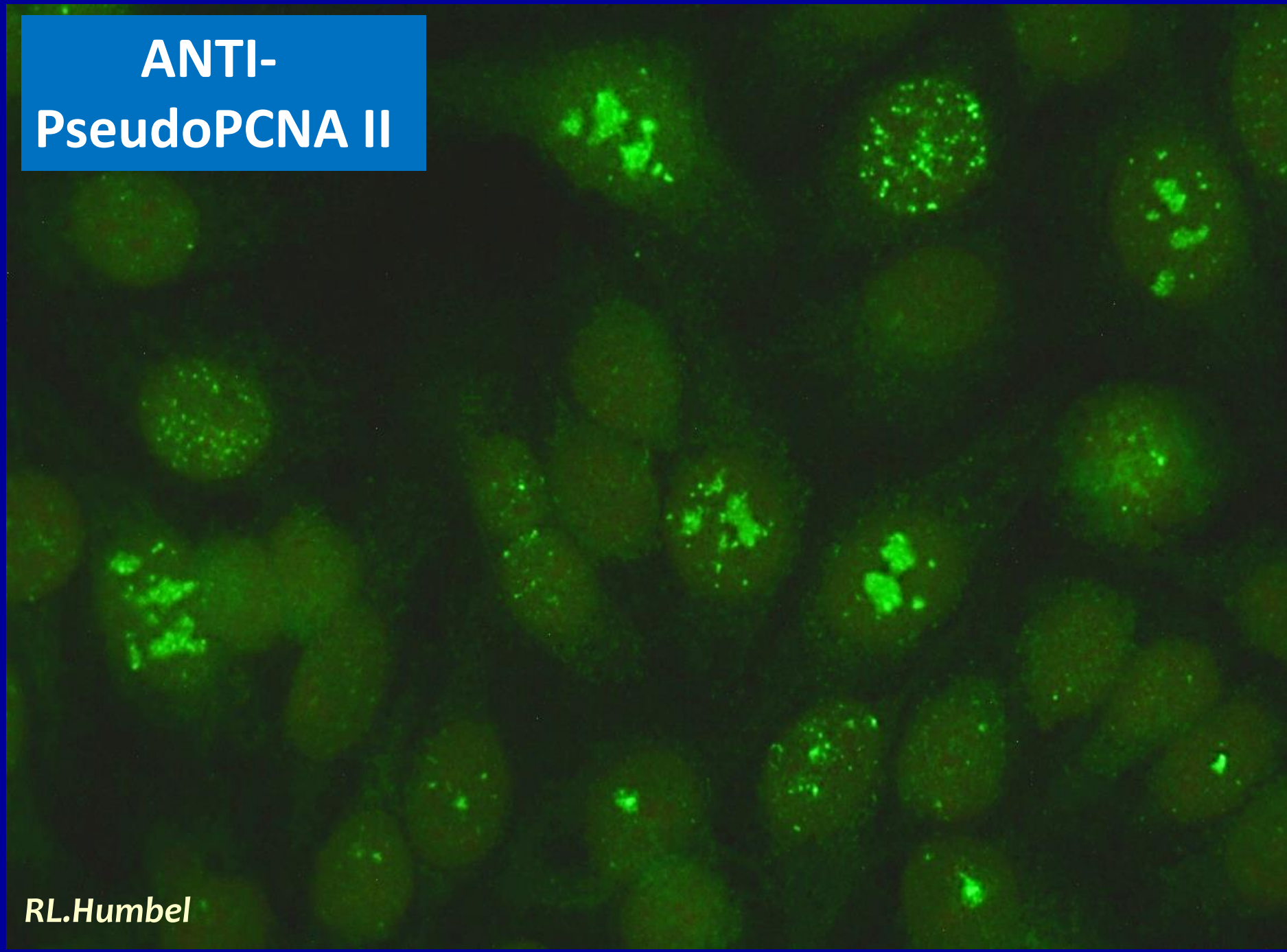

MENARINI
RL.Humbel


INOVA
RL.Humbel

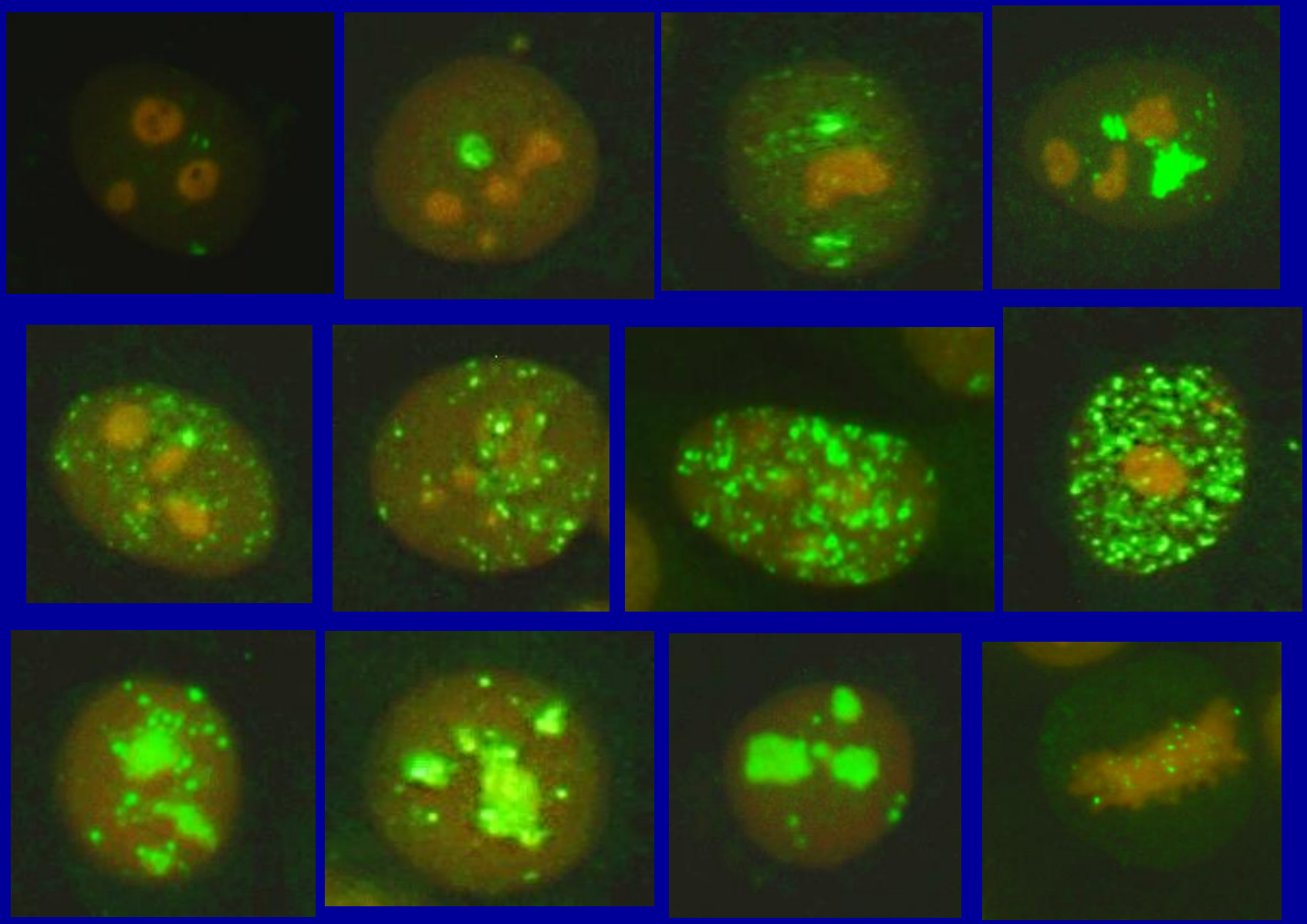

PLEOMORPHIC
ANTI-
PseudoPCNA I
RL.Humbel


ANTI-
PseudoPCNA I
RL.Humbel

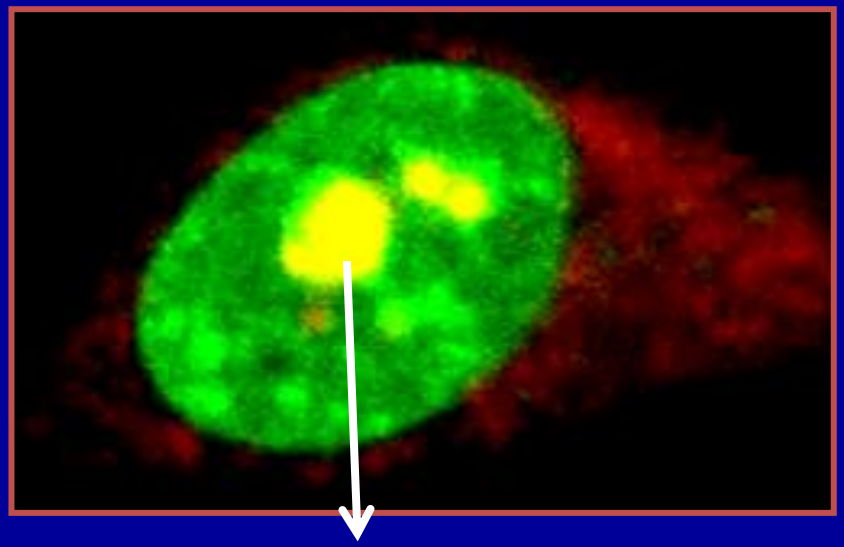

PLEOMORPHIC
ANTI-
PseudoPCNA II
RL.Humbel


ANTI-
PseudoPCNA II
RL.Humbel

ANTI- PseudoPCNA II



RL.Humbel



NUCLEOLI

HOMOGENEOUS

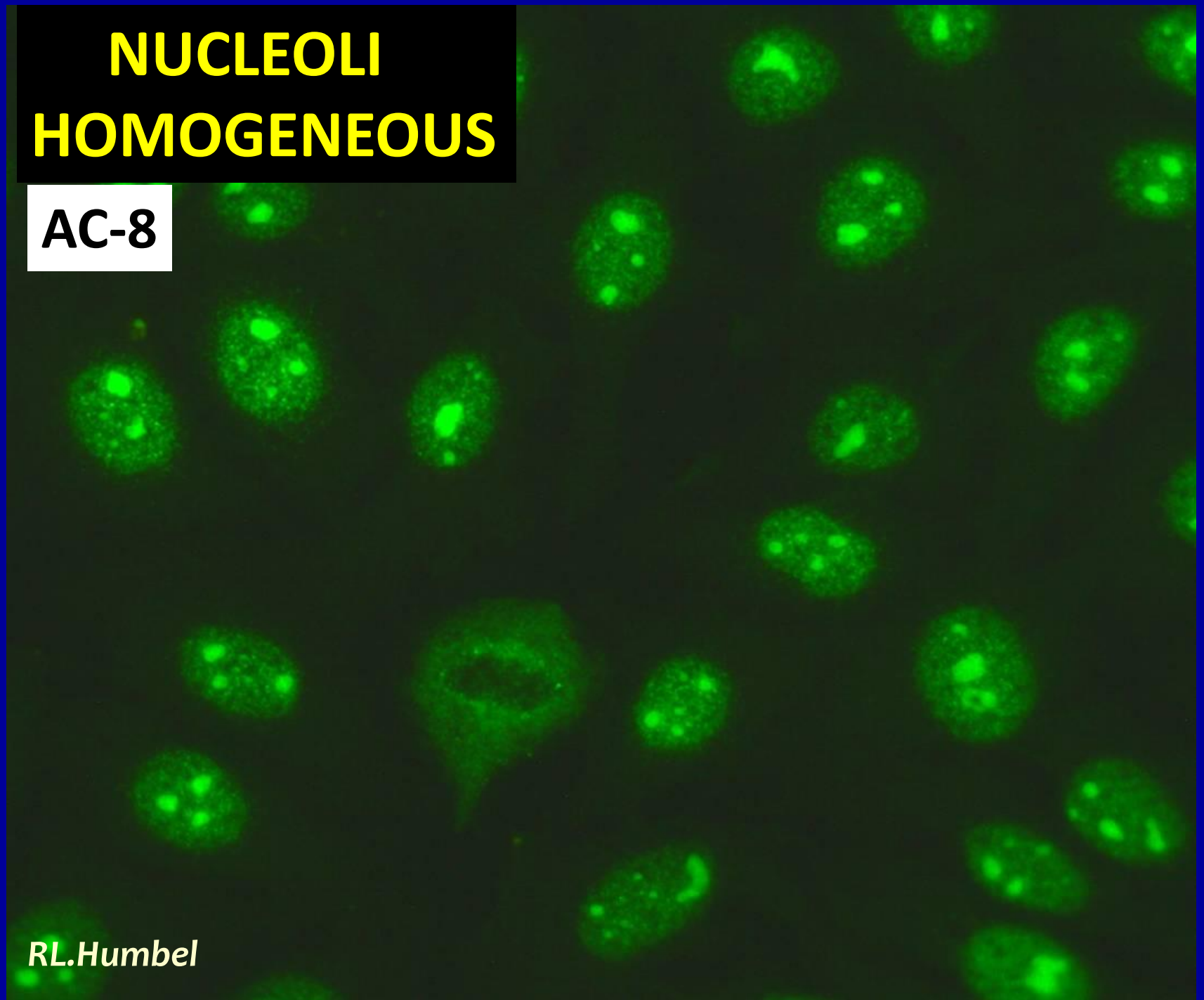
AC-8

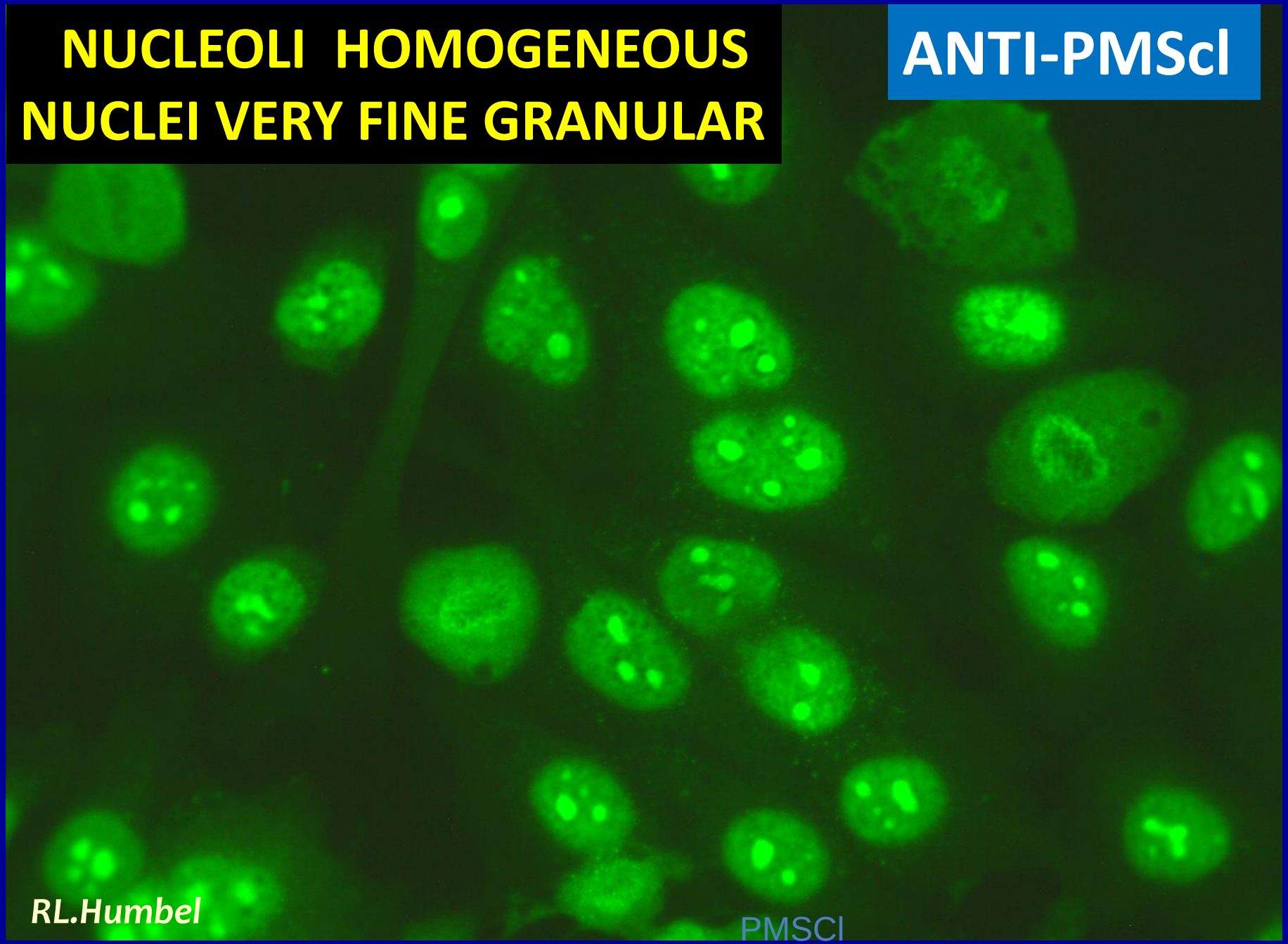
CLUMPY

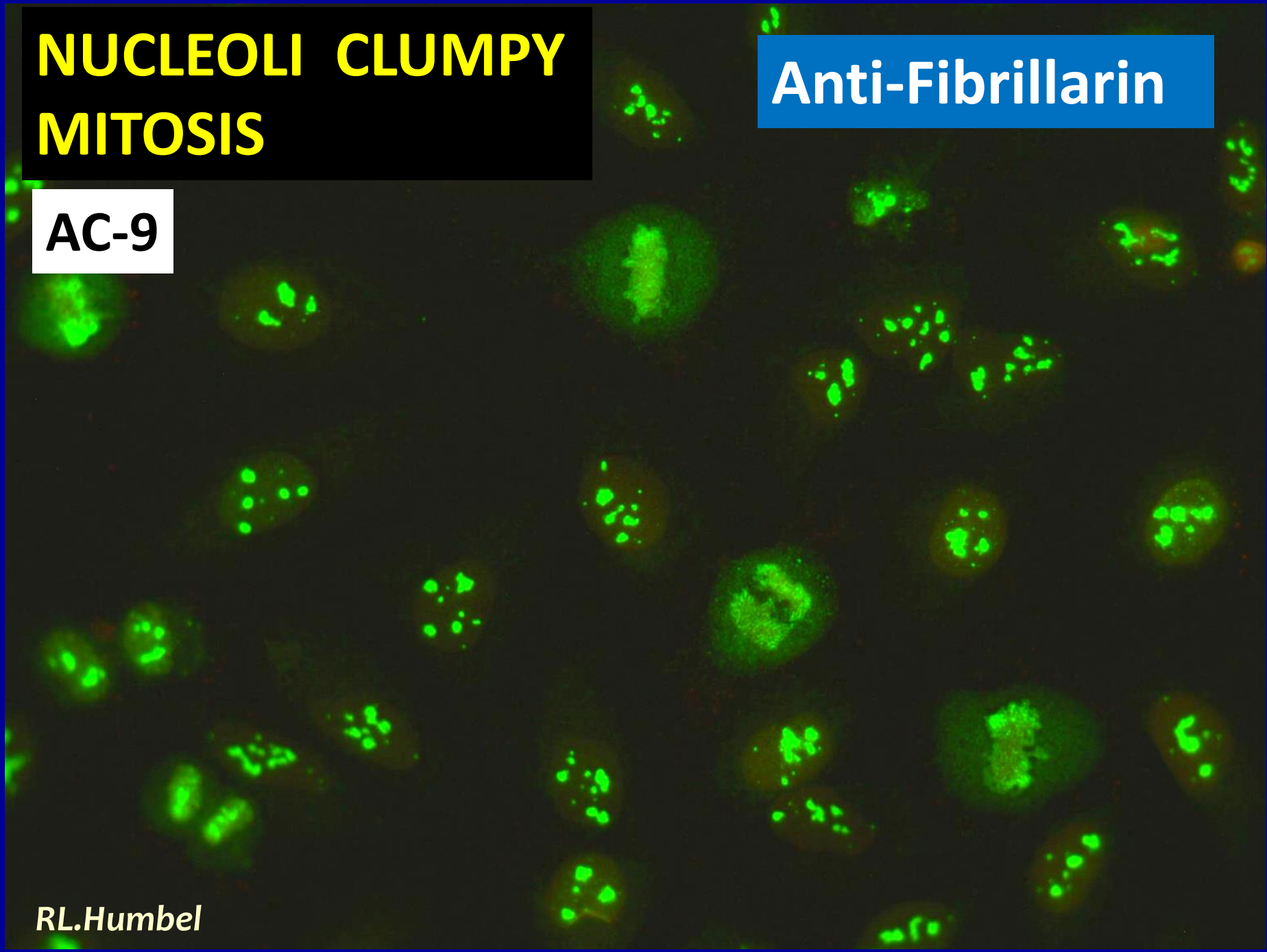
AC-9

GRANULAR
PUNCTATE

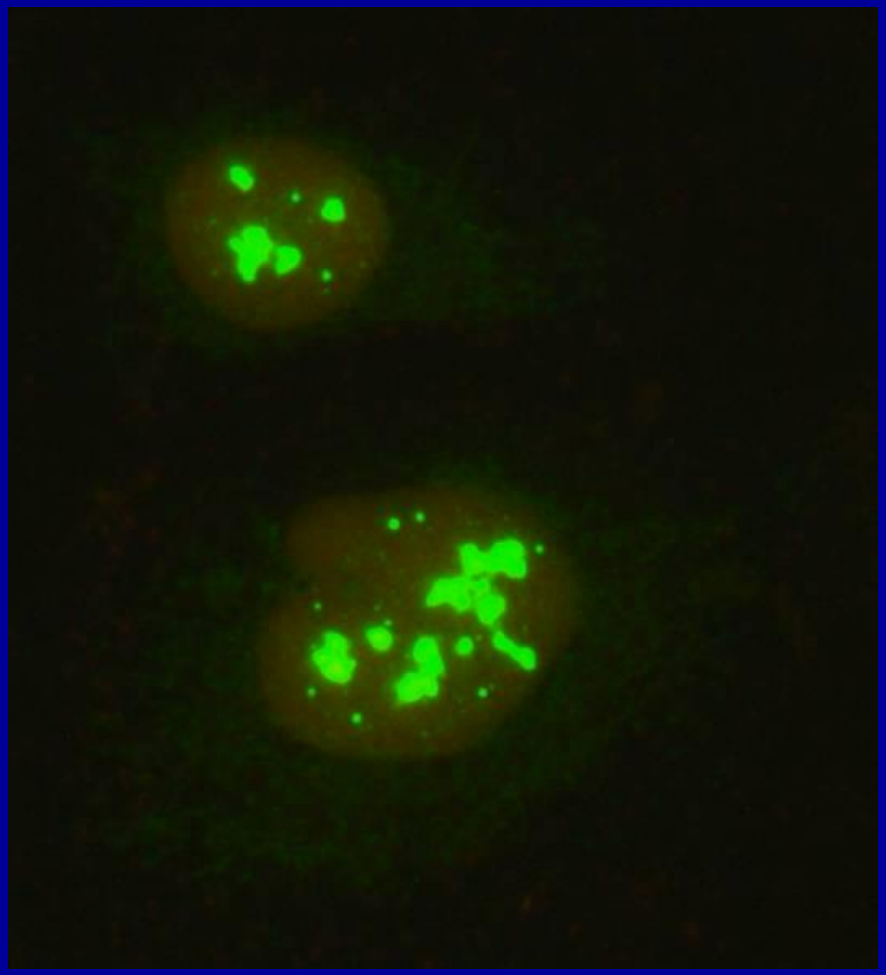
AC-10

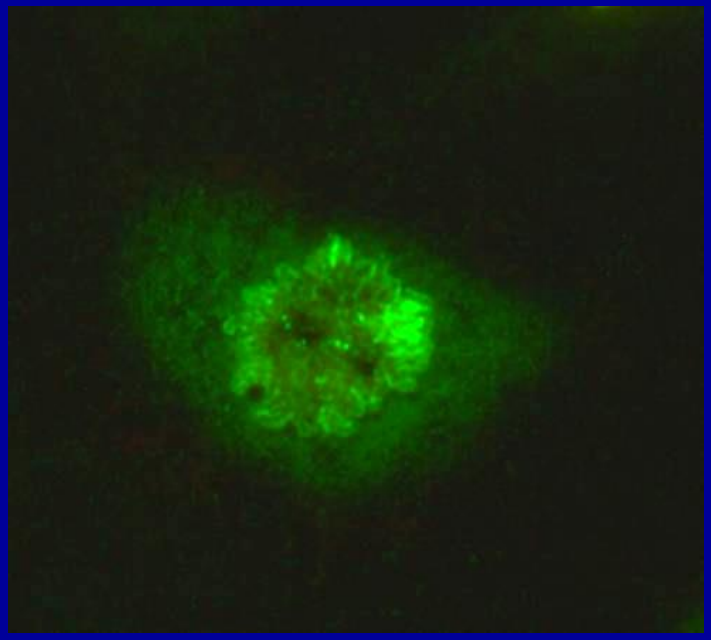

NUCLEOLI
HOMOGENEOUS
AC-8
RL.Humbel

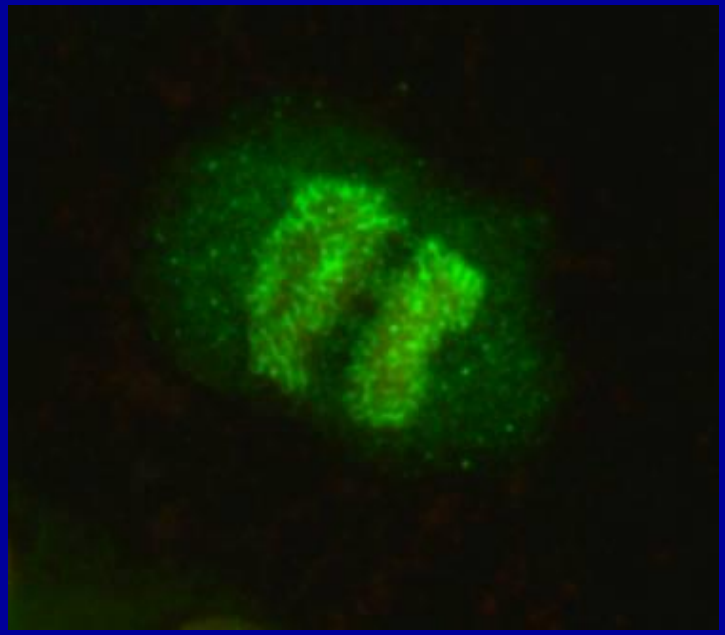

NUCLEOLI HOMOGENEOUS
NUCLEI VERY FINE GRANULAR
ANTI-PMScl
RL.Humbel
PMSCl


NUCLEOLI CLUMPY
MITOSIS
Anti-Fibrillarin
AC-9
RL.Humbel

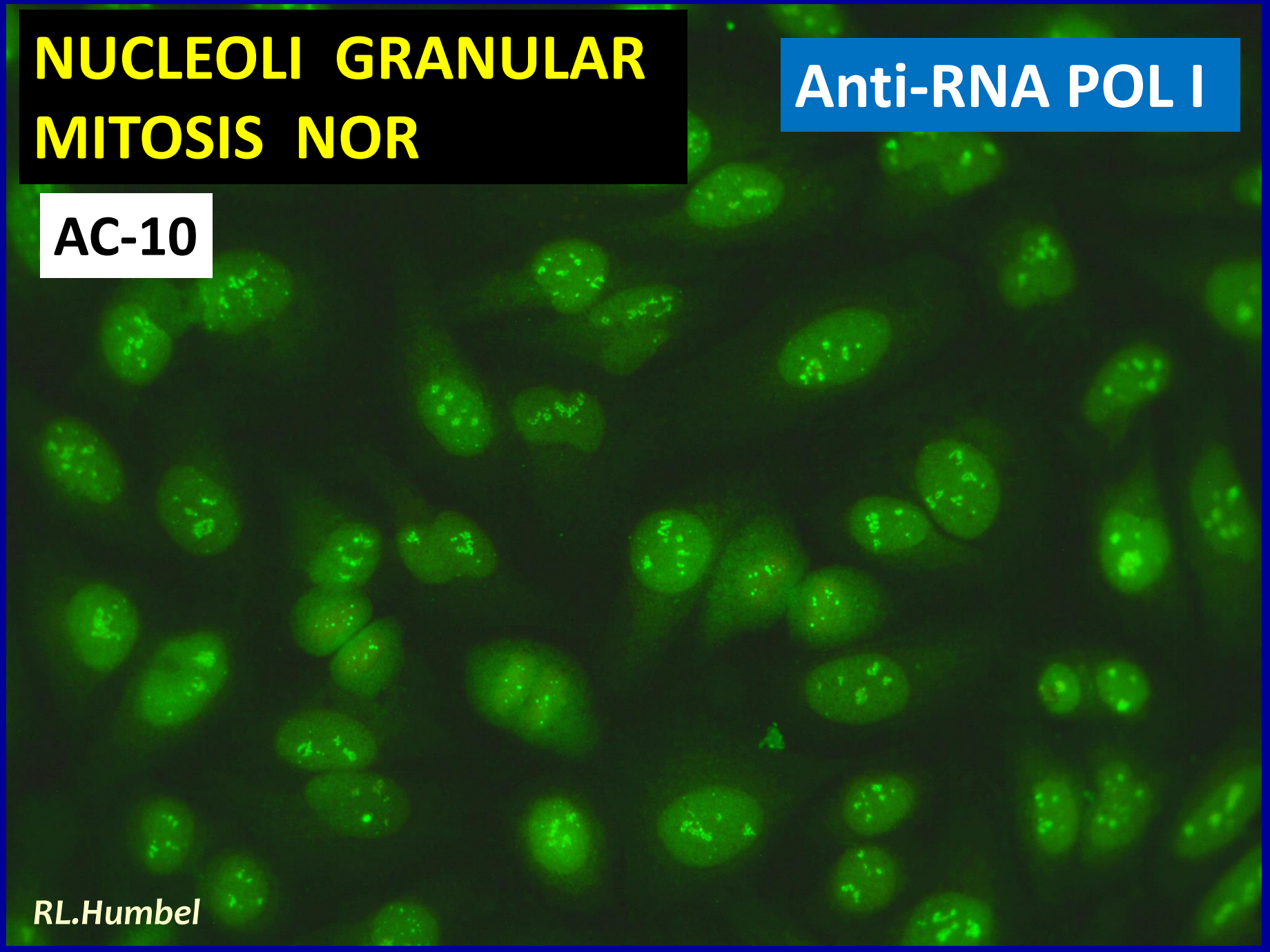
Anti-Fibrillarin

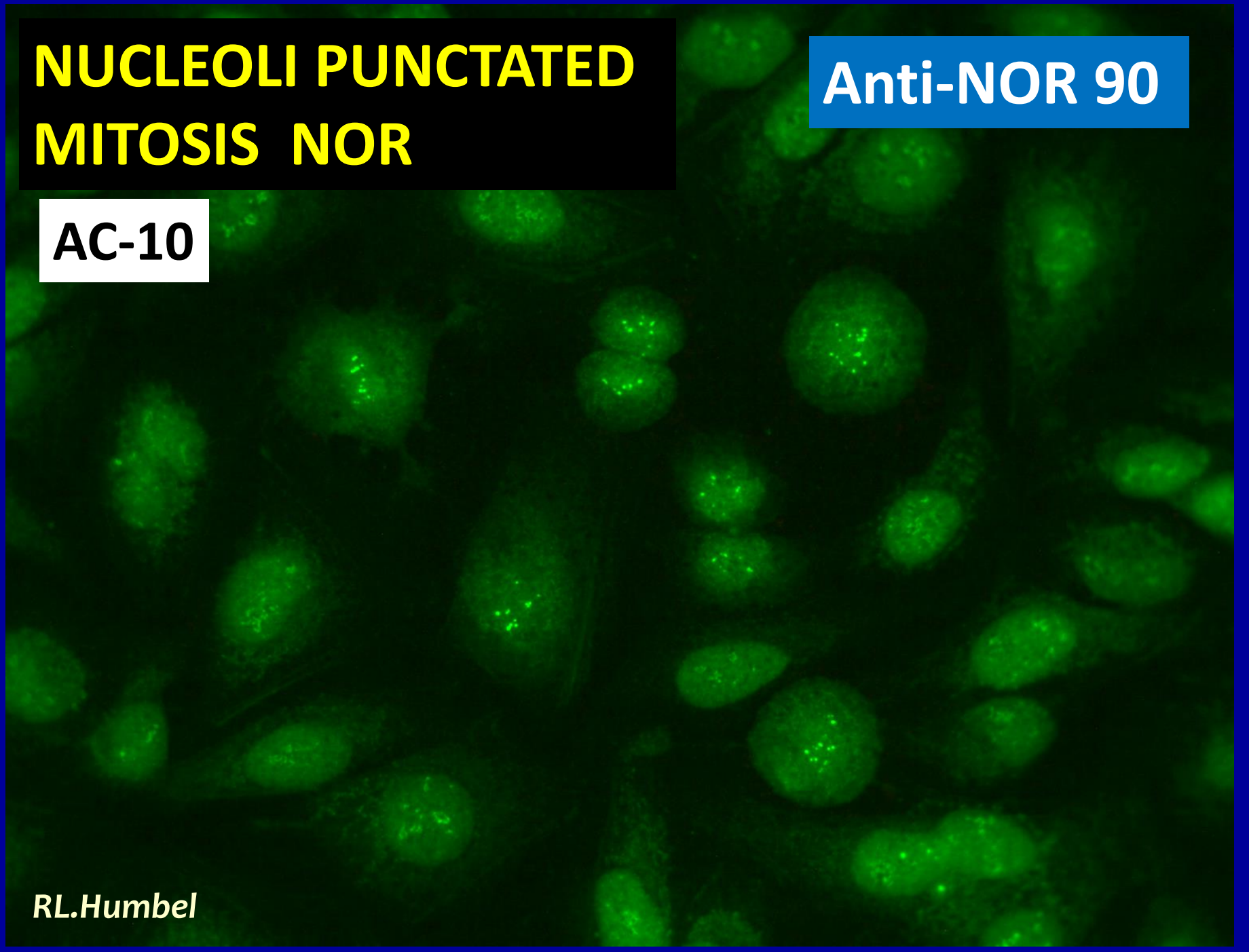


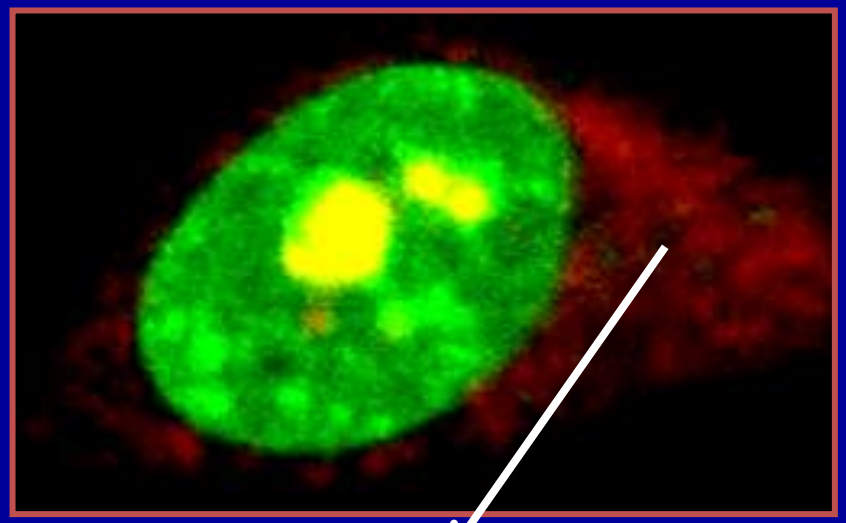




RL.Humbel


NUCLEOLI GRANULAR
MITOSIS NOR
Anti-RNA POL I
AC-10
RL.Humbel


NUCLEOLI PUNCTATED
MITOSIS NOR
Anti-NOR 90
AC-10
RL.Humbel



CYTOPLASM

SPECKLED/GRANULAR

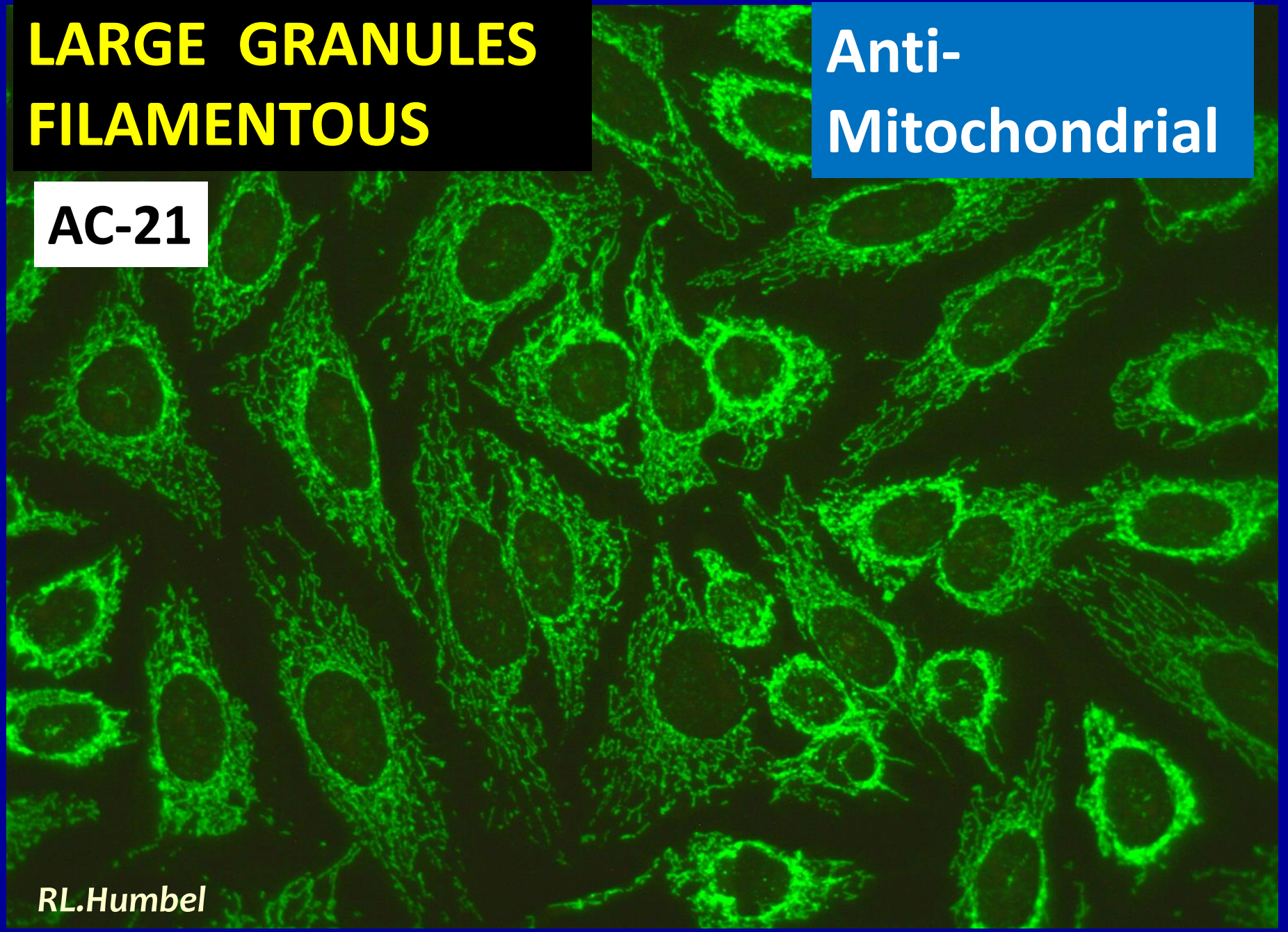
- Large Granules — AC-21
- Discrete Dots — AC-18
- Fine Granules — AC-20
- Dense Fine Granules — AC-19

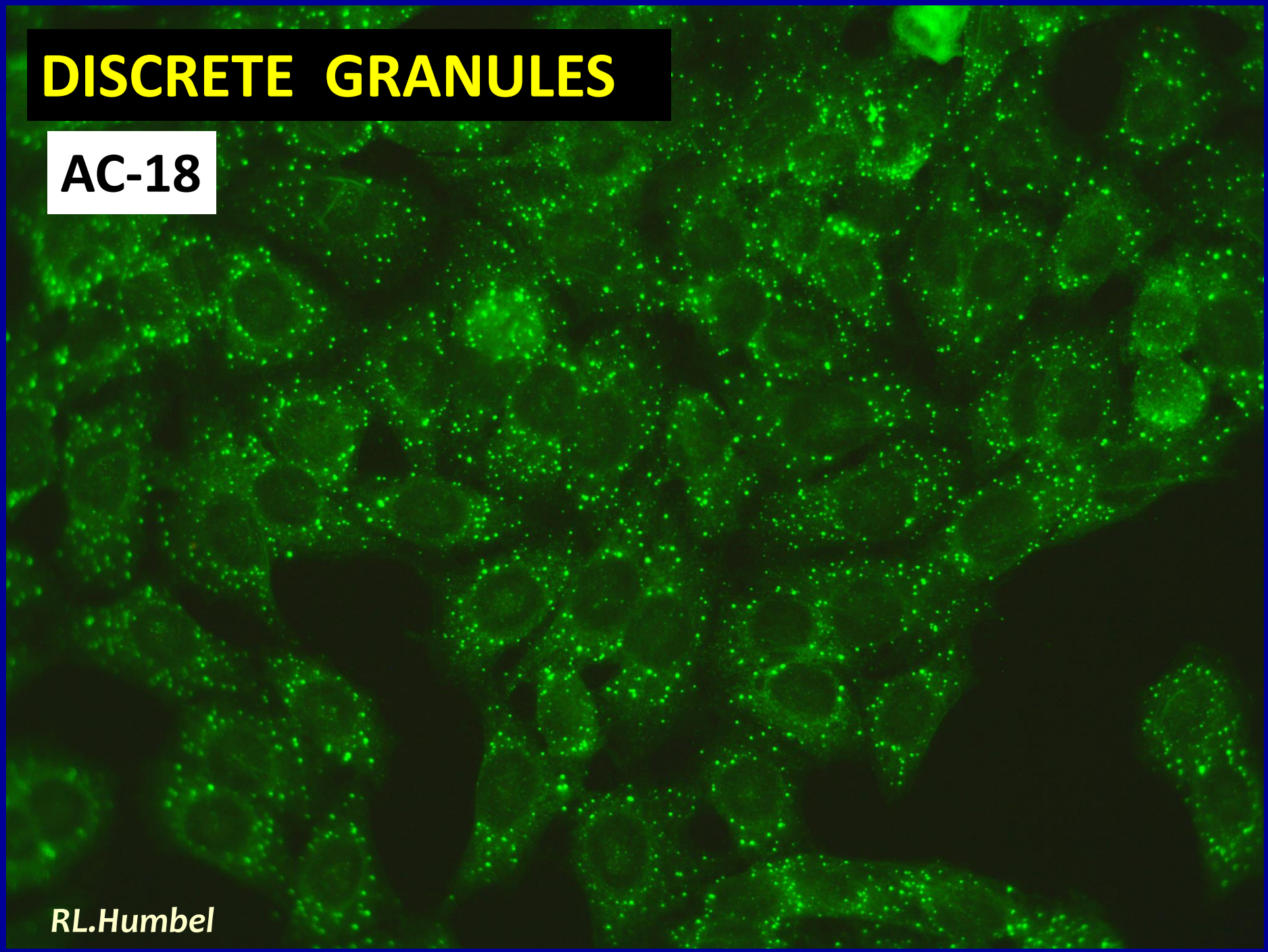
INCLUSIONS

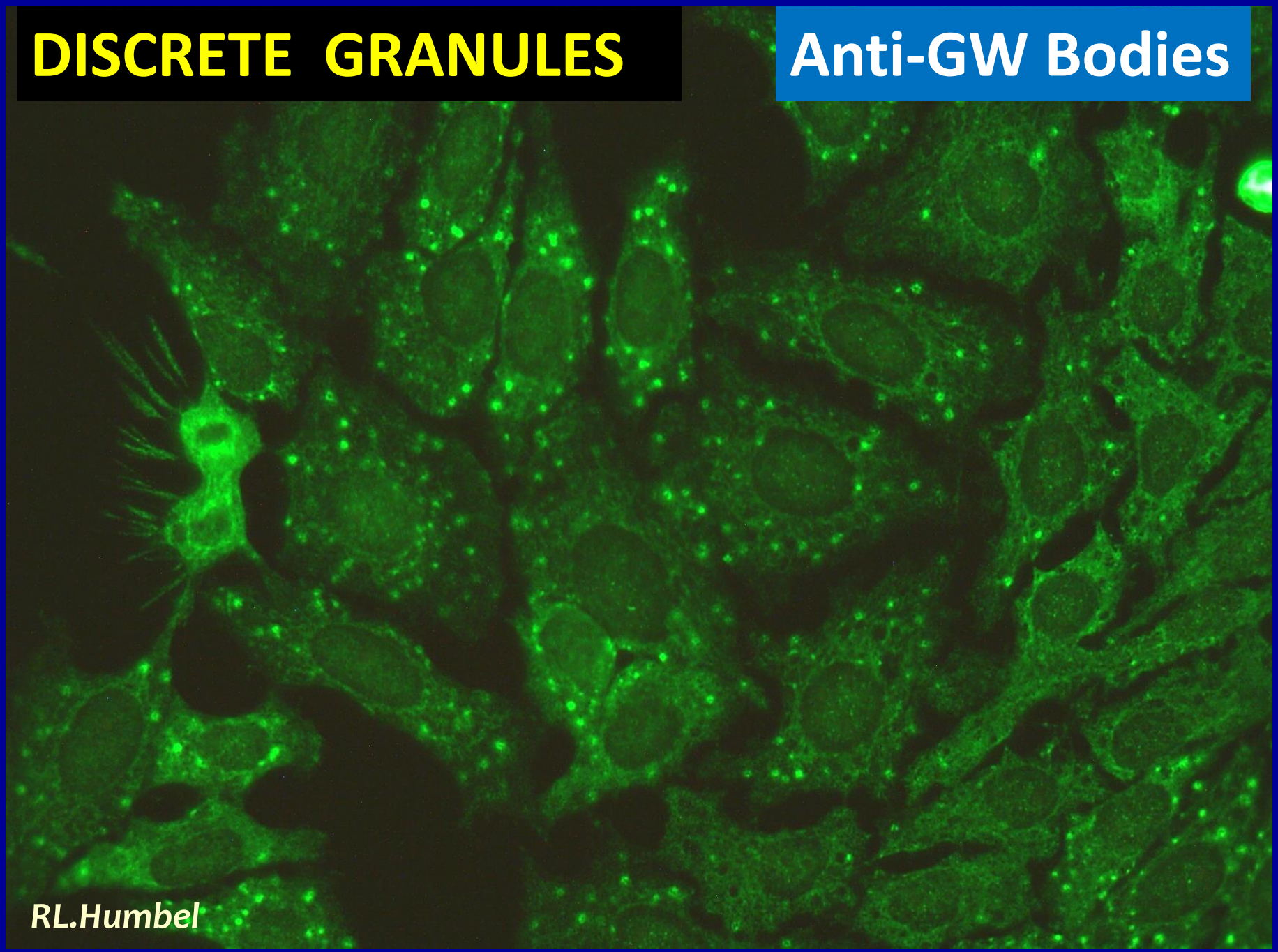
- Golgi — AC-22
- Nematine RR — AC-23

FILAMENTS

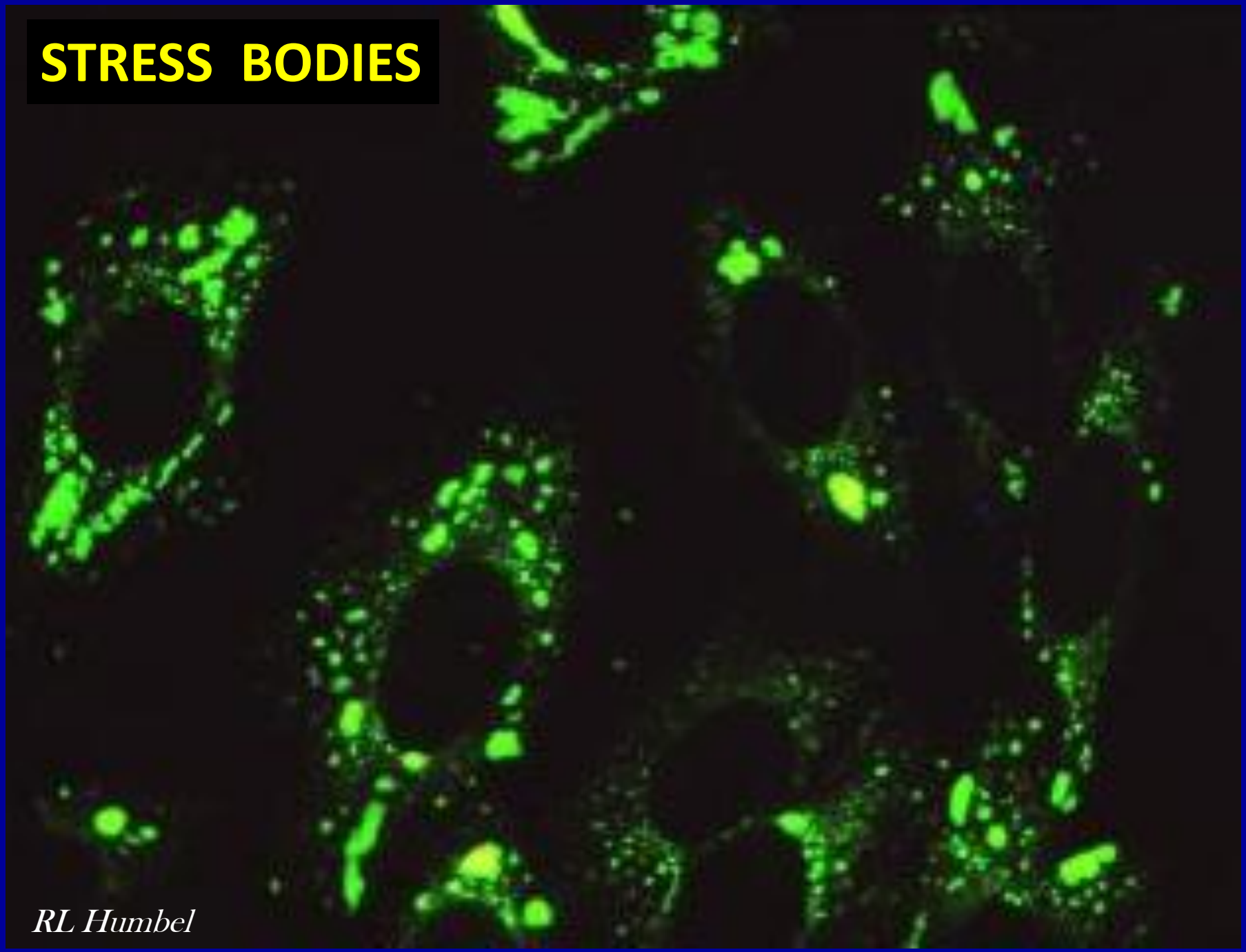
- Linear / Cables — AC-15
- Radial Filaments — AC-16
- Fine Long Filaments — Sequential — AC-17


LARGE GRANULES
FILAMENTOUS
AC-21
Anti-
Mitochondrial
RL.Humbel

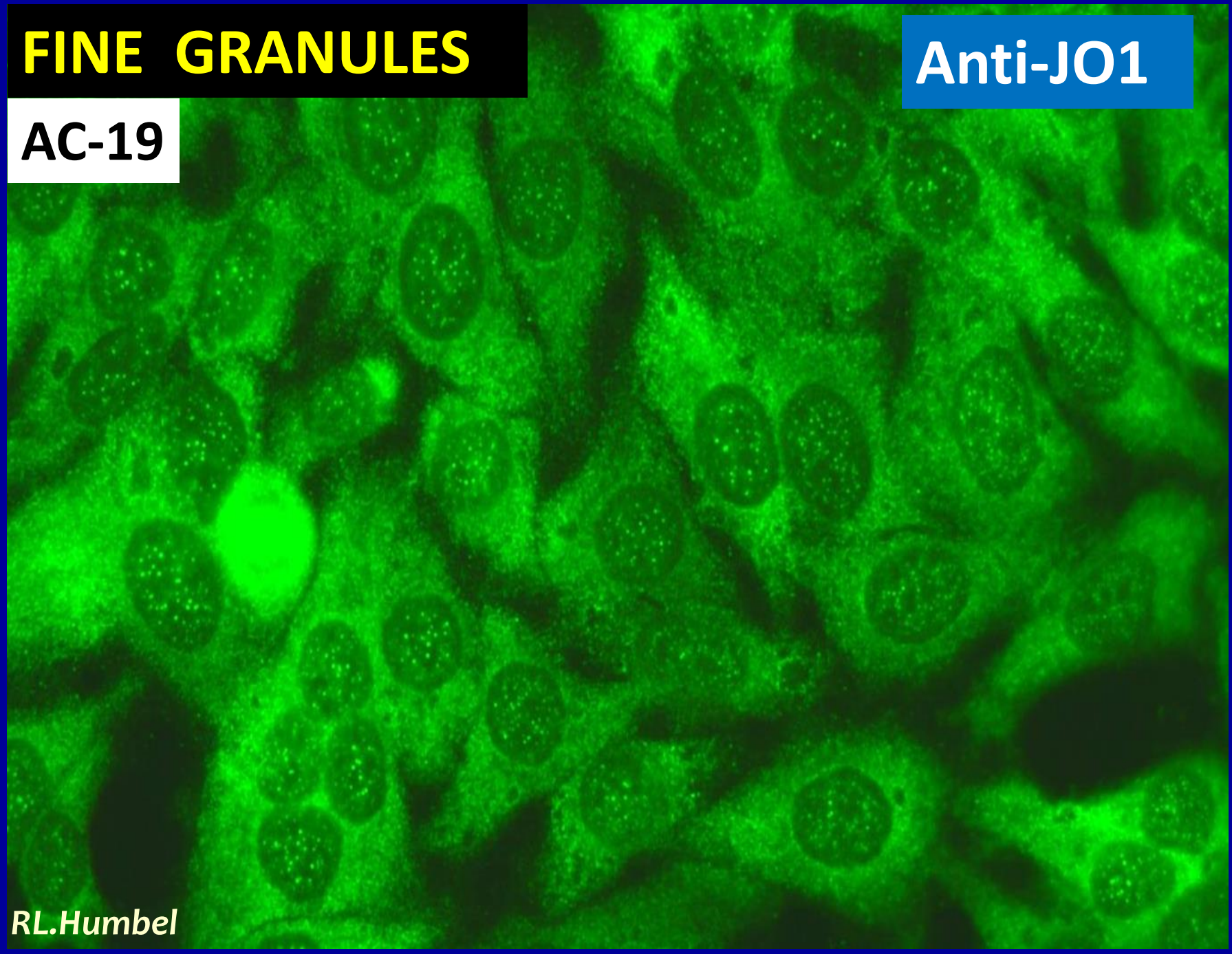

DISCRETE GRANULES
AC-18
RL.Humbel

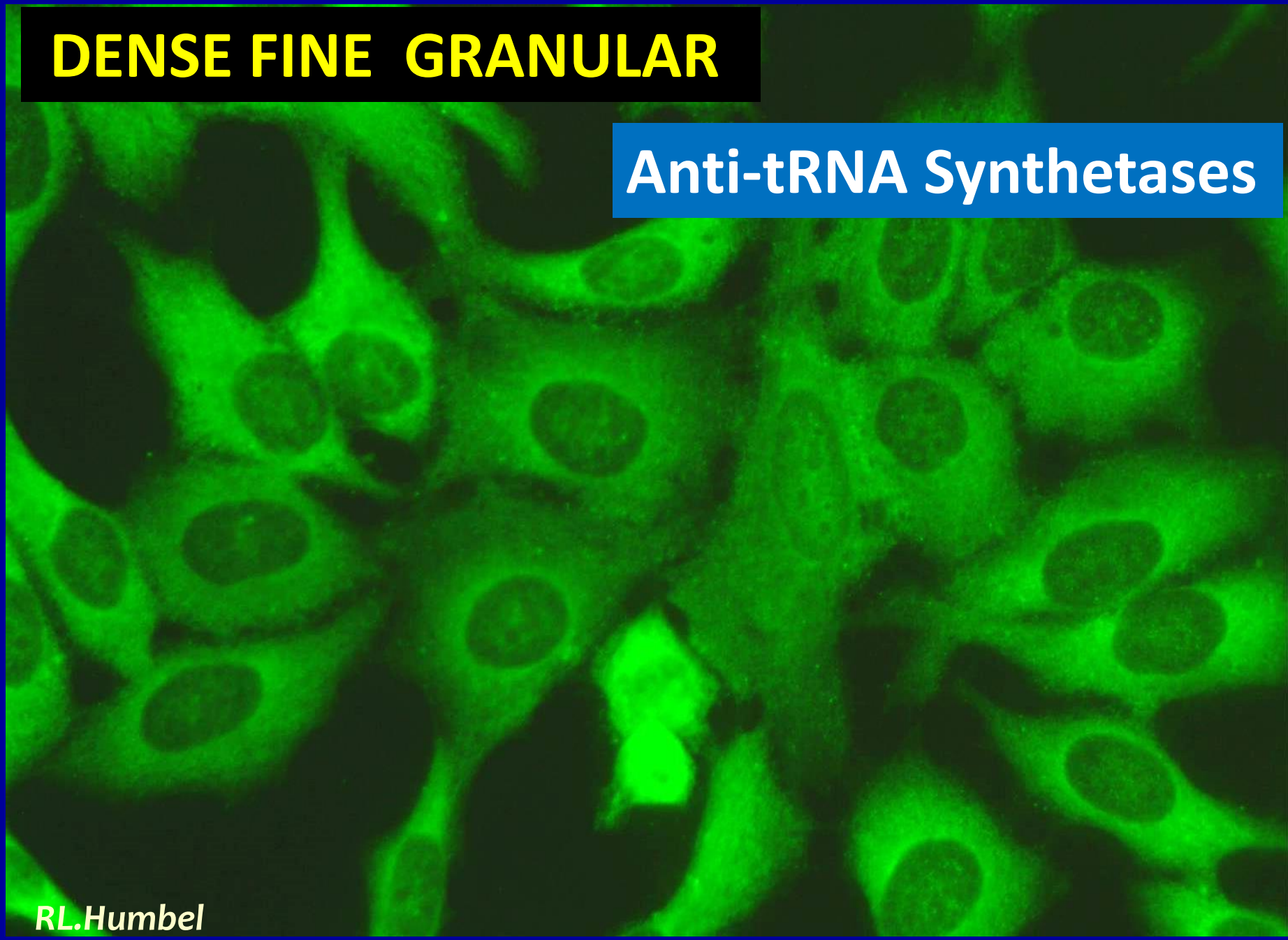

DISCRETE GRANULES
Anti-GW Bodies
RL.Humbel

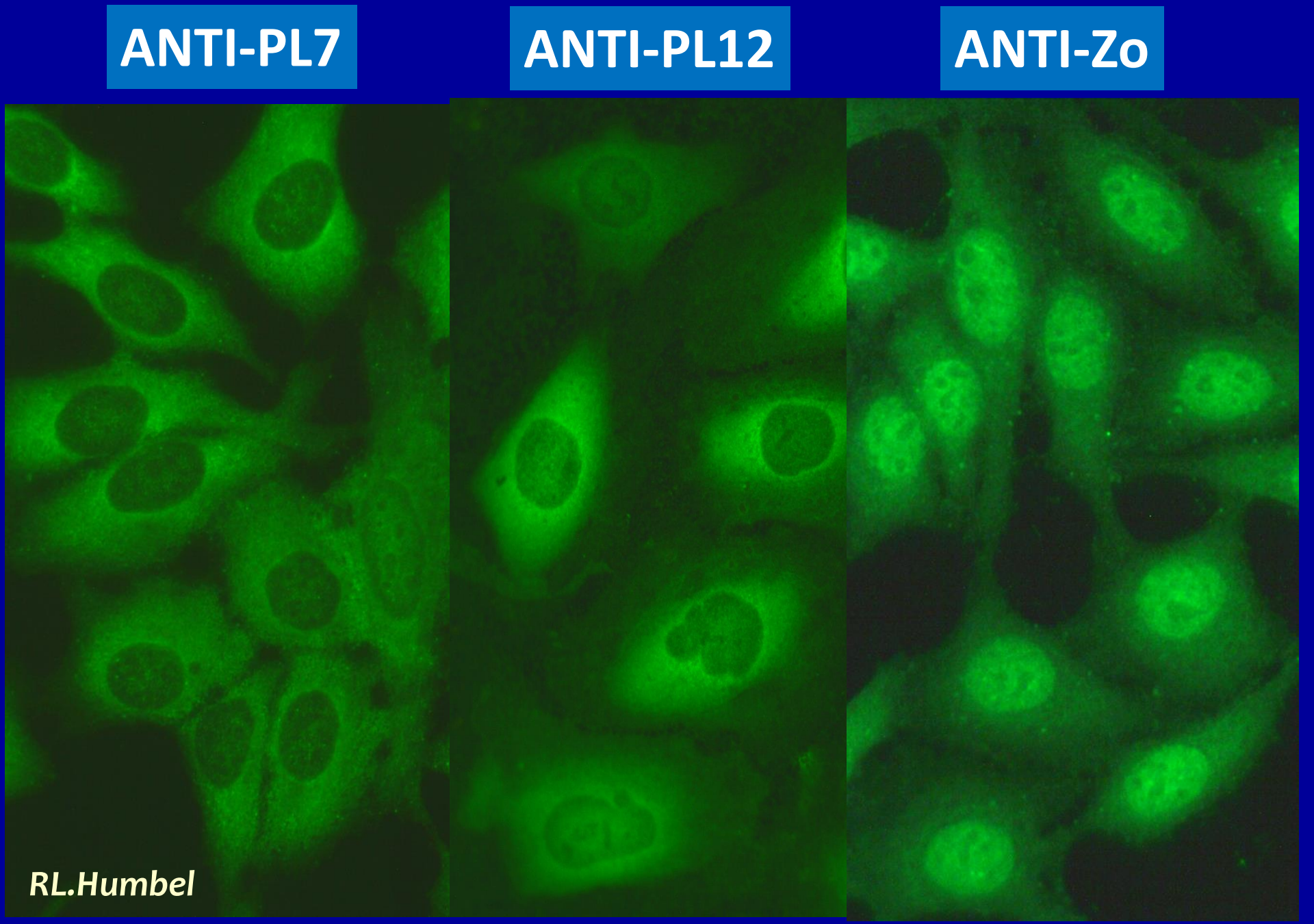
STRESS BODIES

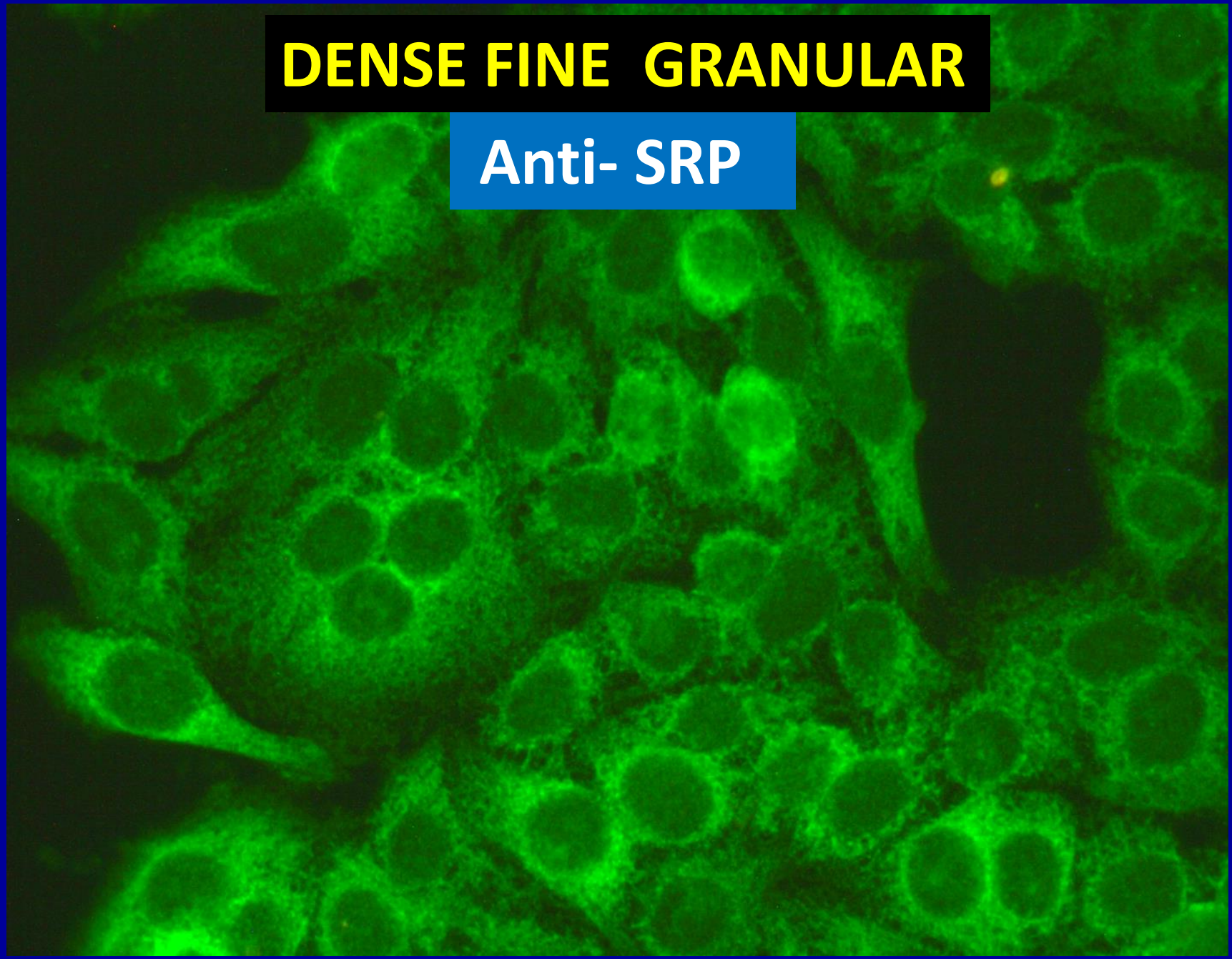


RL Humbel


FINE GRANULES
AC-19
Anti-JO1
RL.Humbel

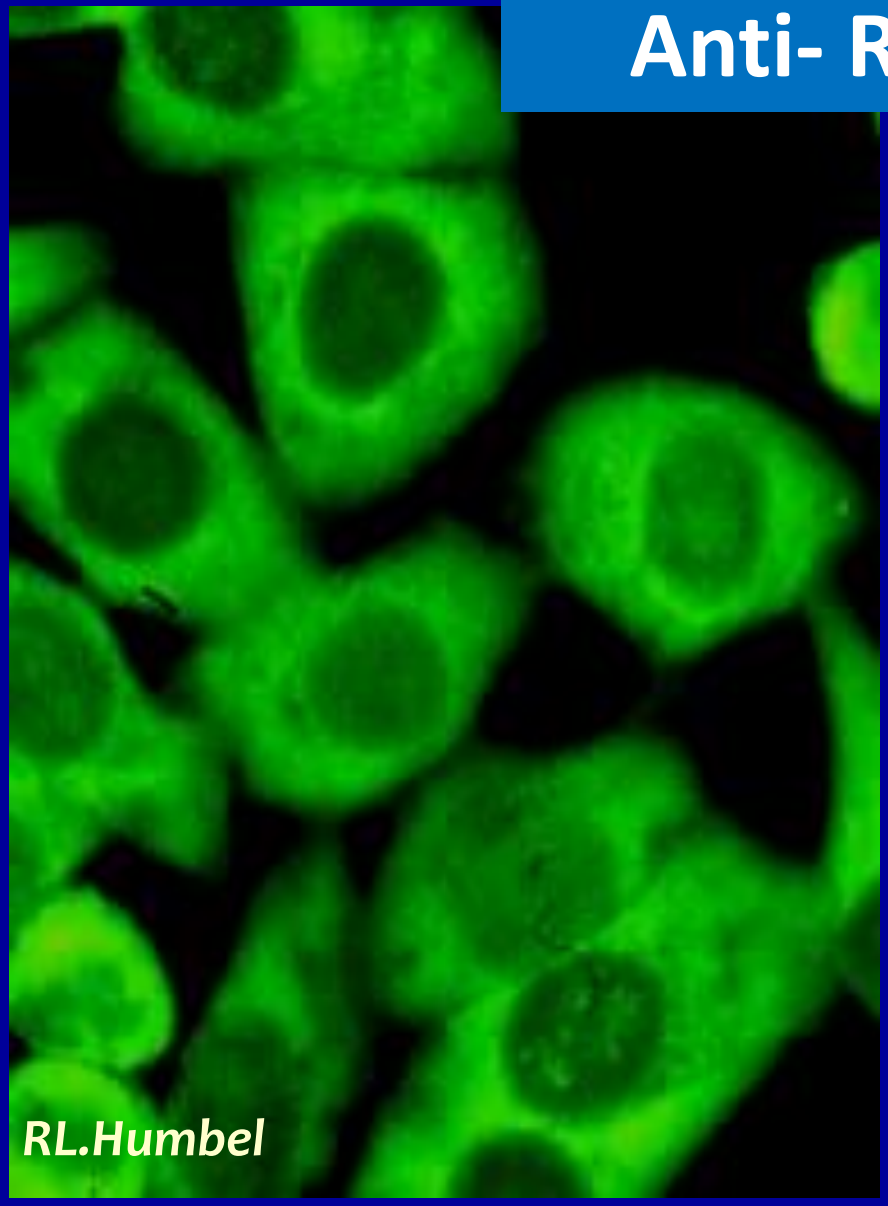

DENSE FINE GRANULAR
Anti-tRNA Synthetases
RL.Humbel

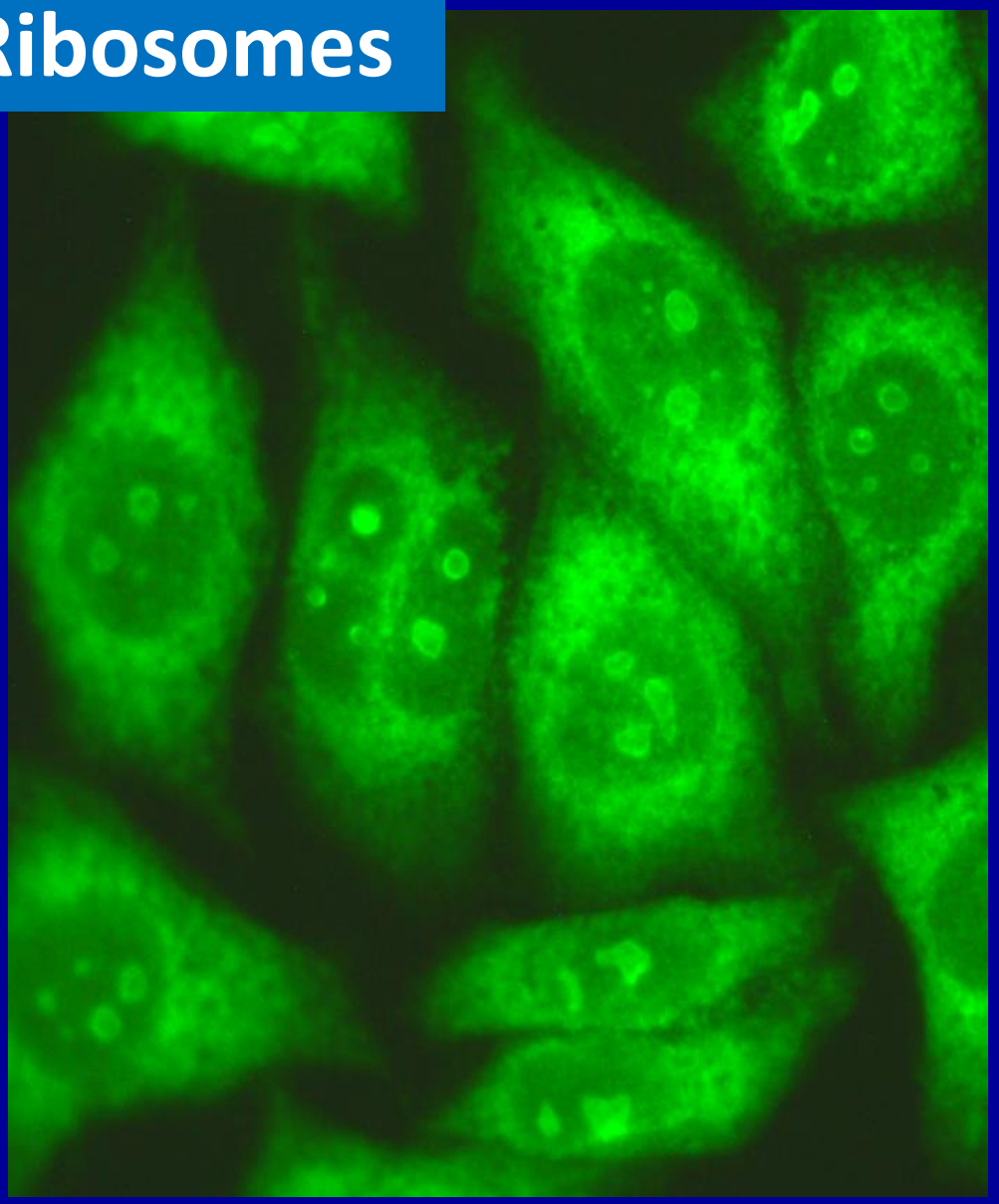

ANTI-PL7
ANTI-PL12
ANTI-Zo
RL.Humbel

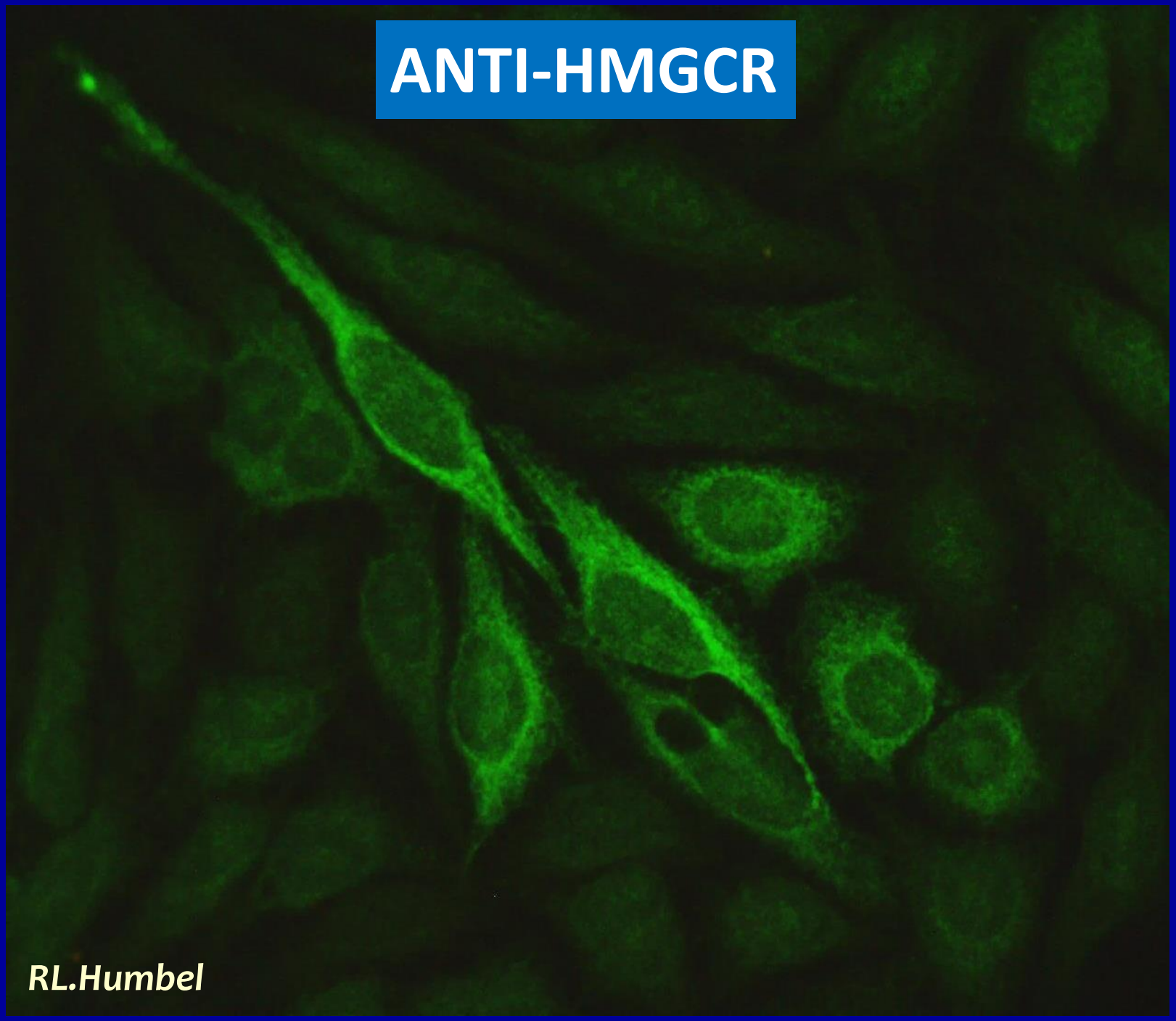

DENSE FINE GRANULAR
Anti- SRP

VERY DENSE FINE GRANULAR

Anti- Ribosomes



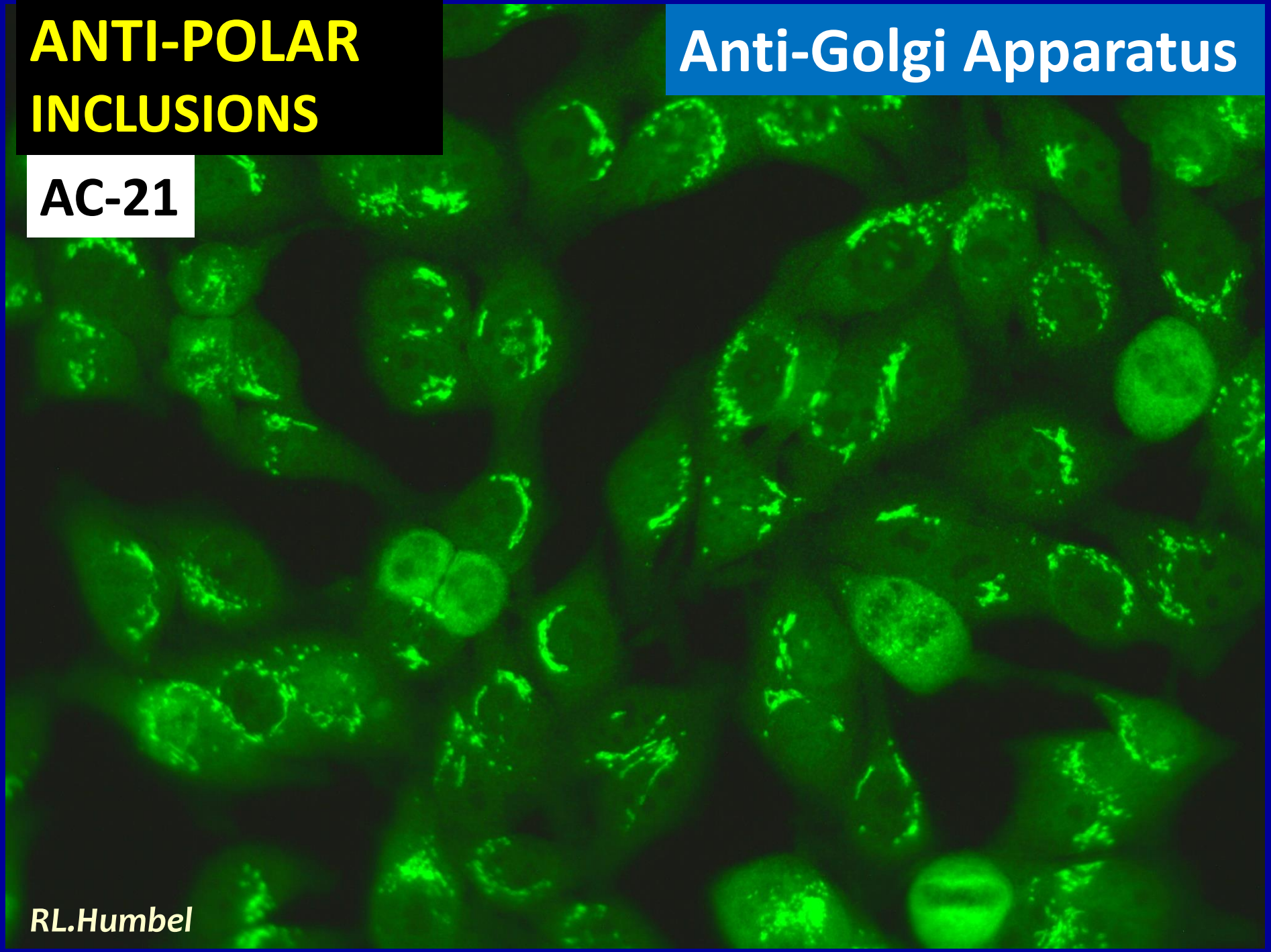



ANTI-HMGCR
RL.Humbel

ANTI-POLAR INCLUSIONS

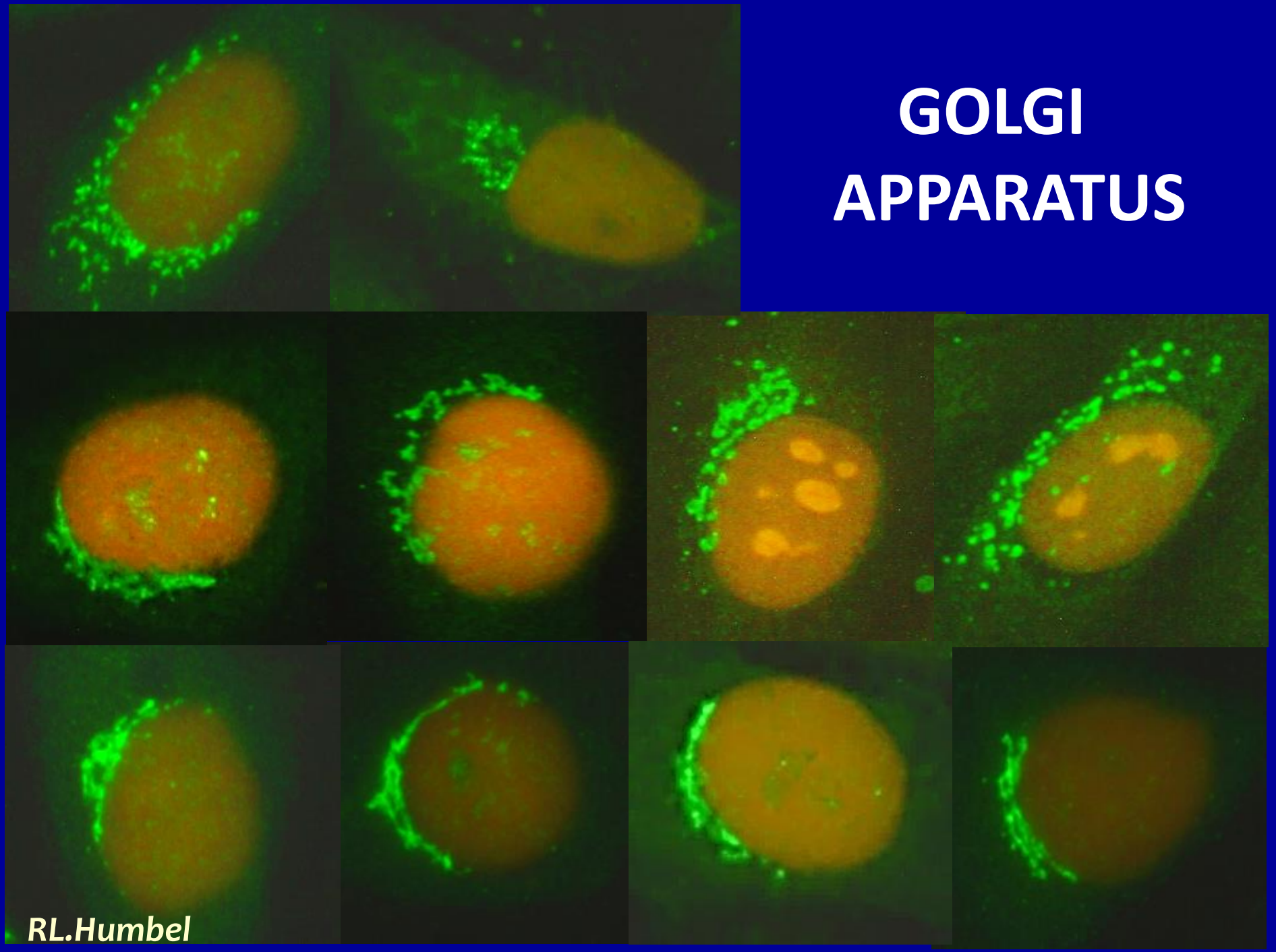
AC-21

Anti-Golgi Apparatus

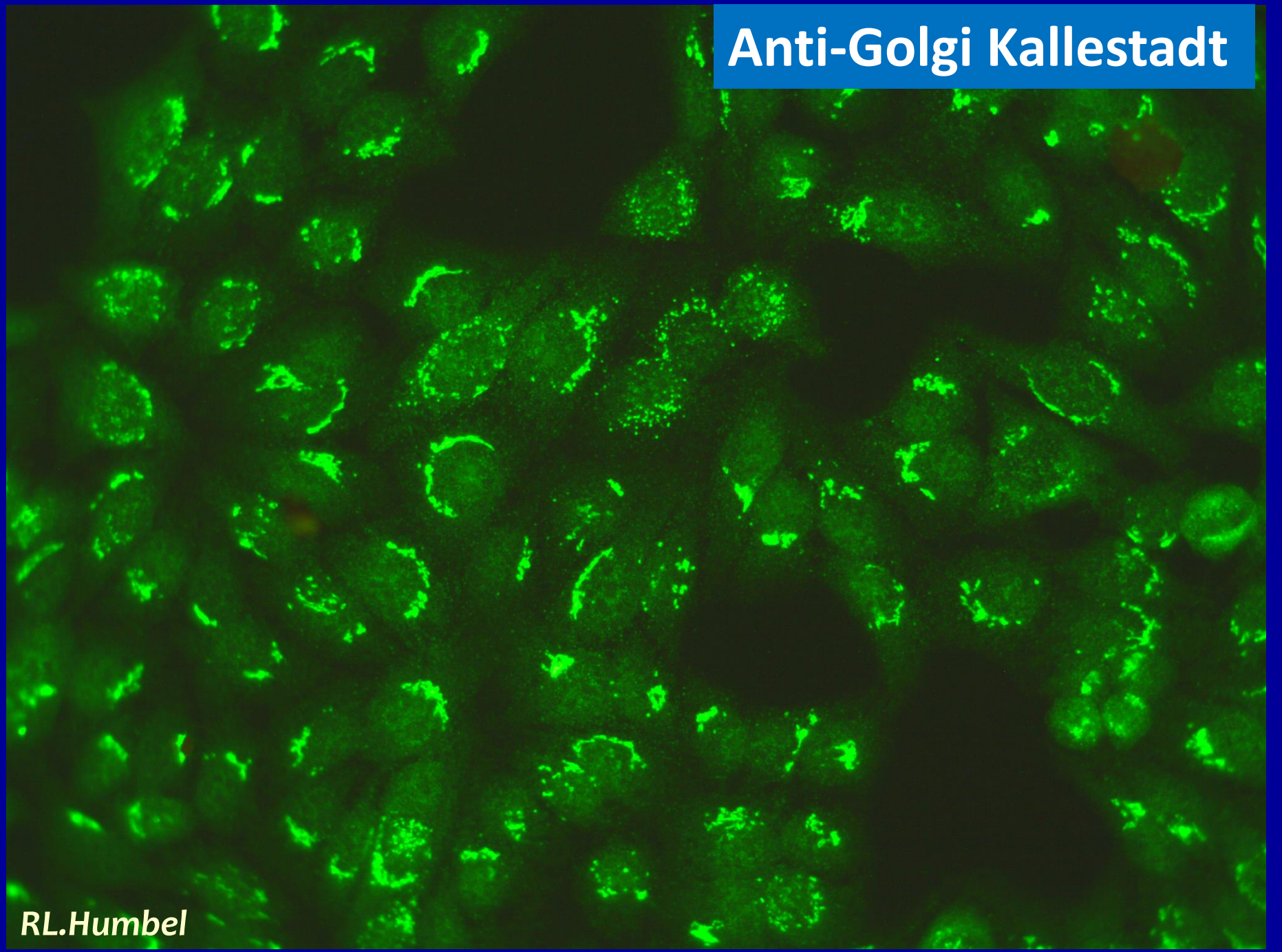


RL.Humbel

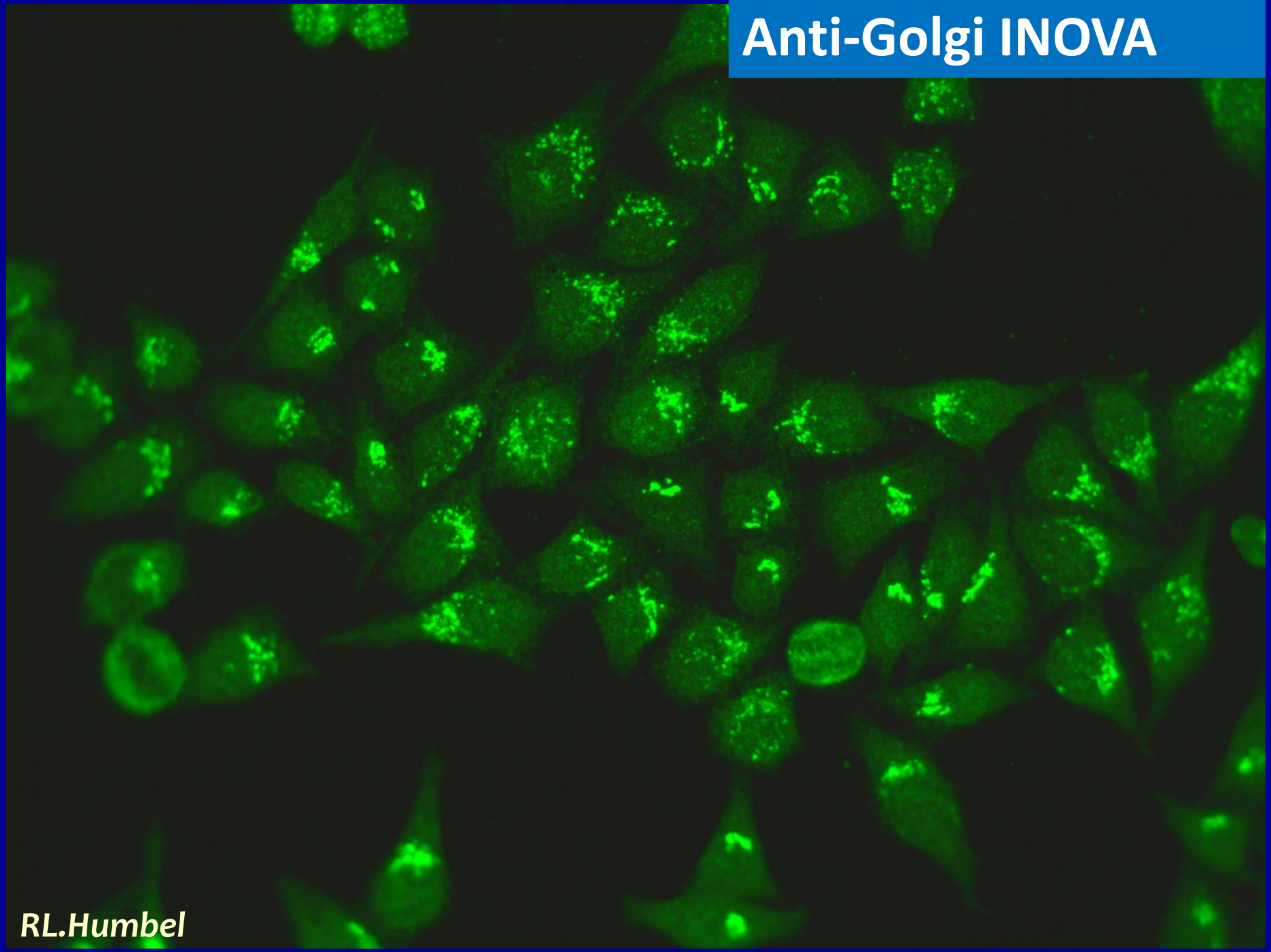
GOLGI APPARATUS



RL.Humbel


Anti-Golgi Kallestadt
RL.Humbel

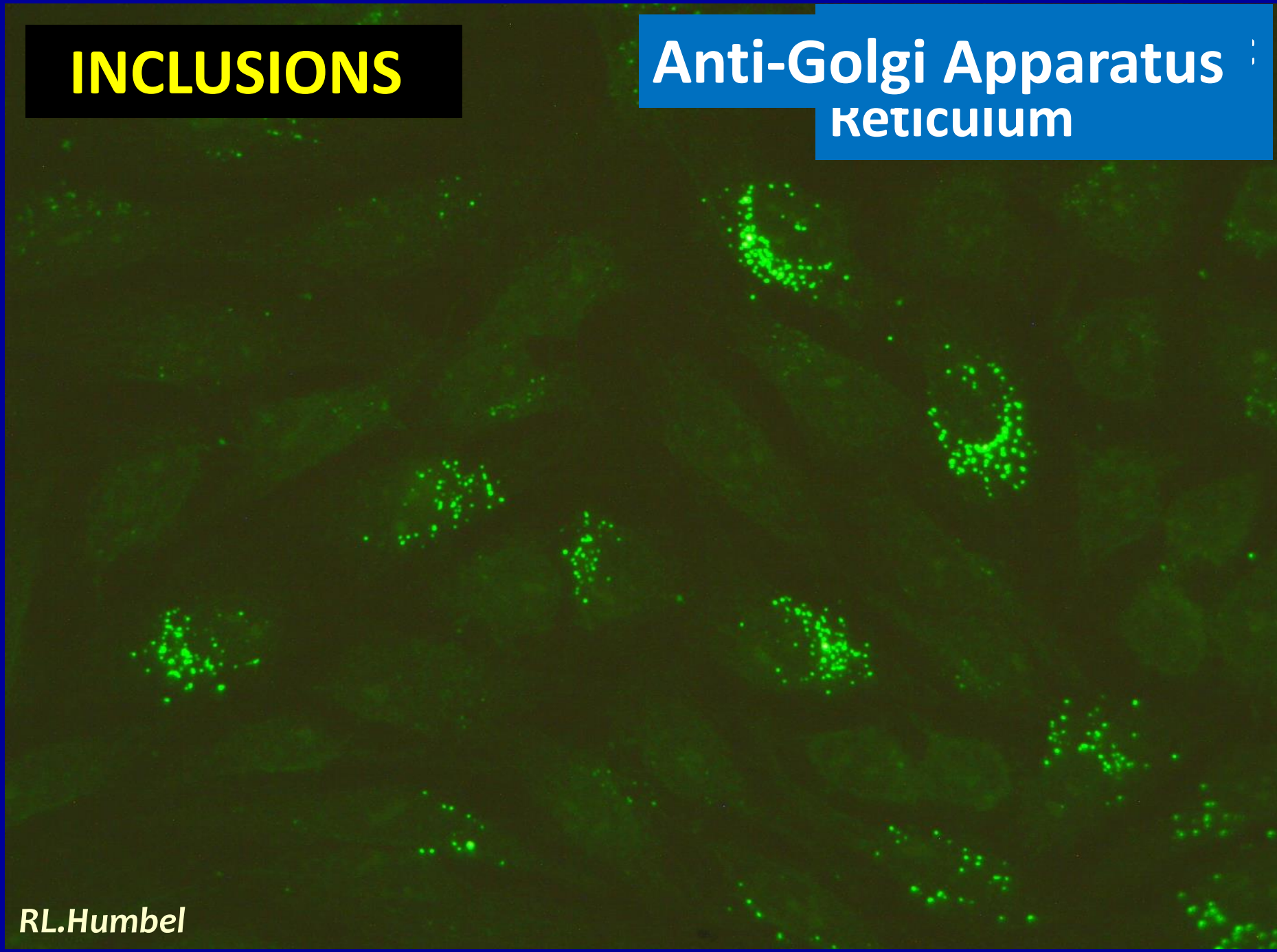
Anti-Golgi INOVA



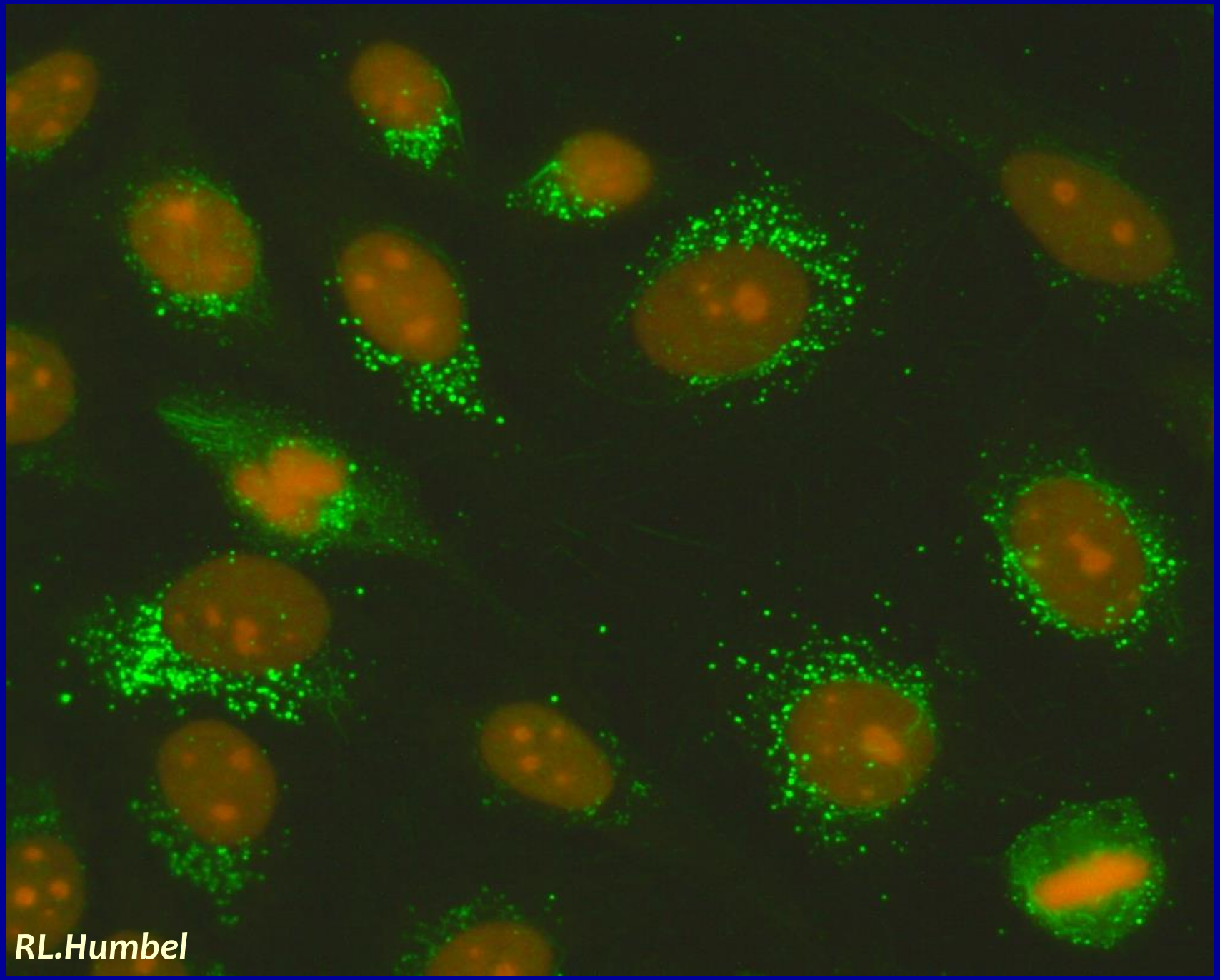
RL.Humbel

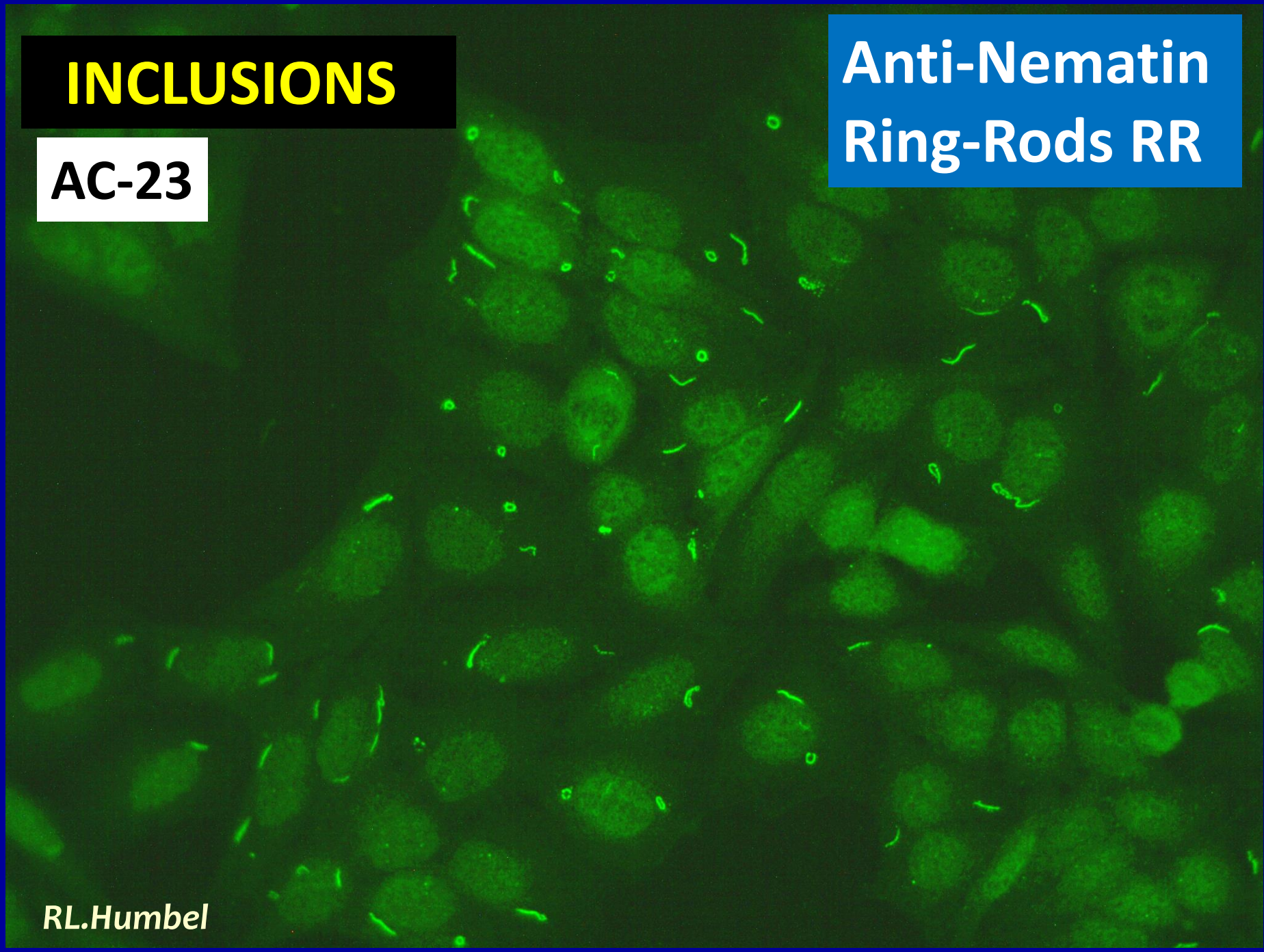
INCLUSIONS

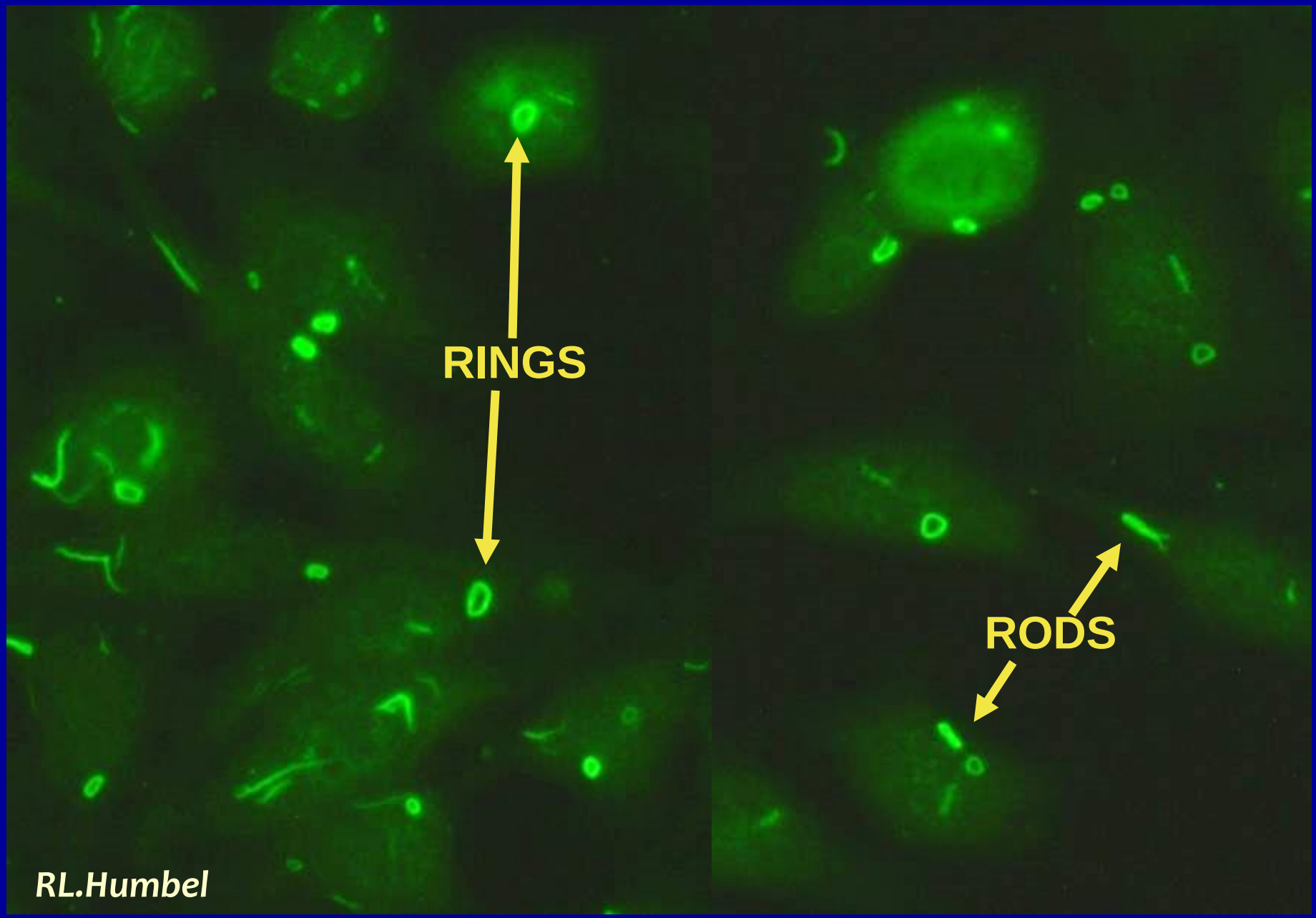
Anti-Golgi Apparatus Reticulum

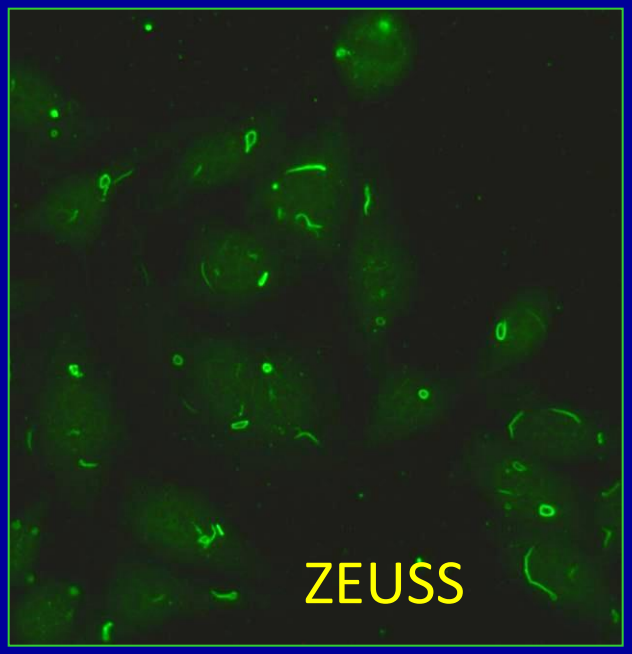


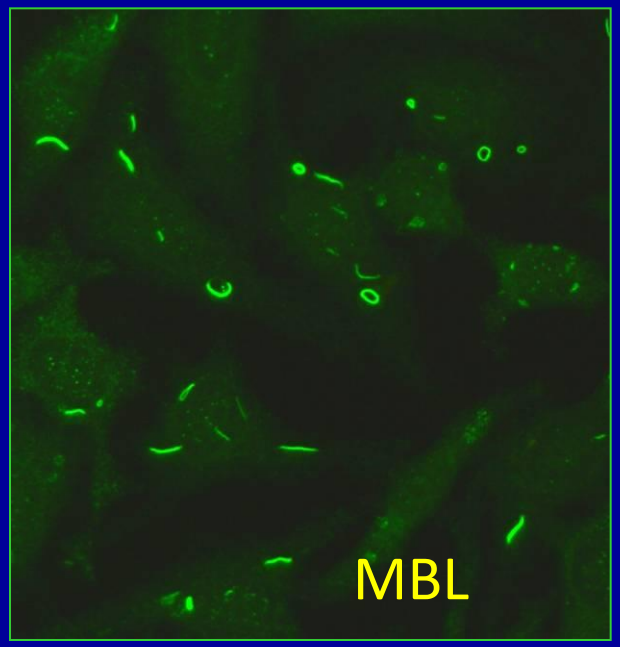
RL.Humbel

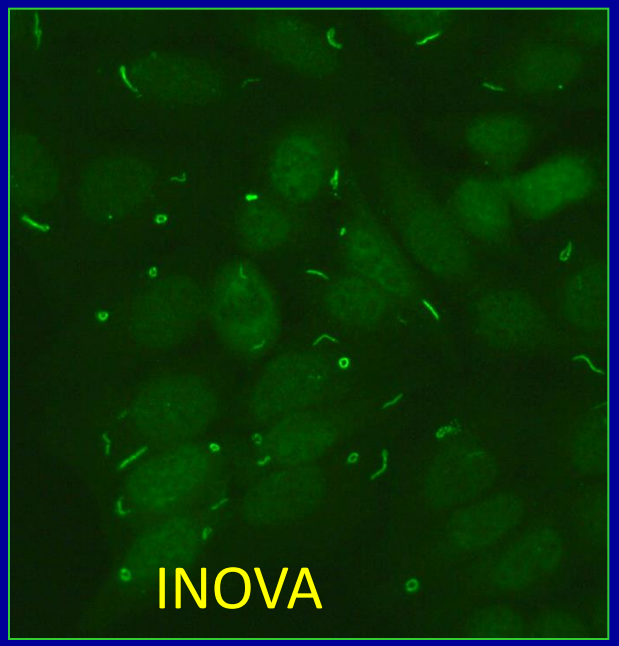

RL.Humbel

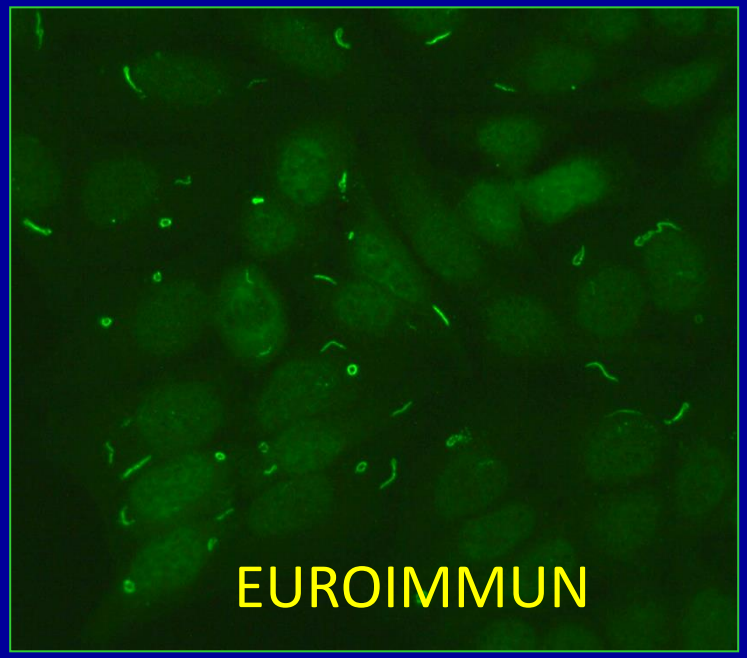

INCLUSIONS
AC-23
Anti-Nematin
Ring-Rods RR
RL.Humbel

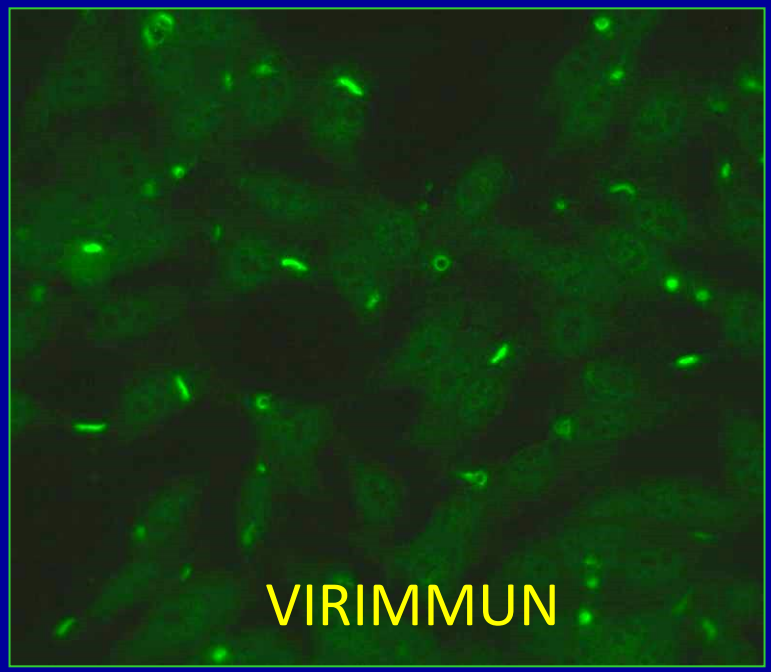

RINGS
RODS
RL.Humbel



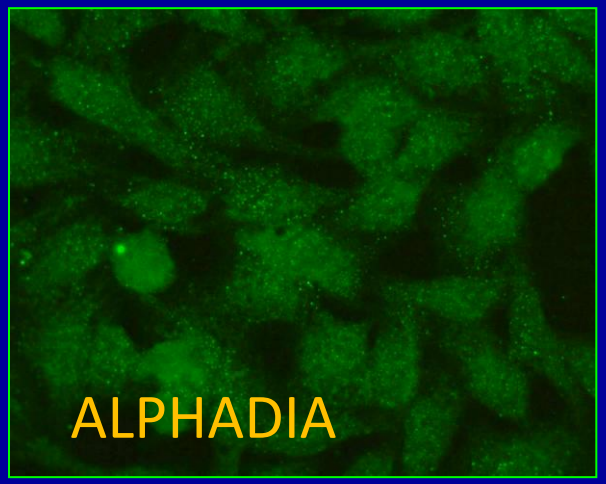




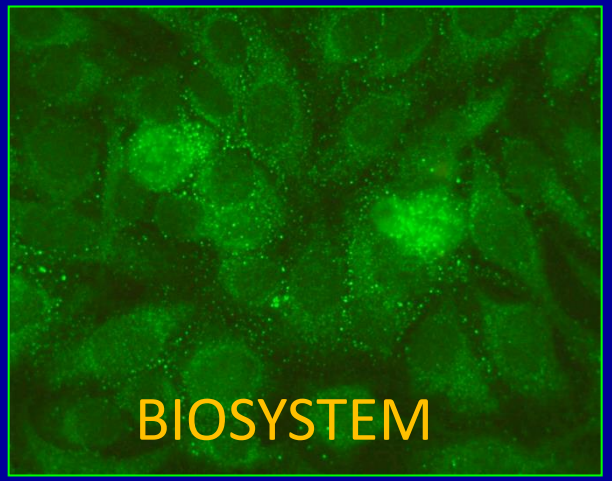




RL.Humbel


ALPHADIA


THE BINDING SITE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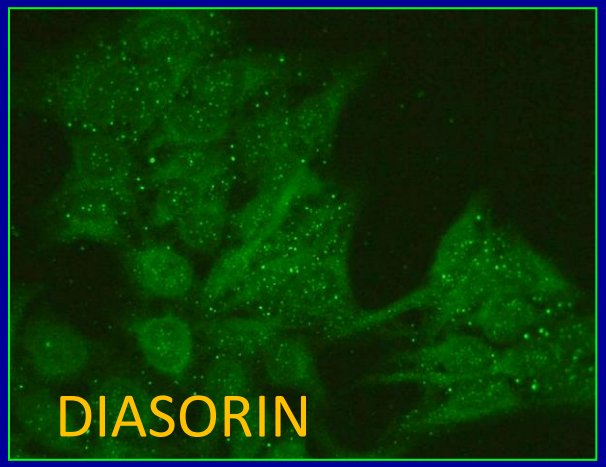
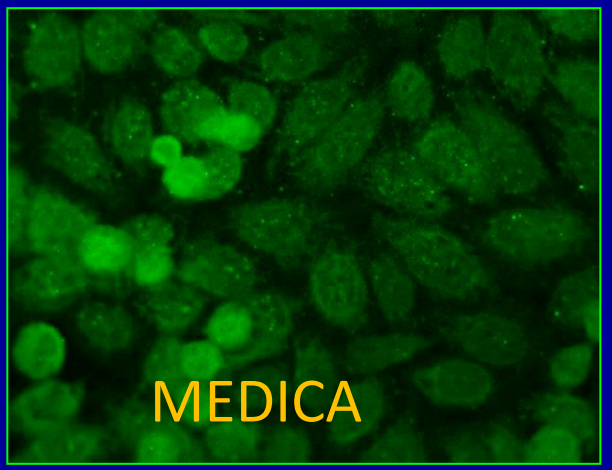
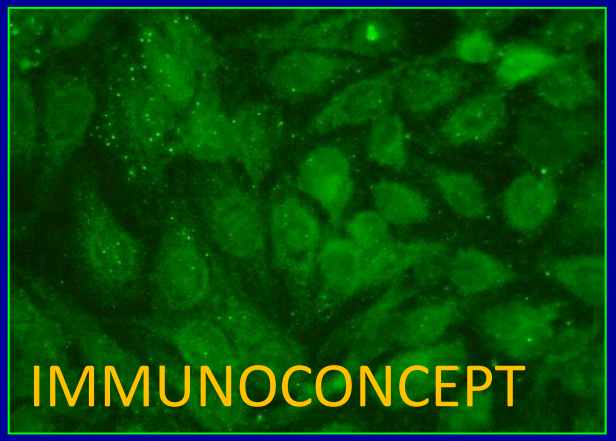
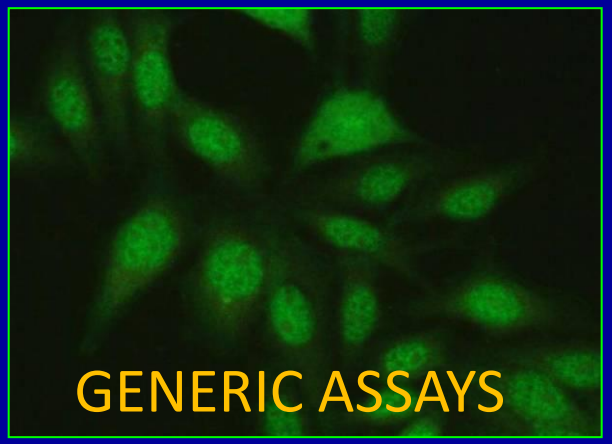

DIASORIN


KALLESTAD

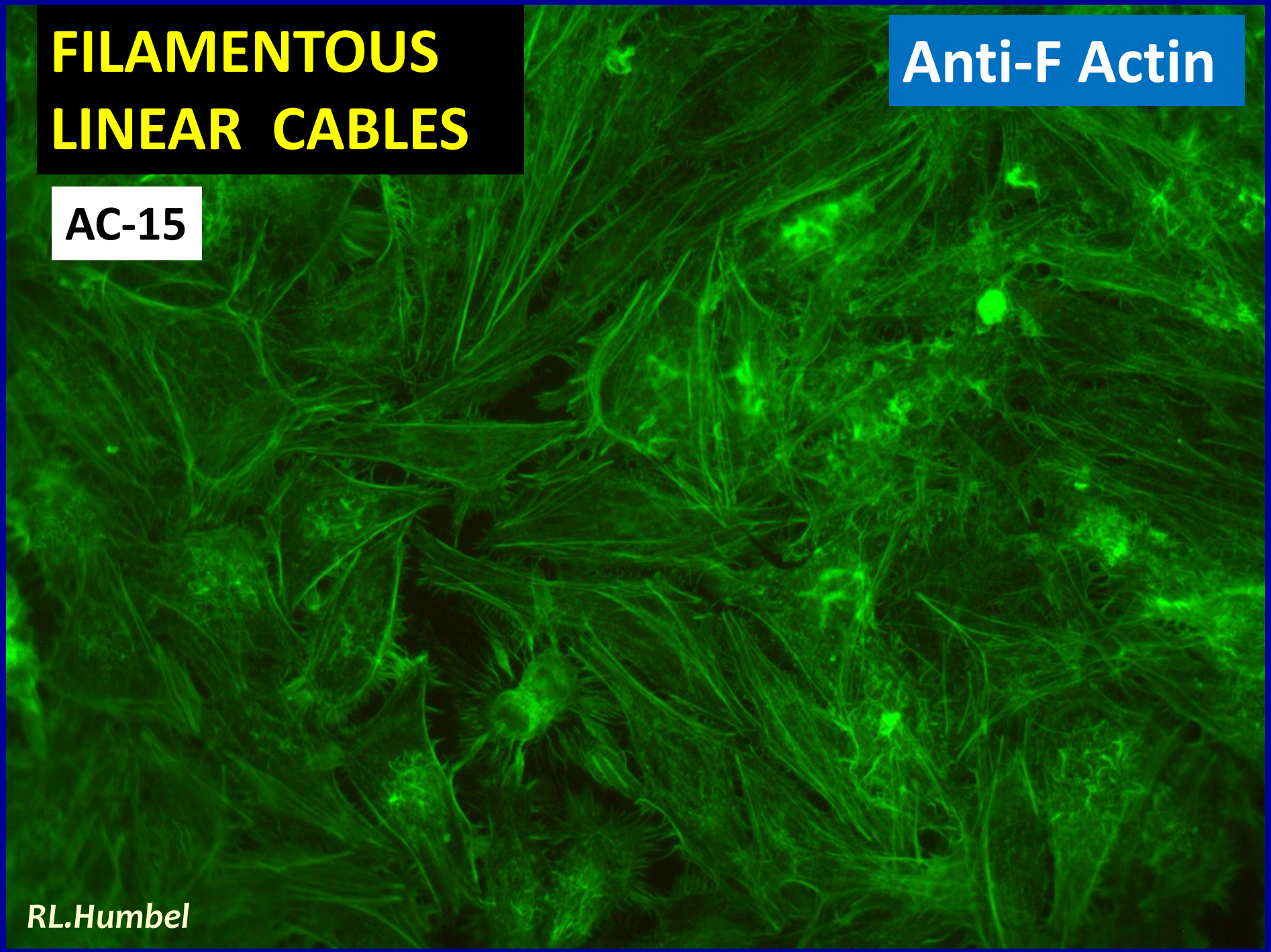

MEDICA

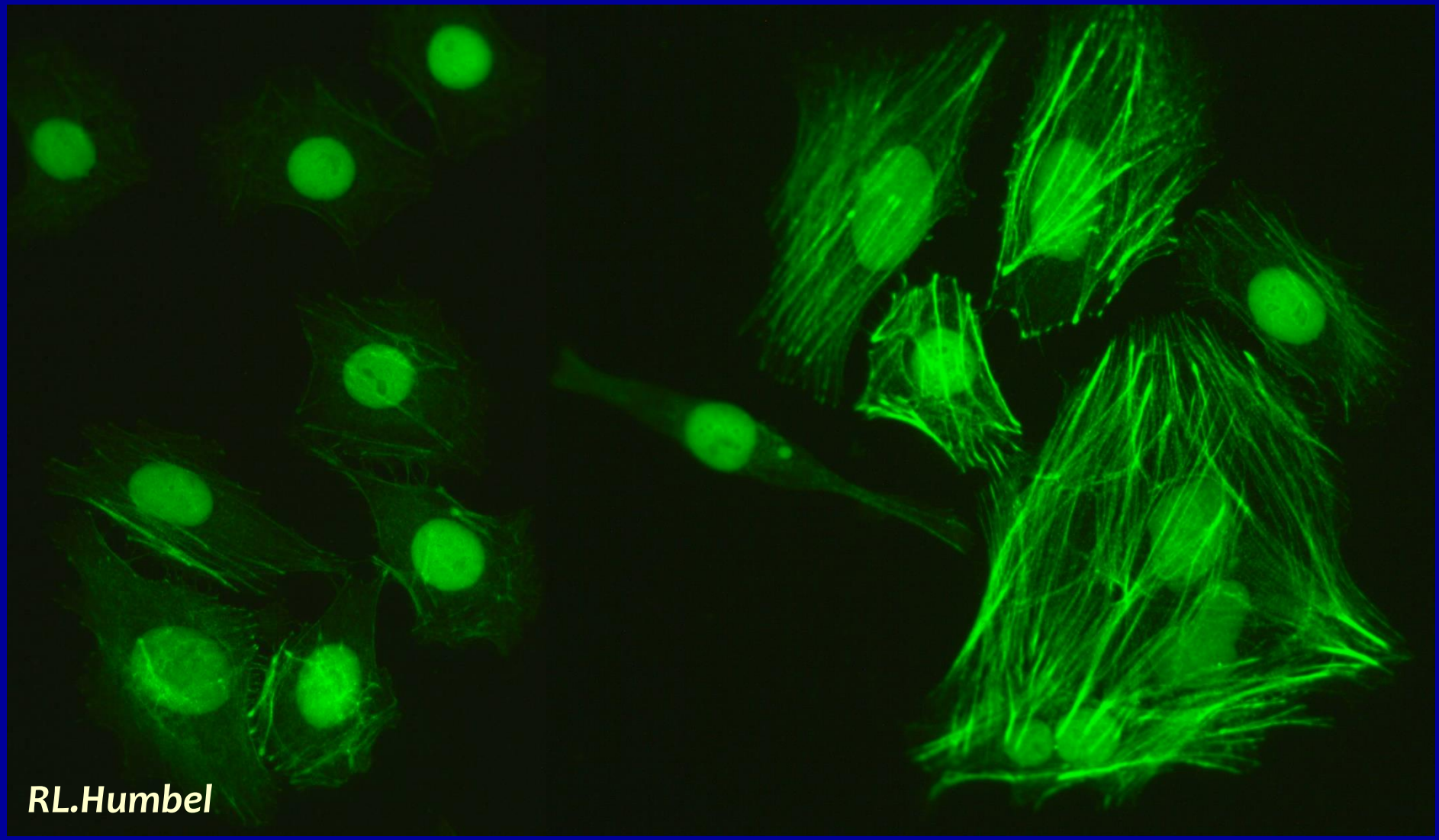

MENARINI


IMMUNOCONCEPT


GENERIC ASSAYS

RL.Humbel

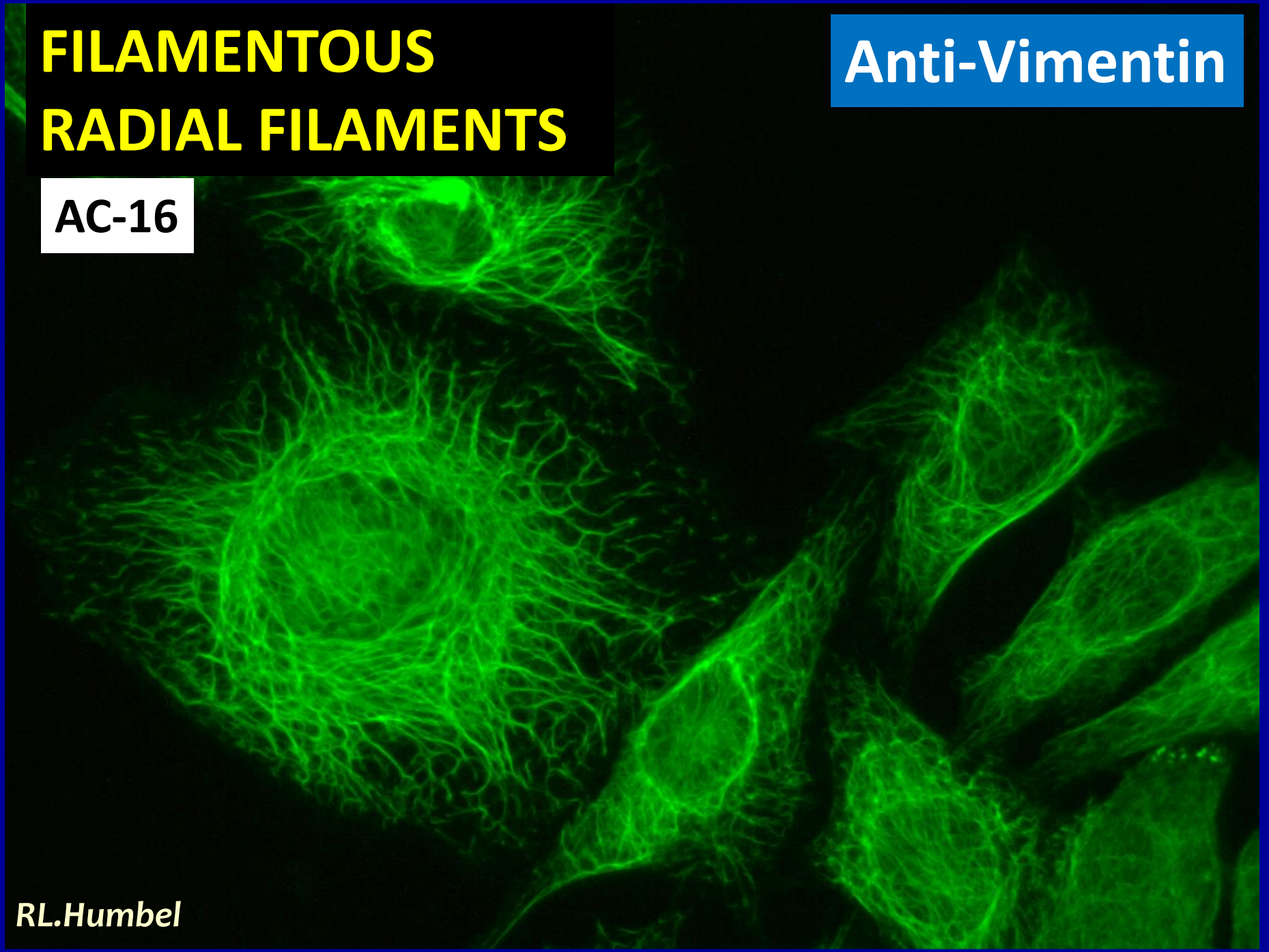

FILAMENTOUS
LINEAR CABLES
AC-15
Anti-F Actin
RL.Humbel


RL.Humbel

FILAMENTOUS RADIAL FILAMENTS

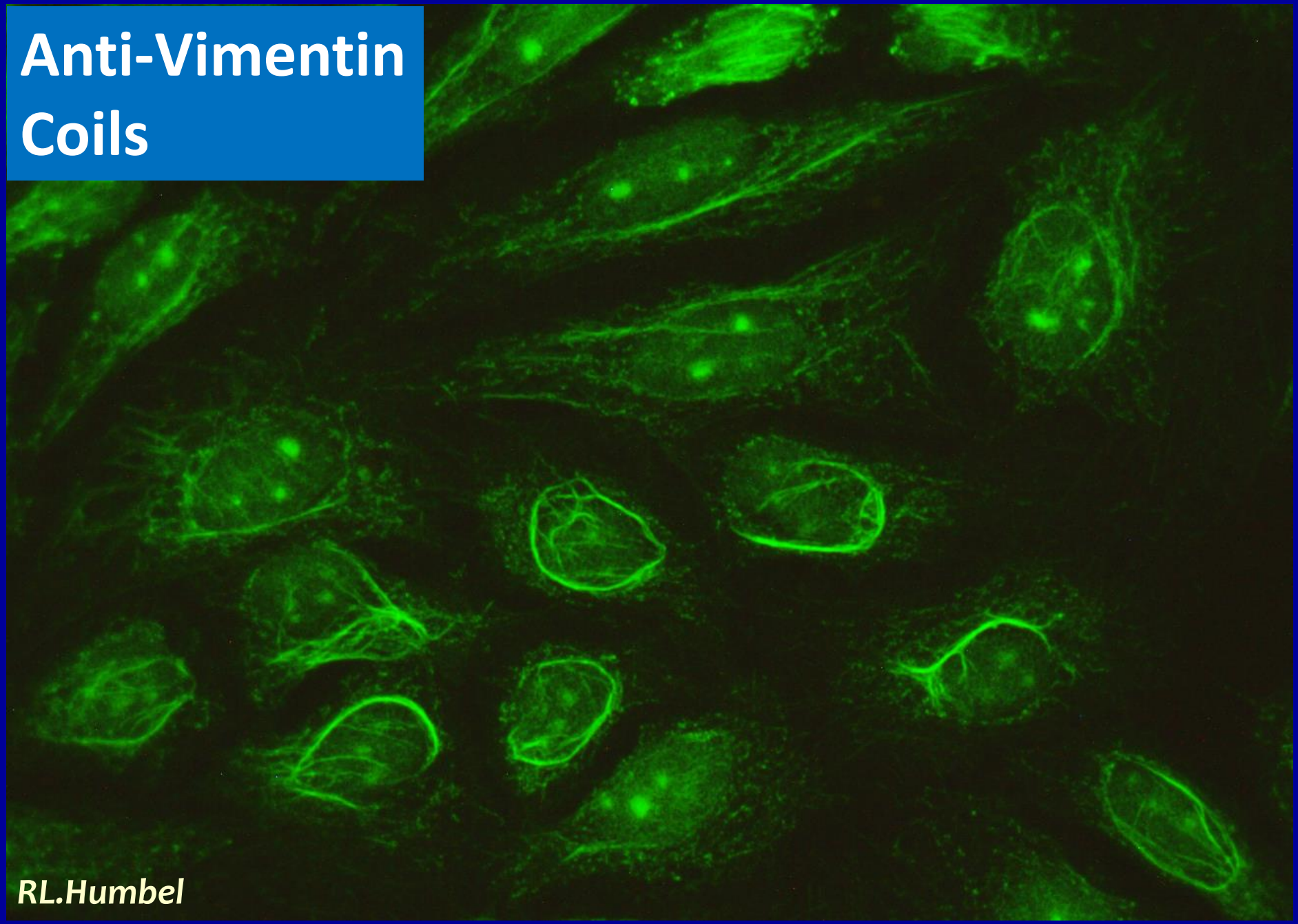
AC-16

Anti-Vimentin

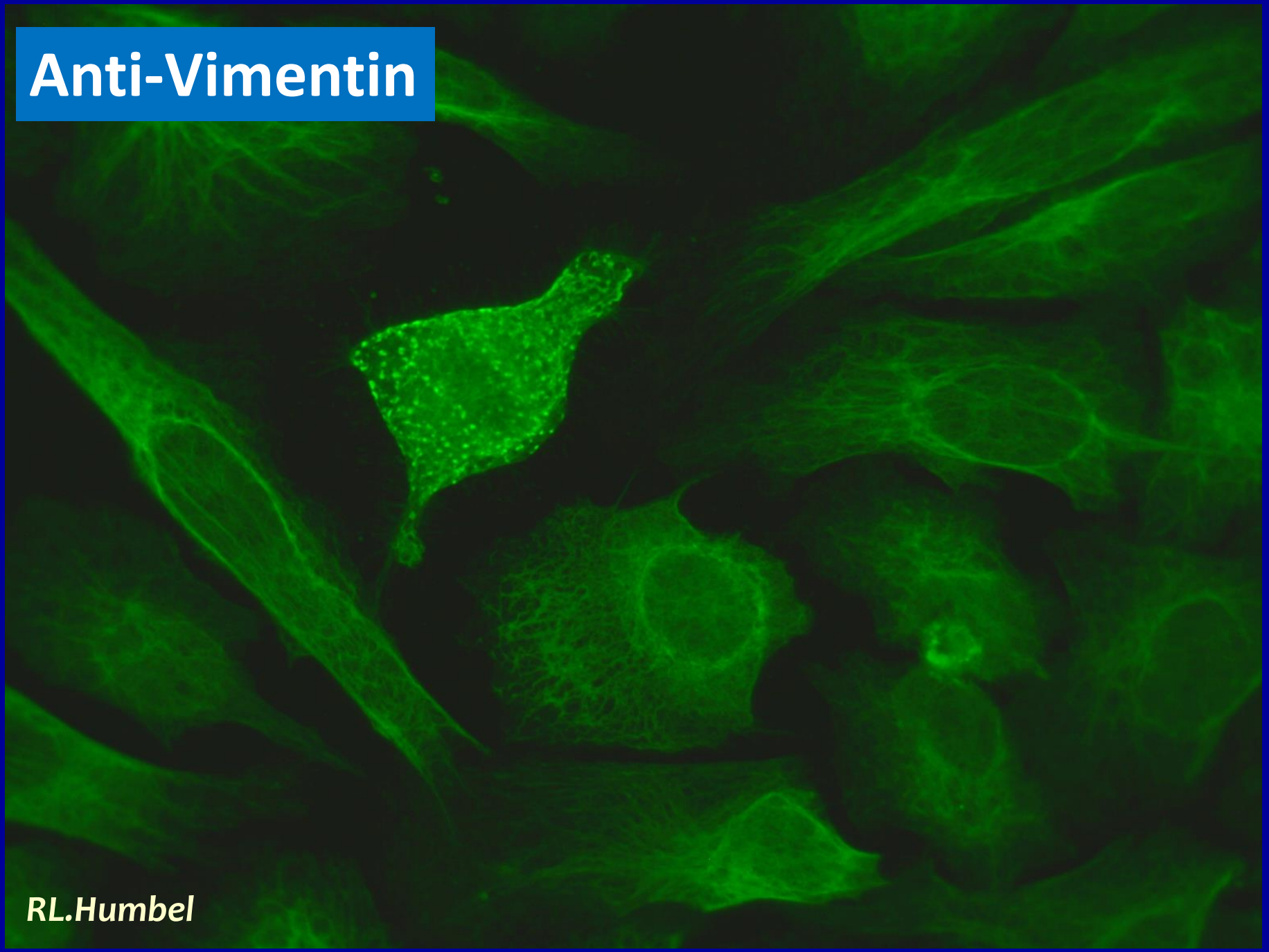


RL.Humbel

Anti-Vimentin Coils

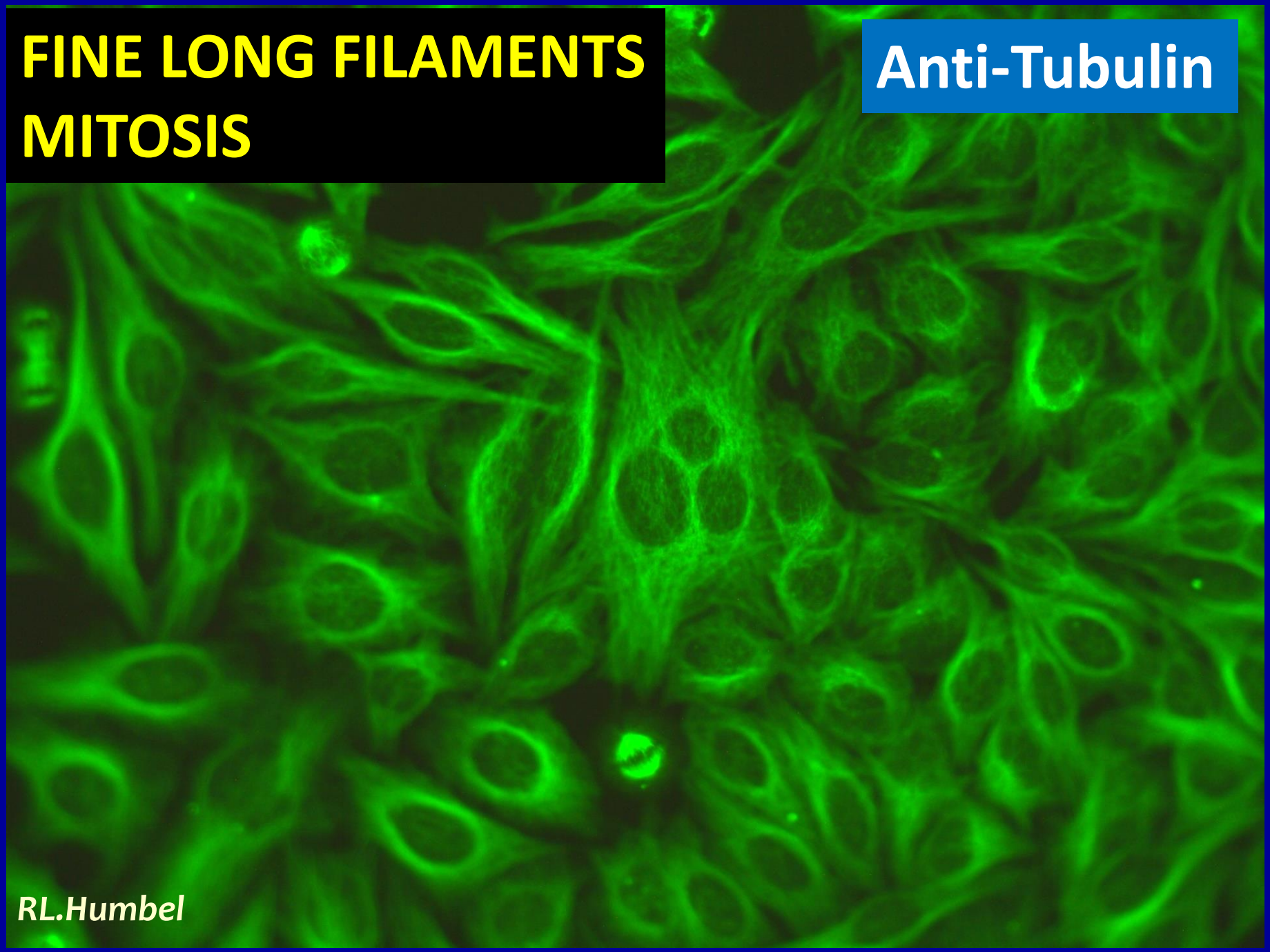


Anti-Vimentin



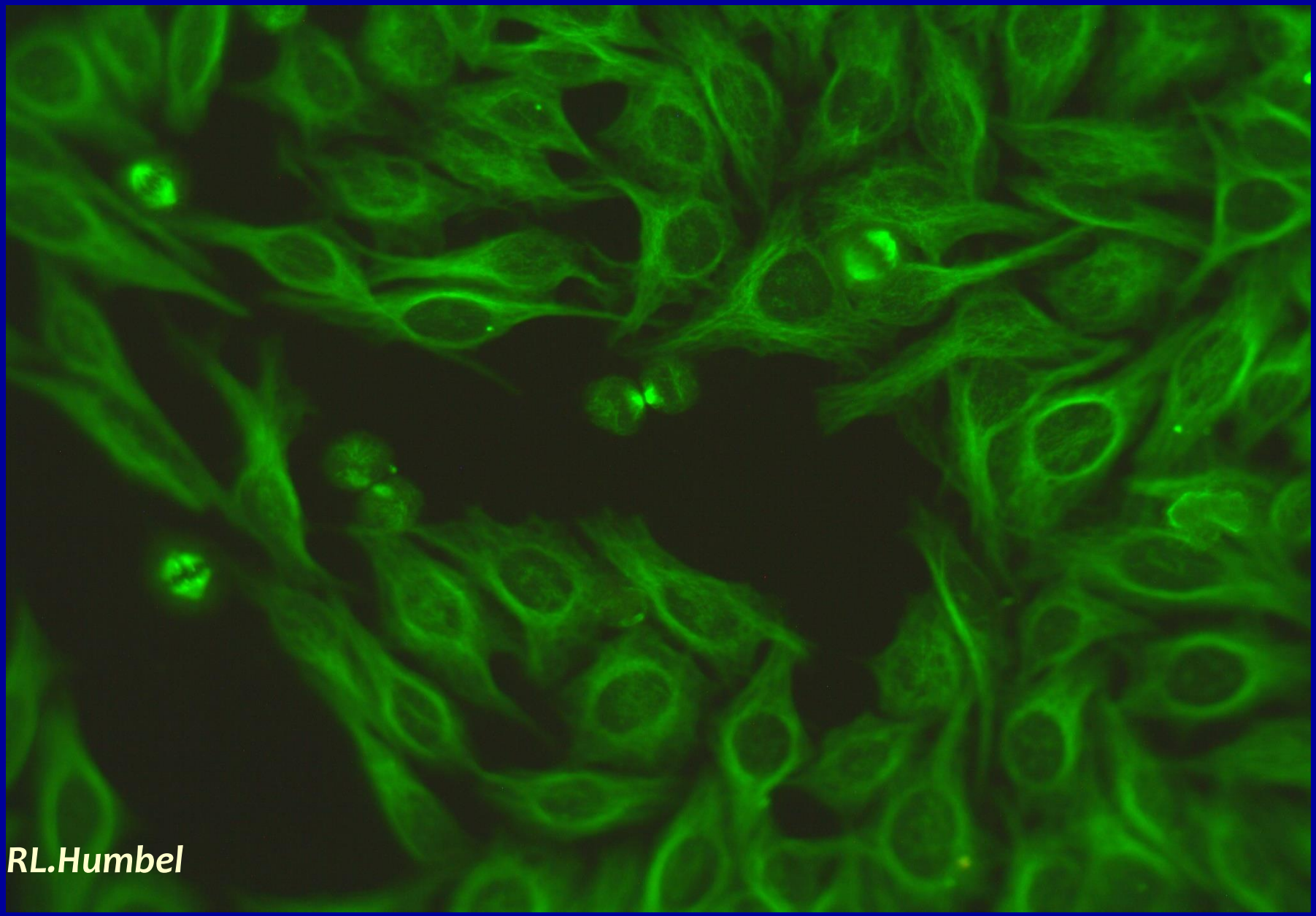
RL.Humbel

FINE LONG FILAMENTS MITOSIS

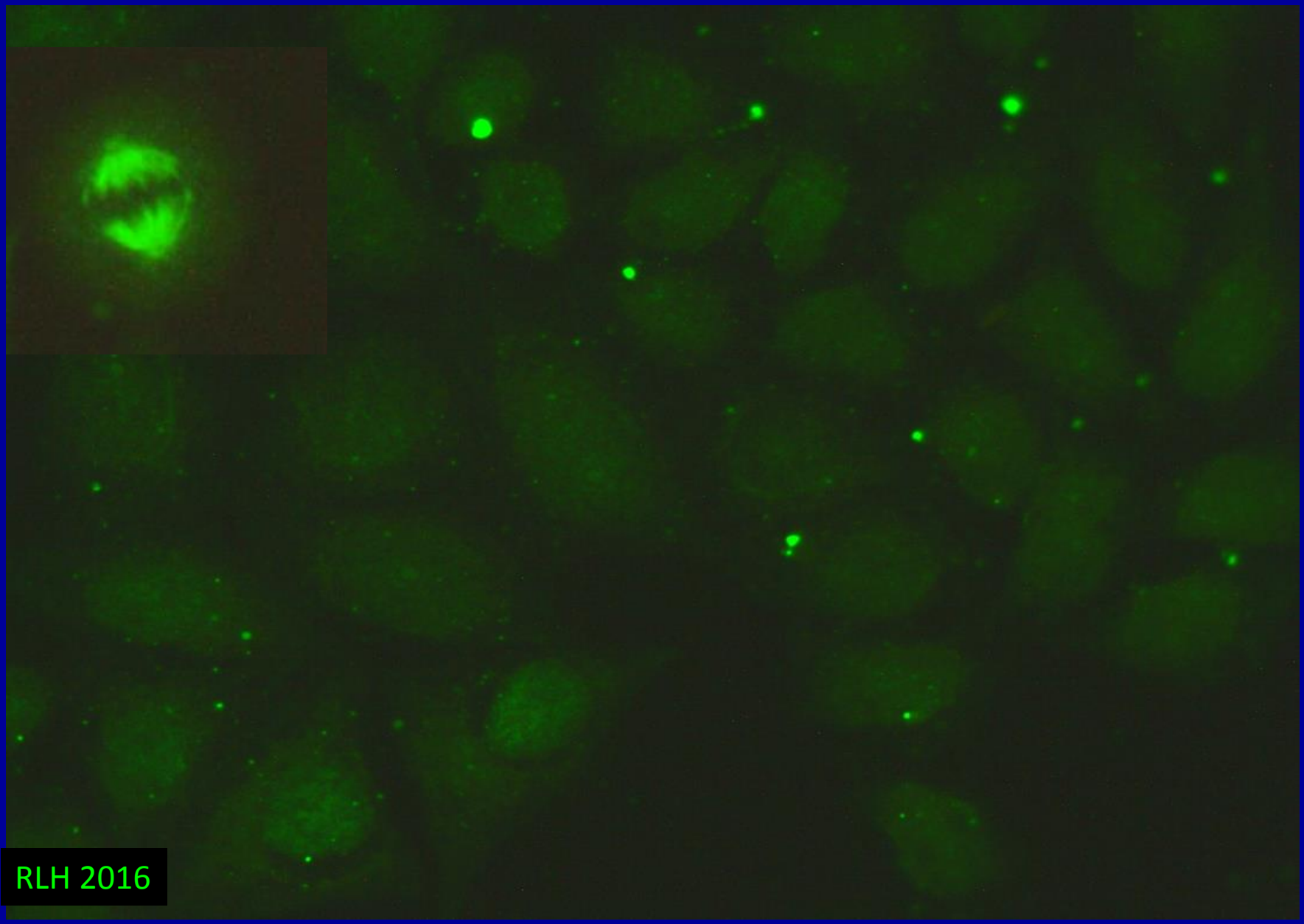


Anti-Tubulin

INOVA



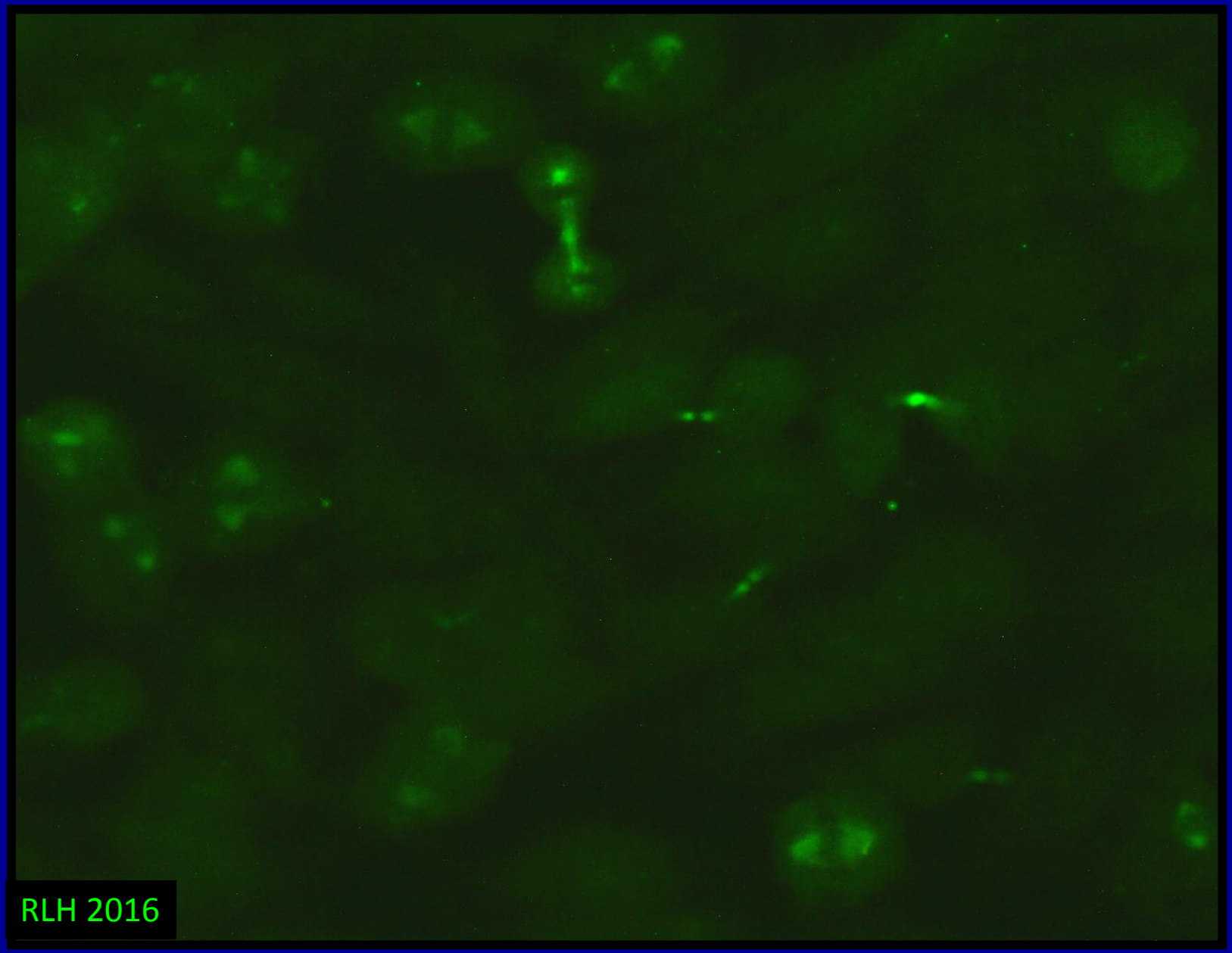
ANTI-TUBULIN *INOVA /ACTIN*



RLH 2016

ANTI-TUBULINE

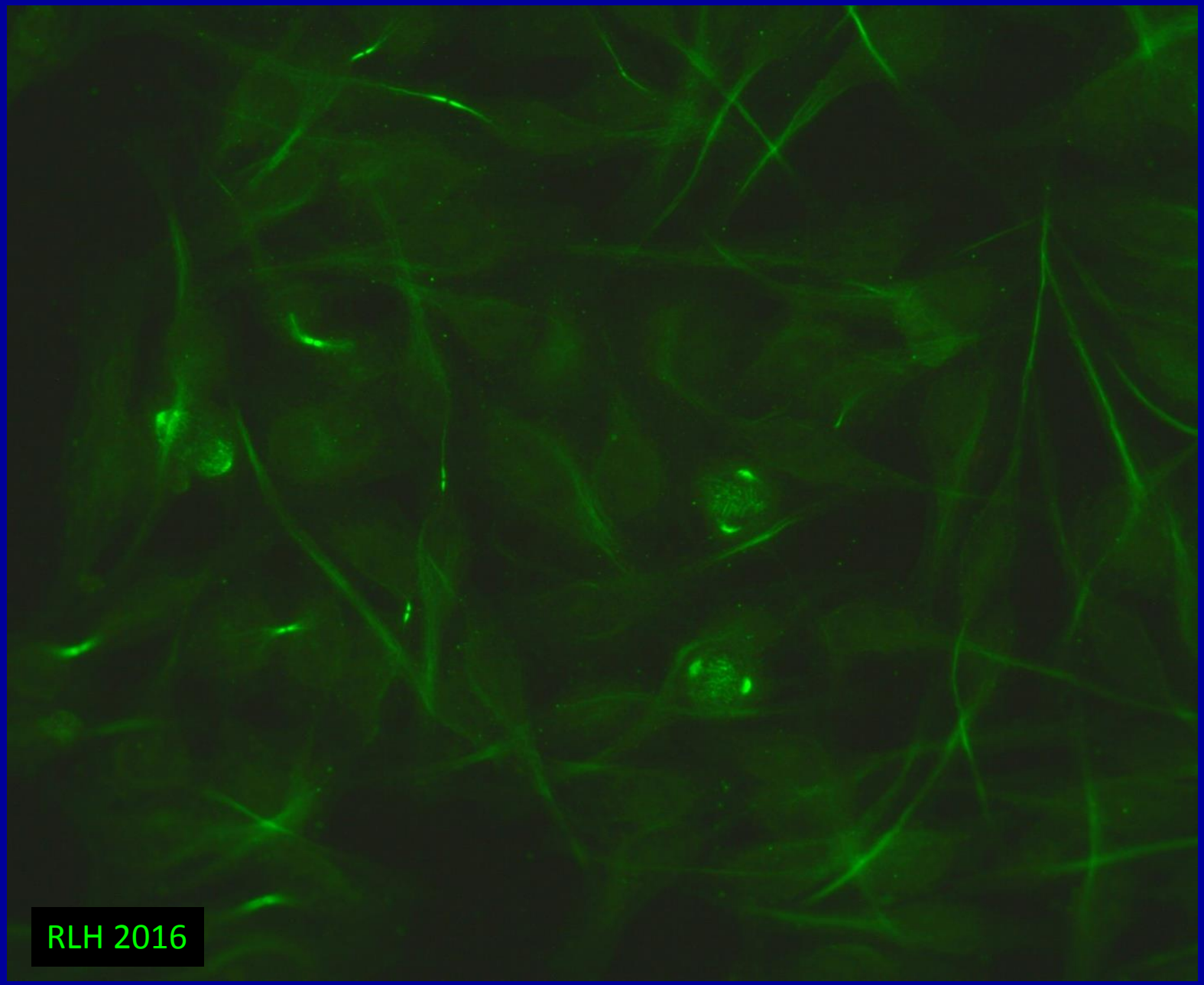
EUROIMMUN 2010



RLH 2016

ANTI-TUBULINE

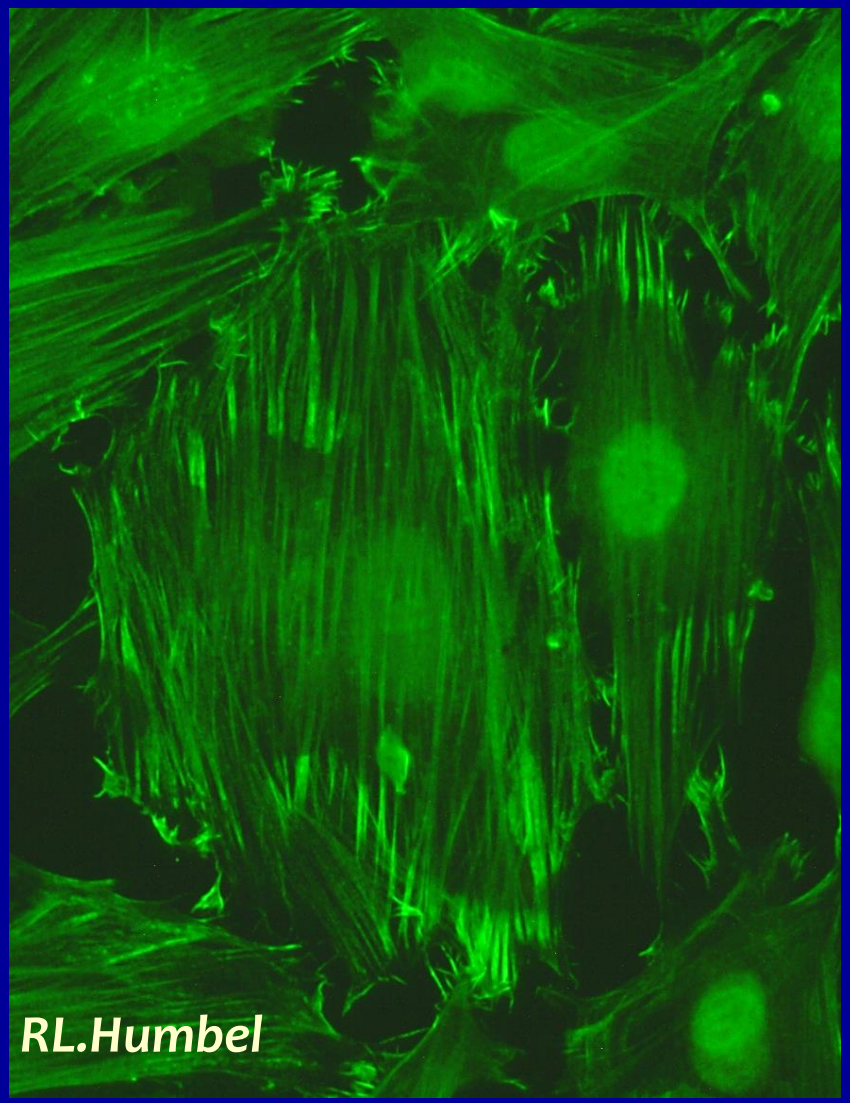
IMMUNOCONCEPT



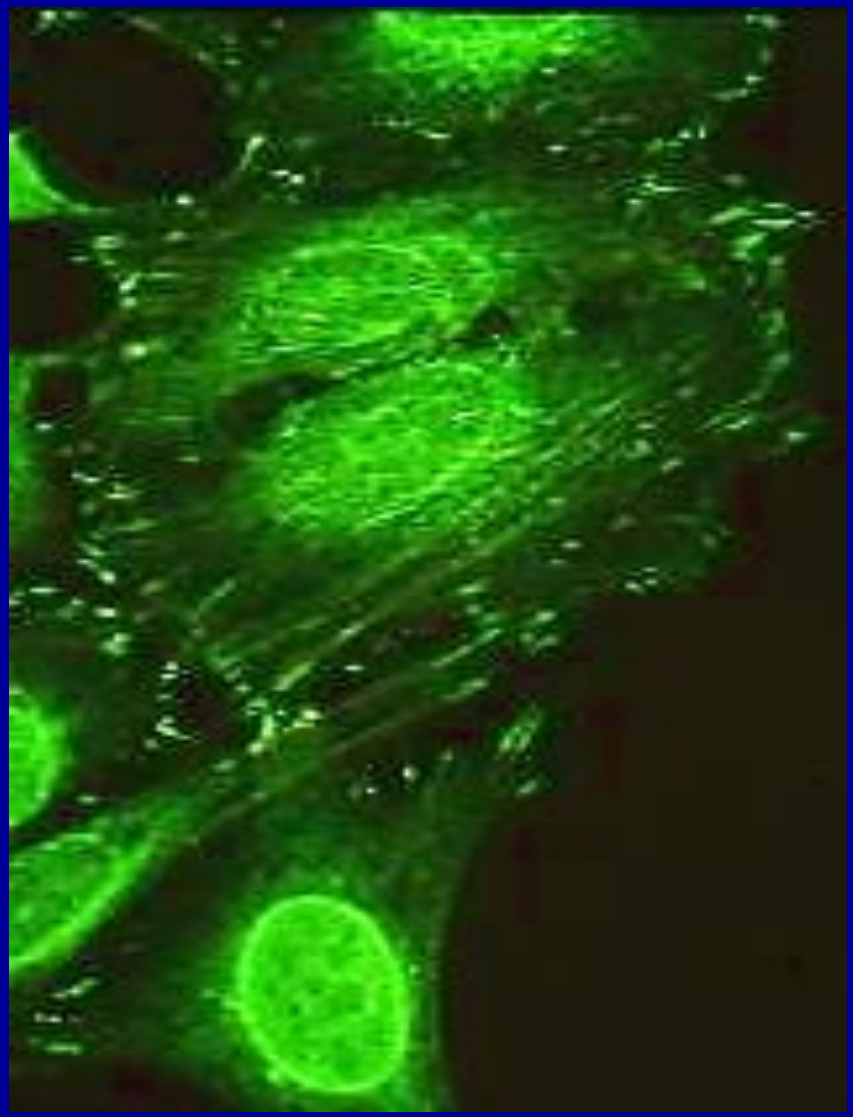
CYTOPLASMIC FIBRILLAR (FILAMENTS)

AC-17

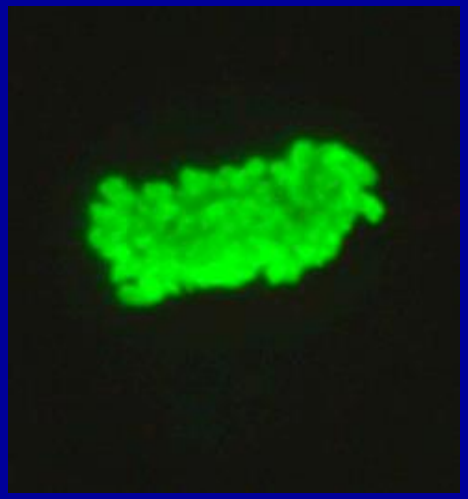
SEGMENTAL

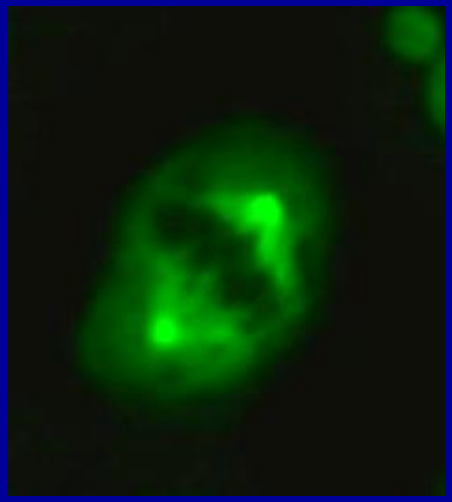


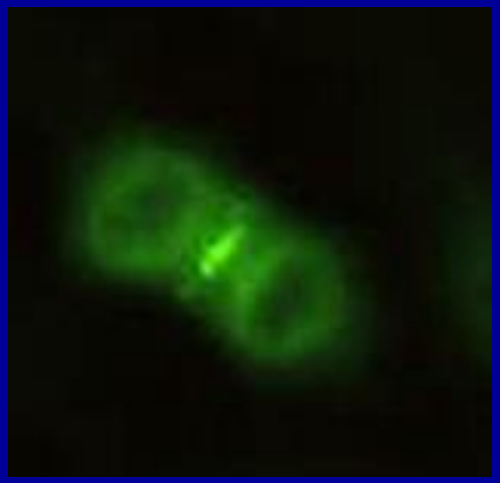
VINCULIN



MITOTIC CELLS







CHROMOSOMES

MITOTIC CHROMOSOME

AC-28

MITOTIC CENTROMERE

CHROMOSOMAL PASSENGER ROTEINS

SPINDLE

CENTROSOME

AC-24

SPINDLE POOL

AC-25 **AC-26**

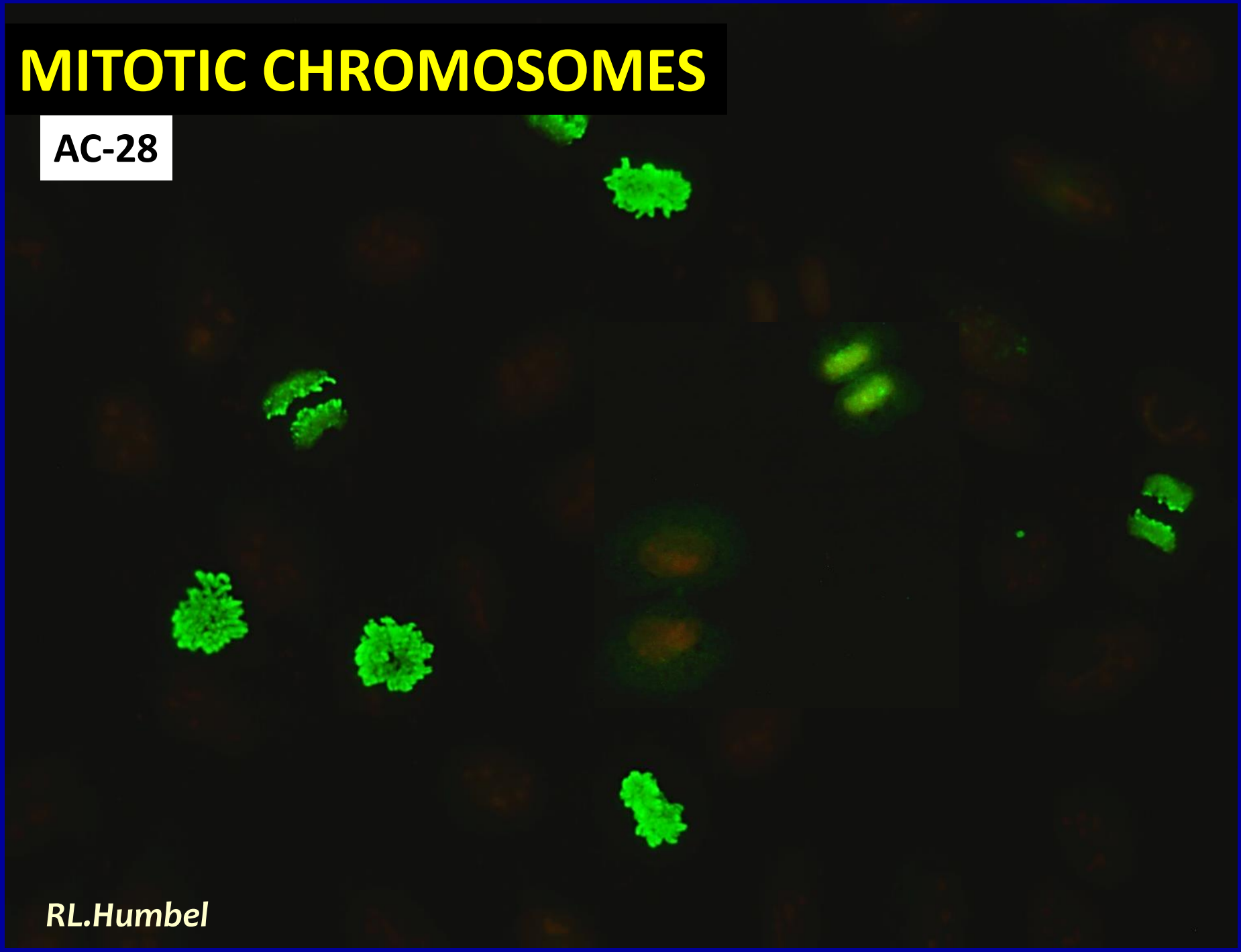
MIDBODY

INTERCELLULAR BRIDGE

AC-27

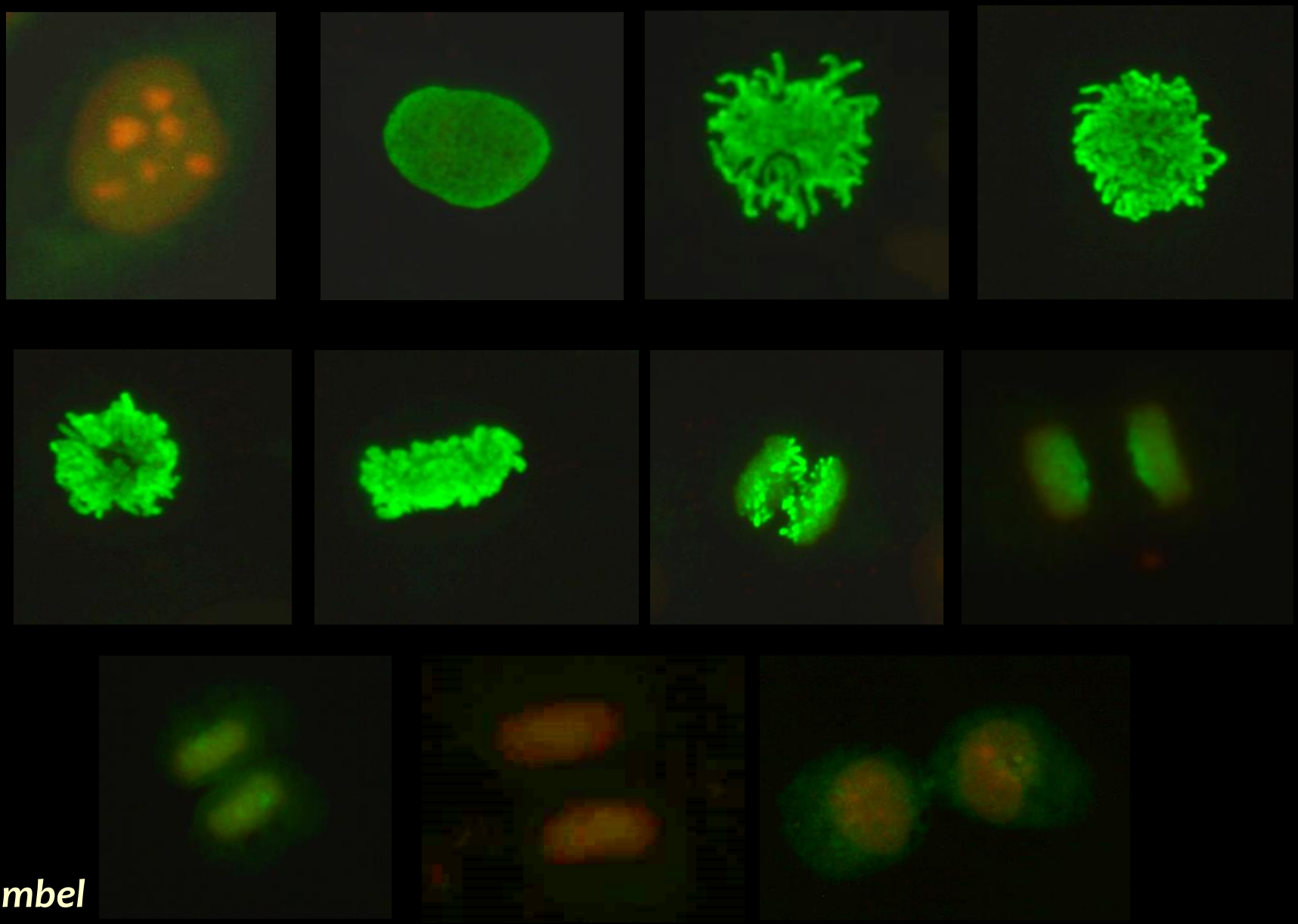
MITOTIC CHROMOSOMES

AC-28

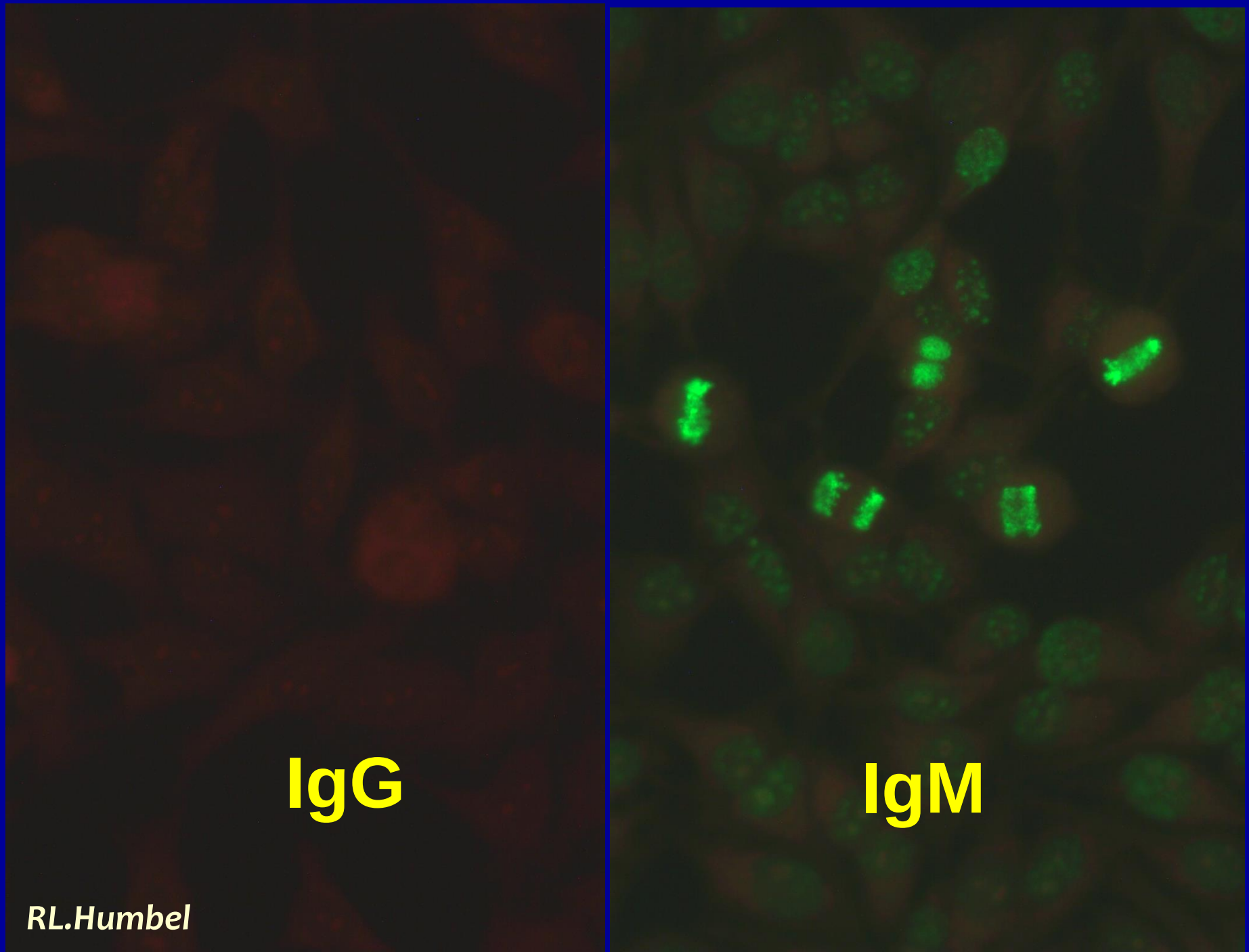


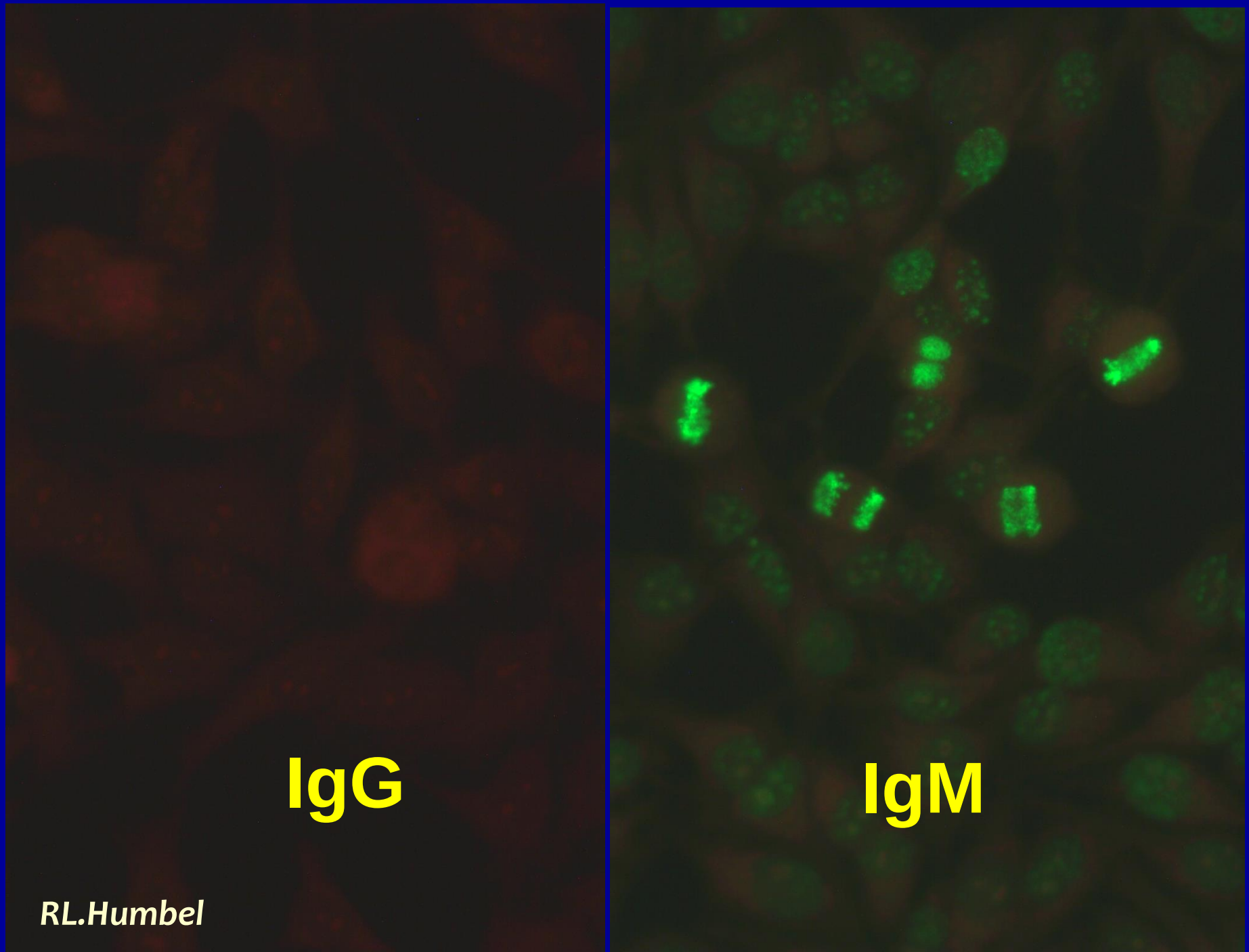
RL.Humbel

ANTIBODIES TO MITOTIC CHROMOSOMES



RL.Humbel

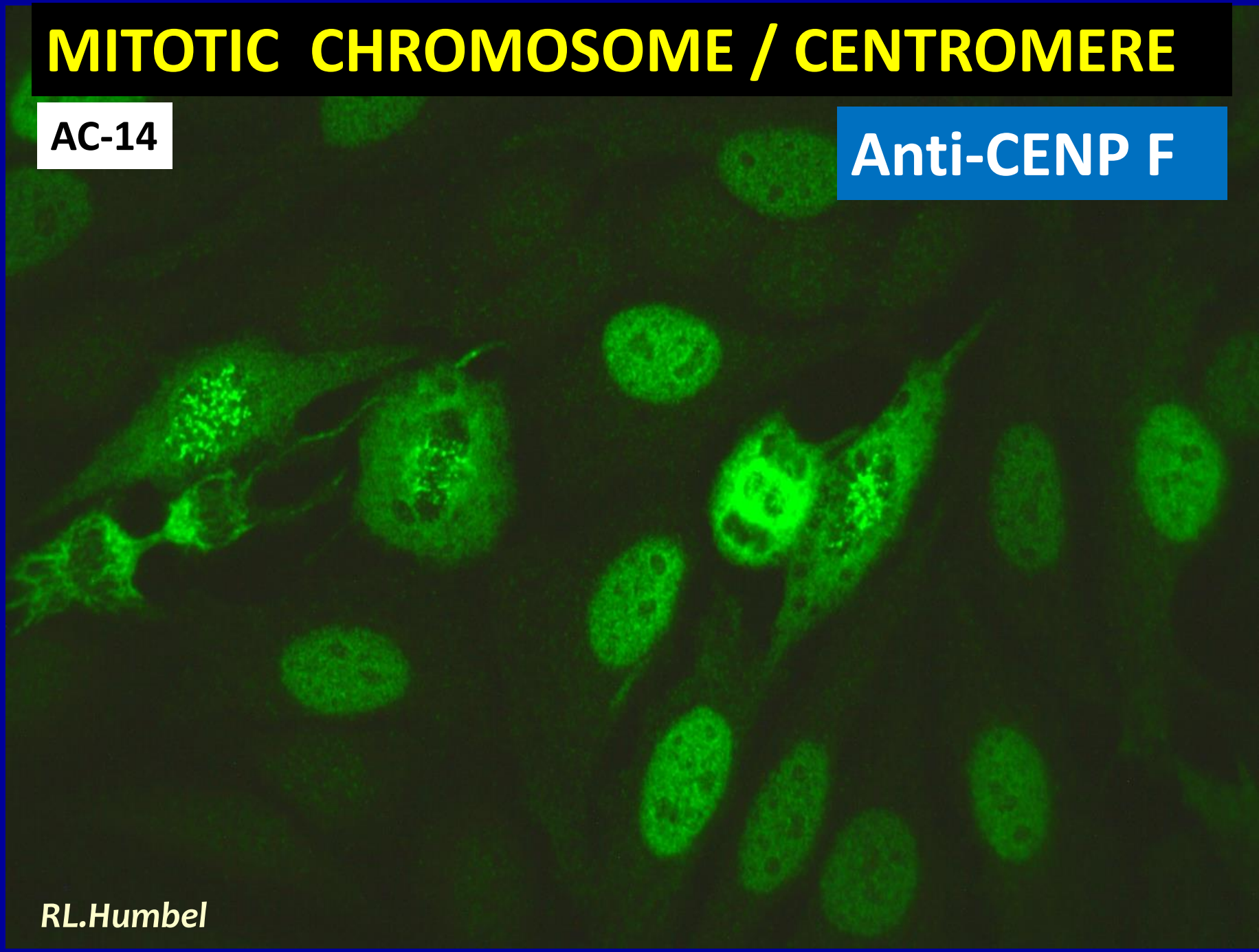

IgG
IgM
RL.Humbel


IgG
IgM
RL.Humbel

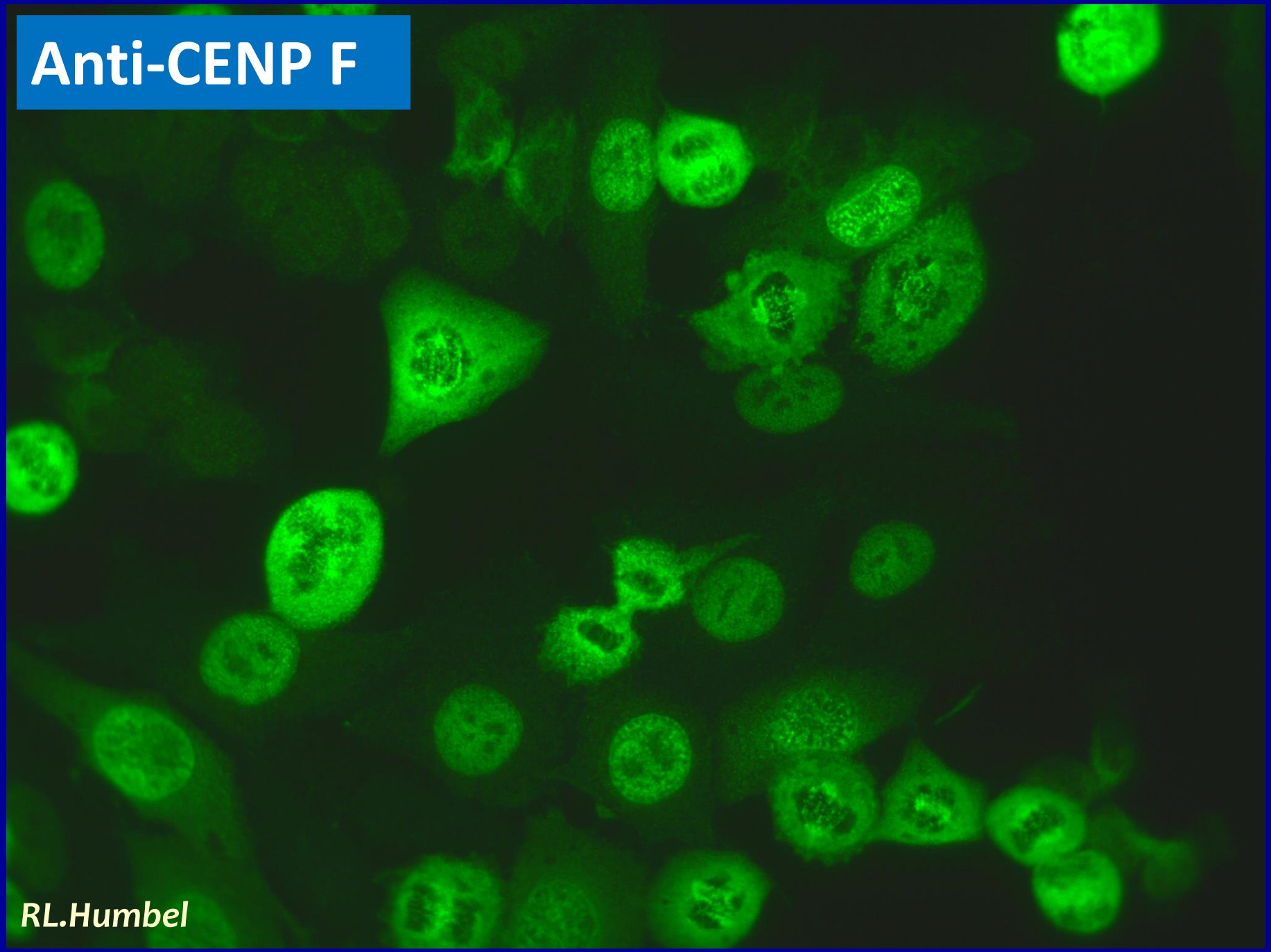
MITOTIC CHROMOSOME / CENTROMERE

AC-14

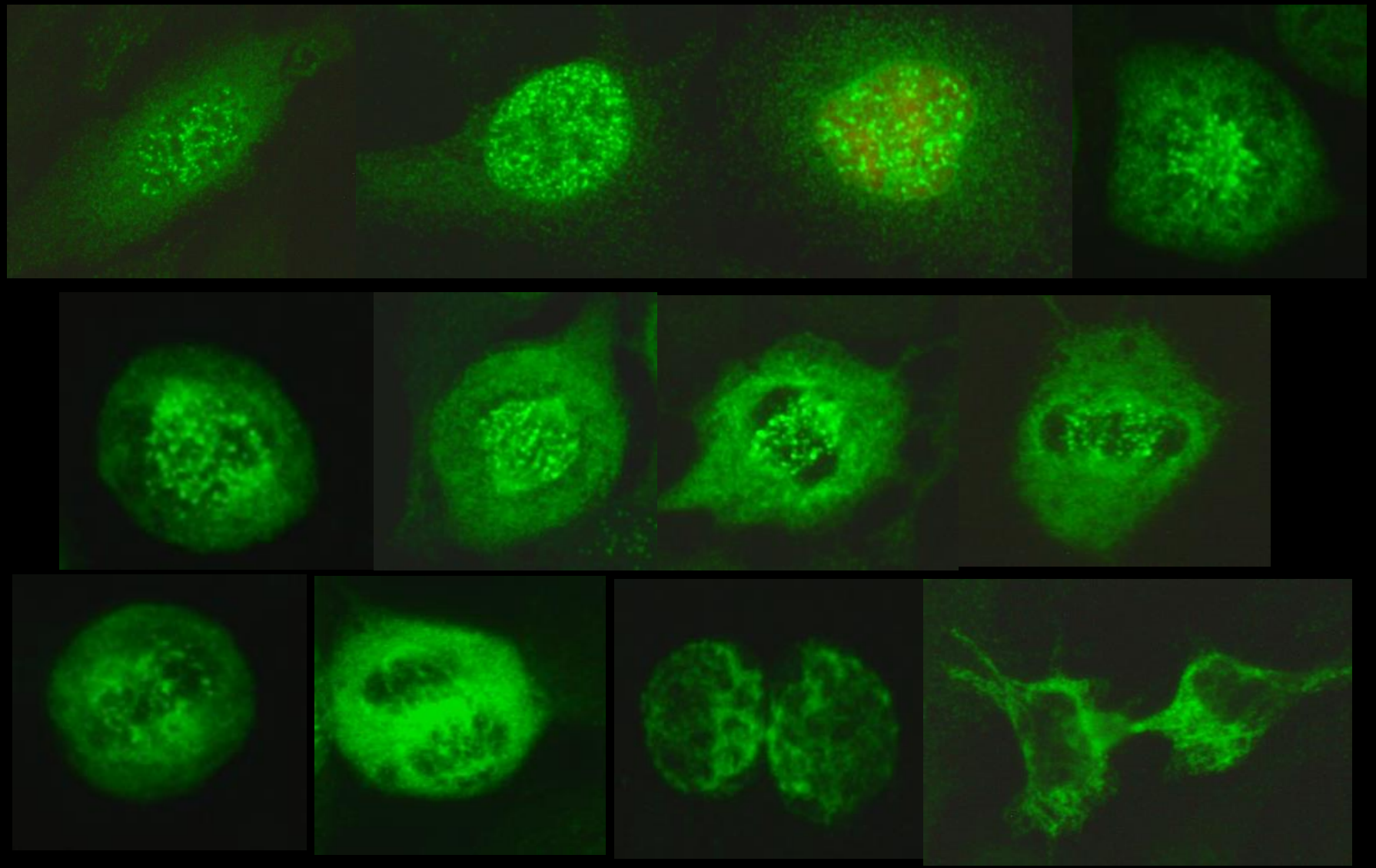
Anti-CENP F



RL.Humbel

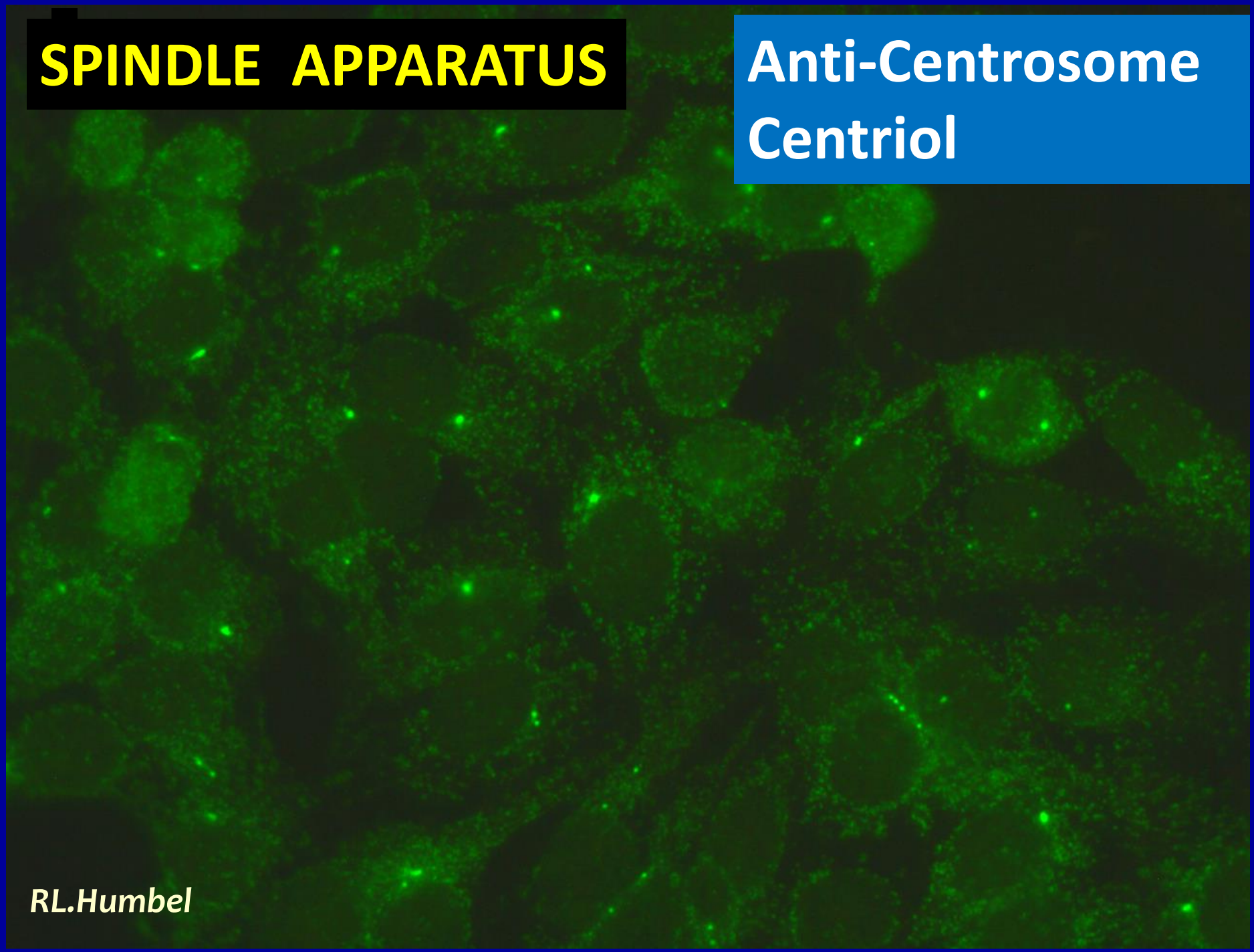

Anti-CENP F
RL.Humbel

MSA3 : ANTI - CENP F

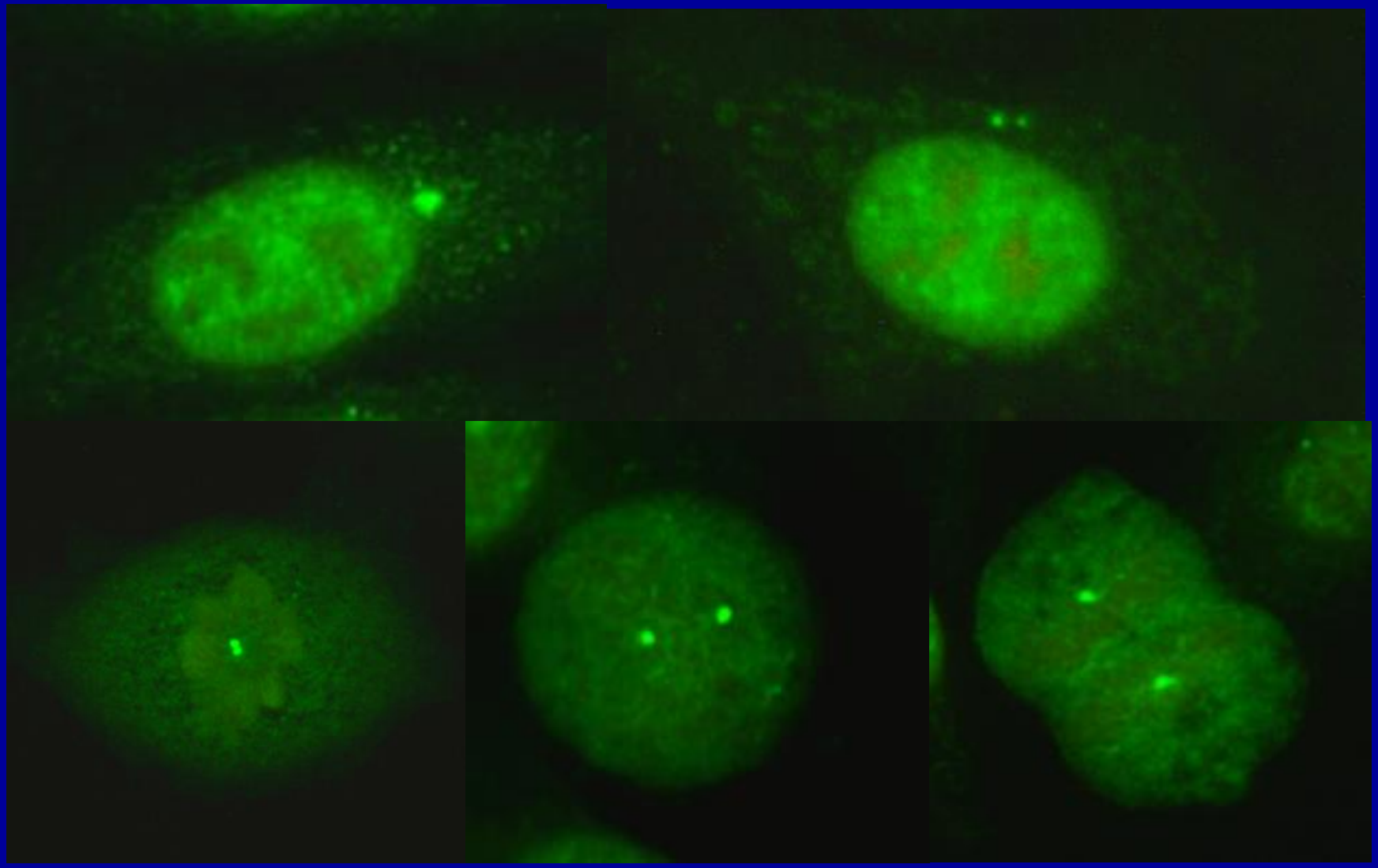


RL.Humbel

SPINDLE APPARATUS

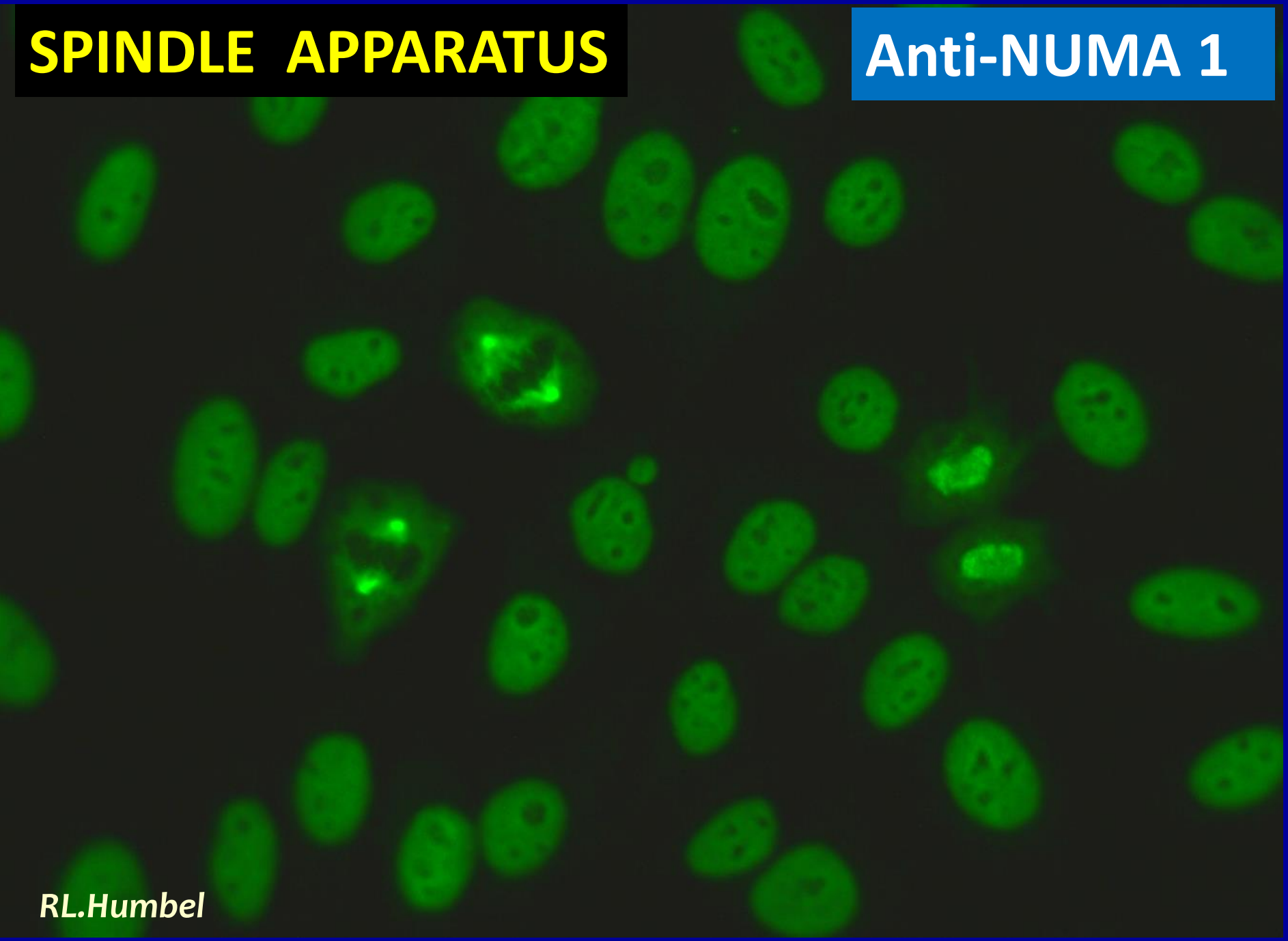


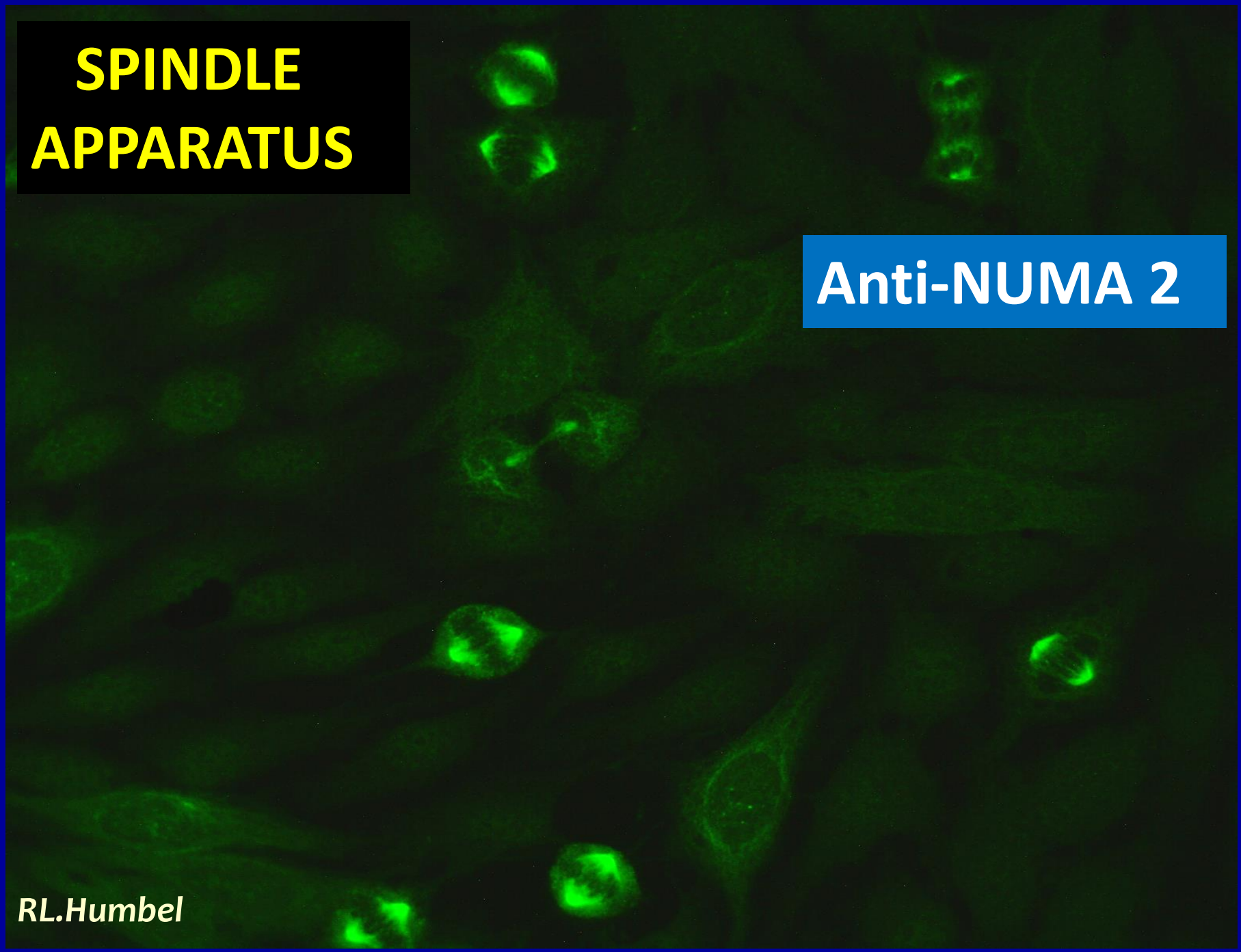
ANTI-CENTROSOME

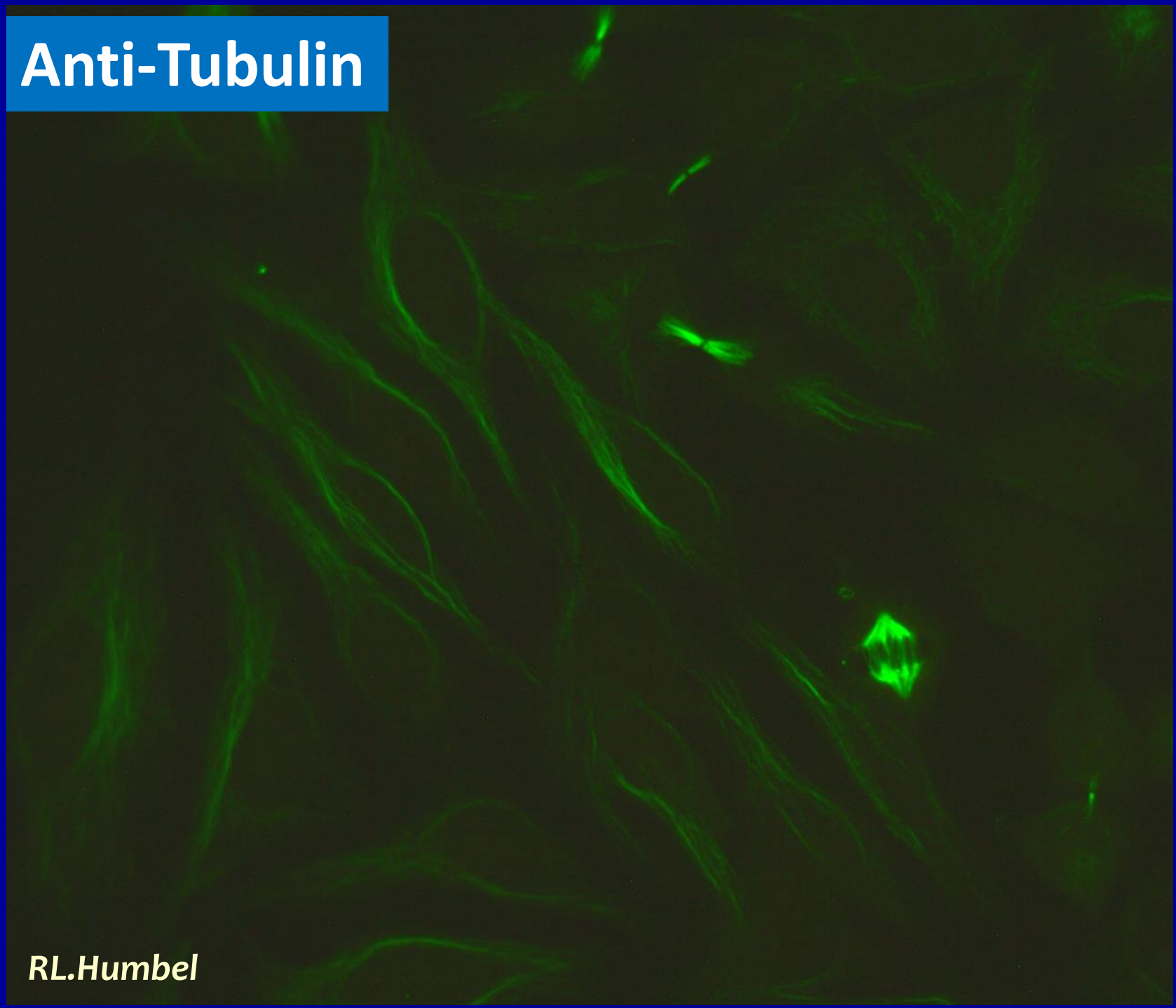


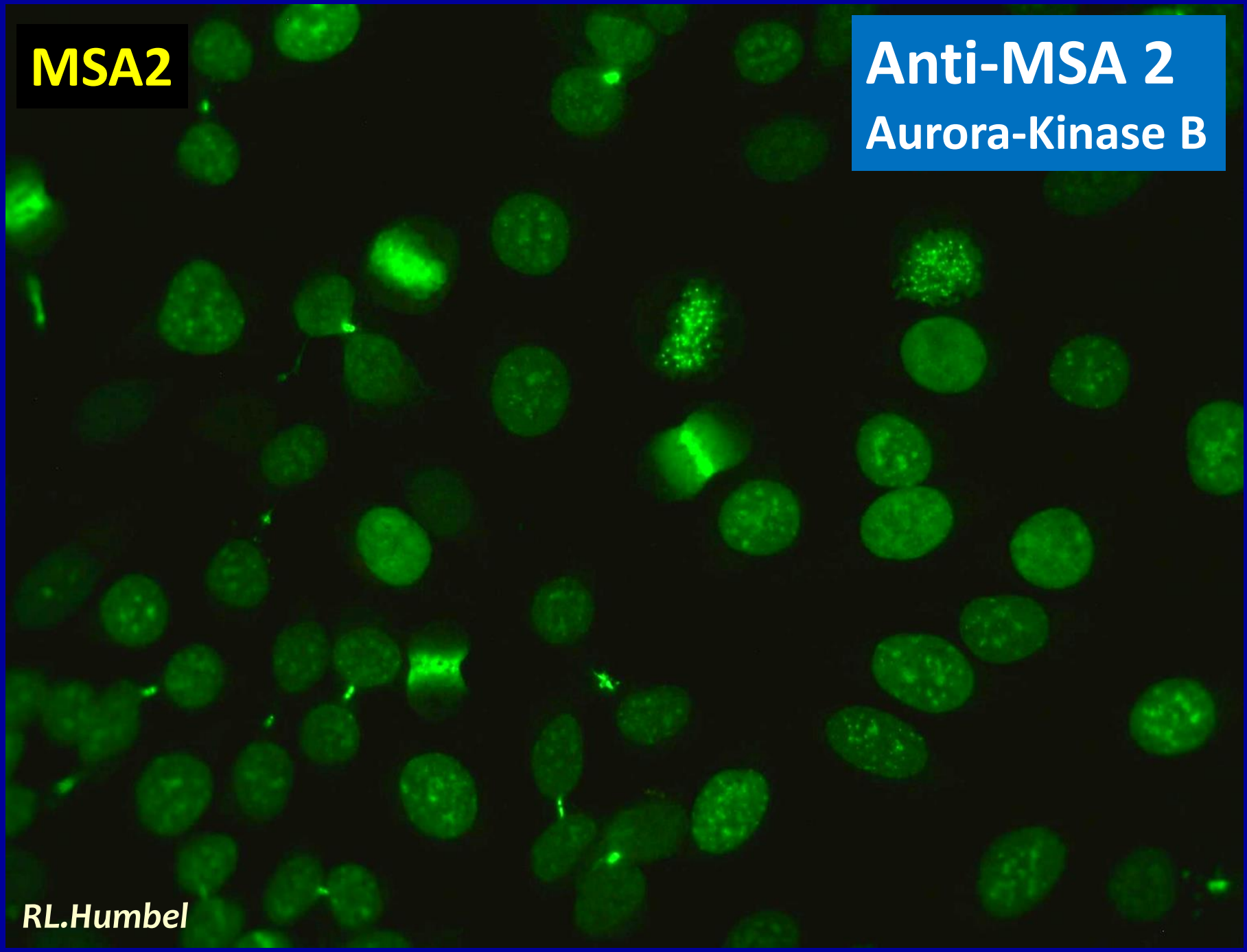
RL.Humbel

SPINDLE APPARATUS

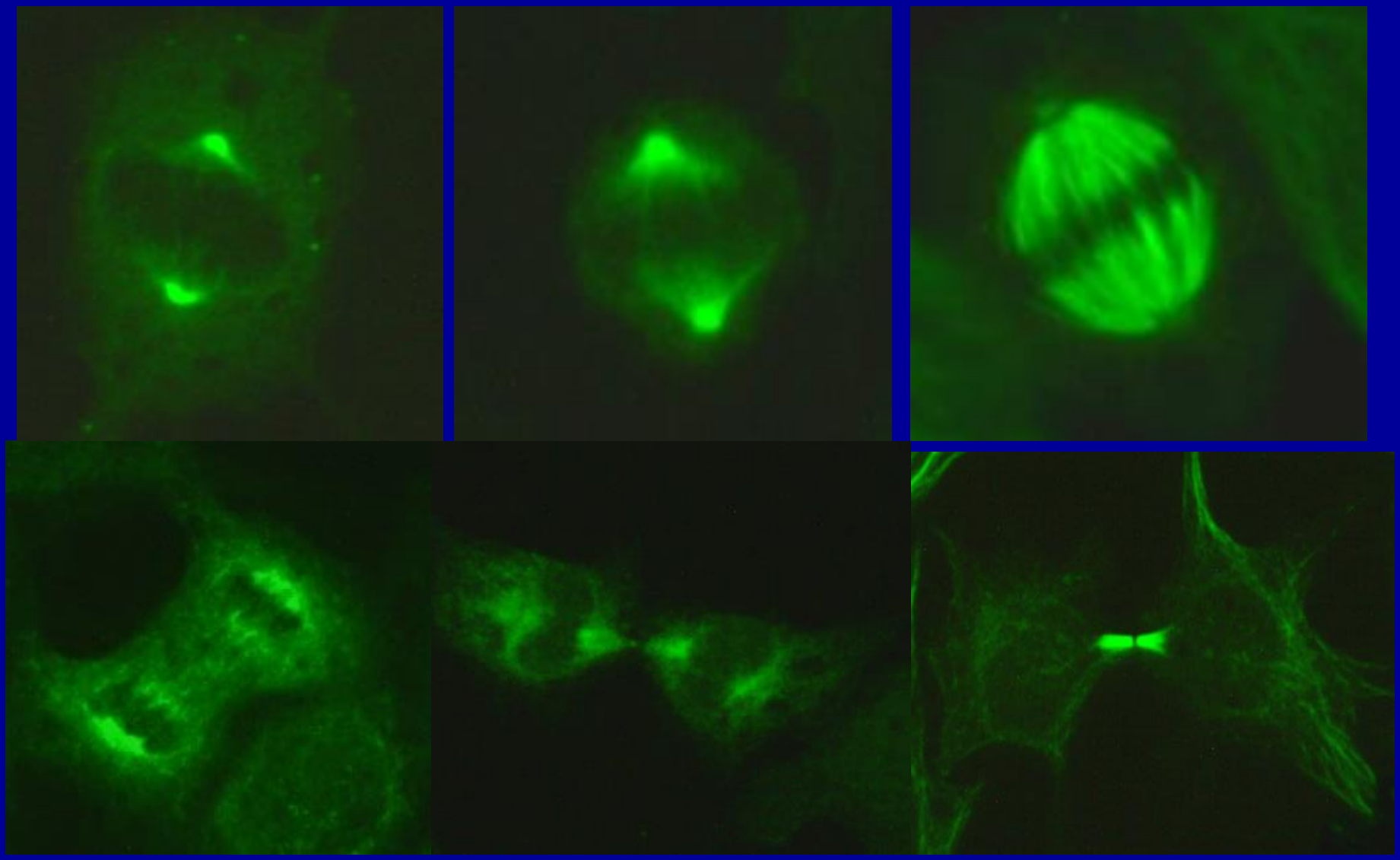



SPINDLE APPARATUS
Anti-NUMA 2
RL.Humbel


Anti-Tubulin
RL.Humbel

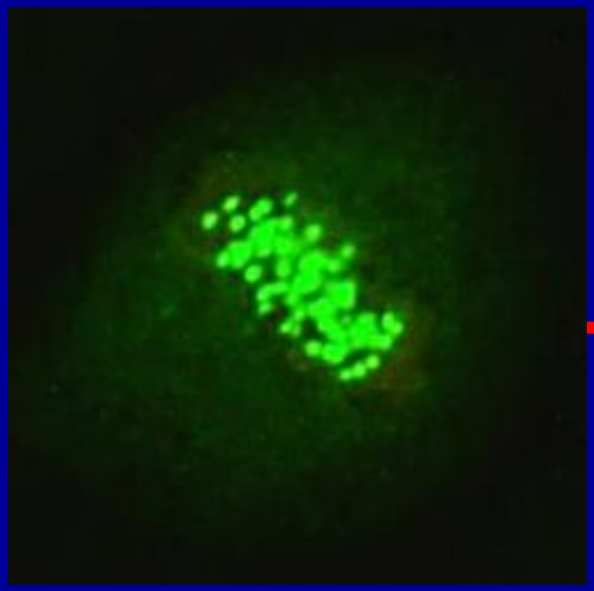

MSA2
Anti-MSA 2
Aurora-Kinase B
RL.Humbel

NUMA 1 NUMA 2 TUBULIN

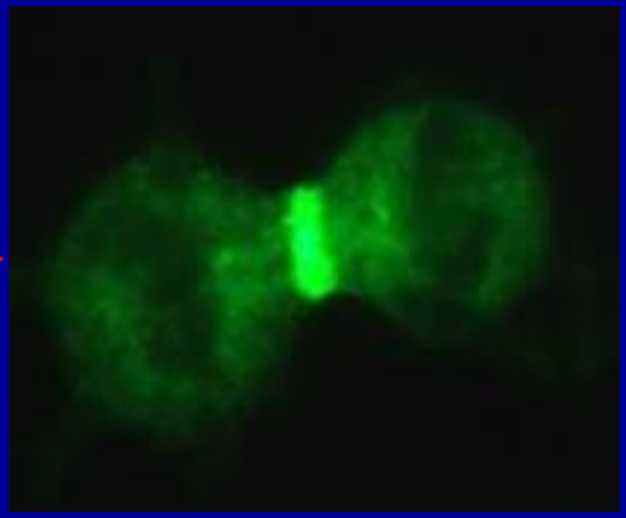


RL.Humbel

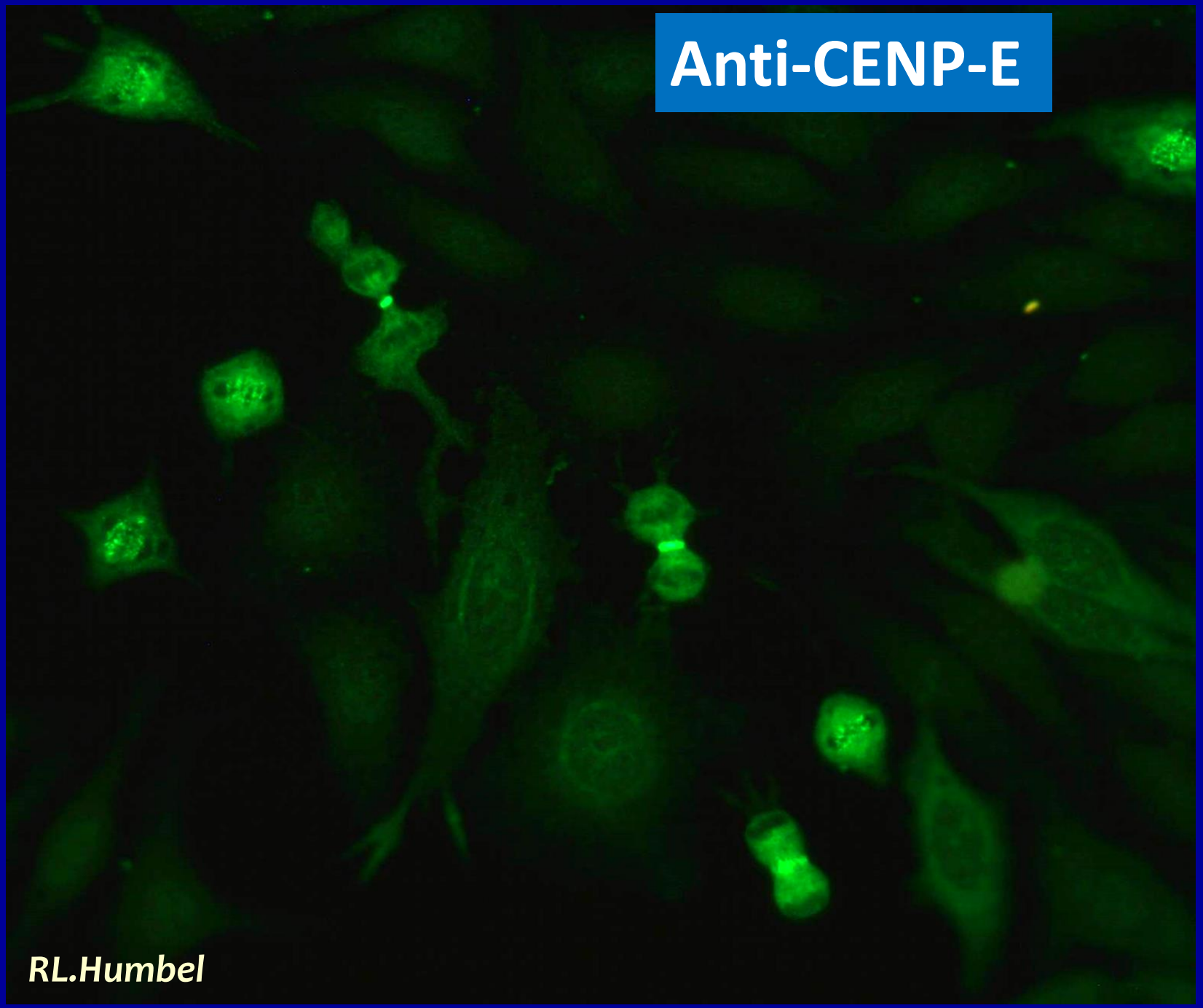
ANTIBODIES TO CHROMOSOMAL PASSENGER PROTEINS



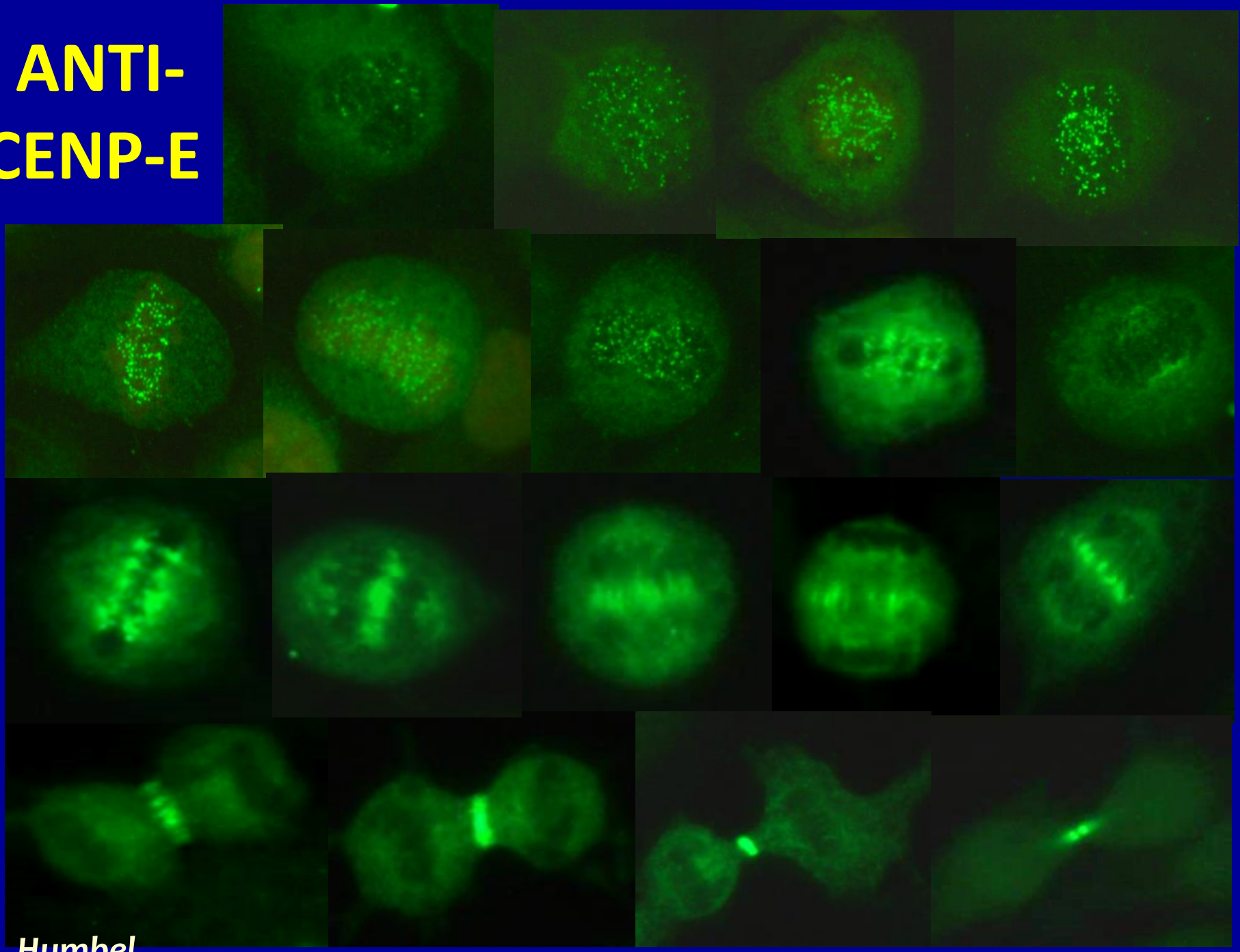
METAPHASE



TELOPHASE

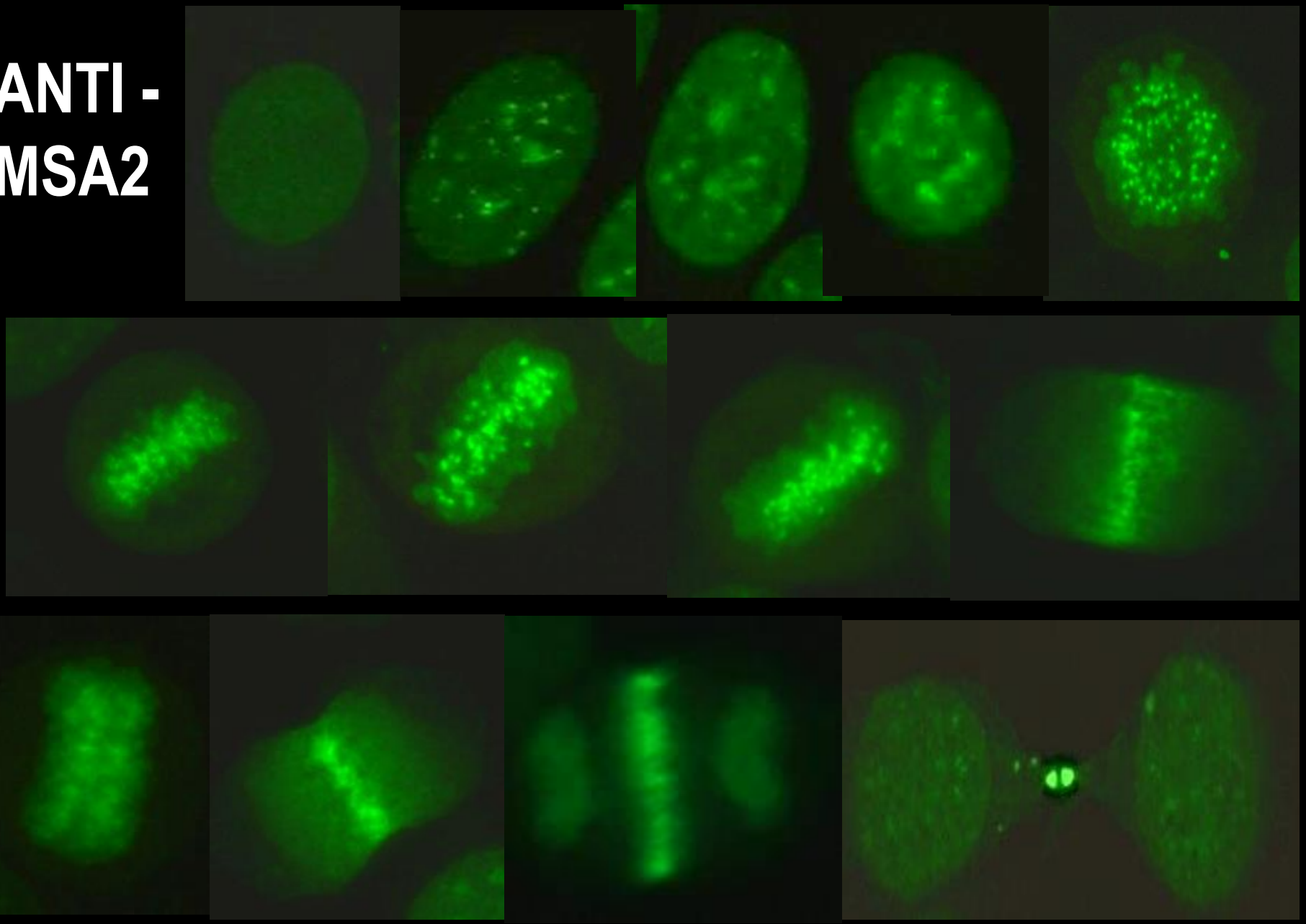

Anti-CENP-E
RL.Humbel

ANTI-CENP-E



RL.Humbel

ANTI - MSA2

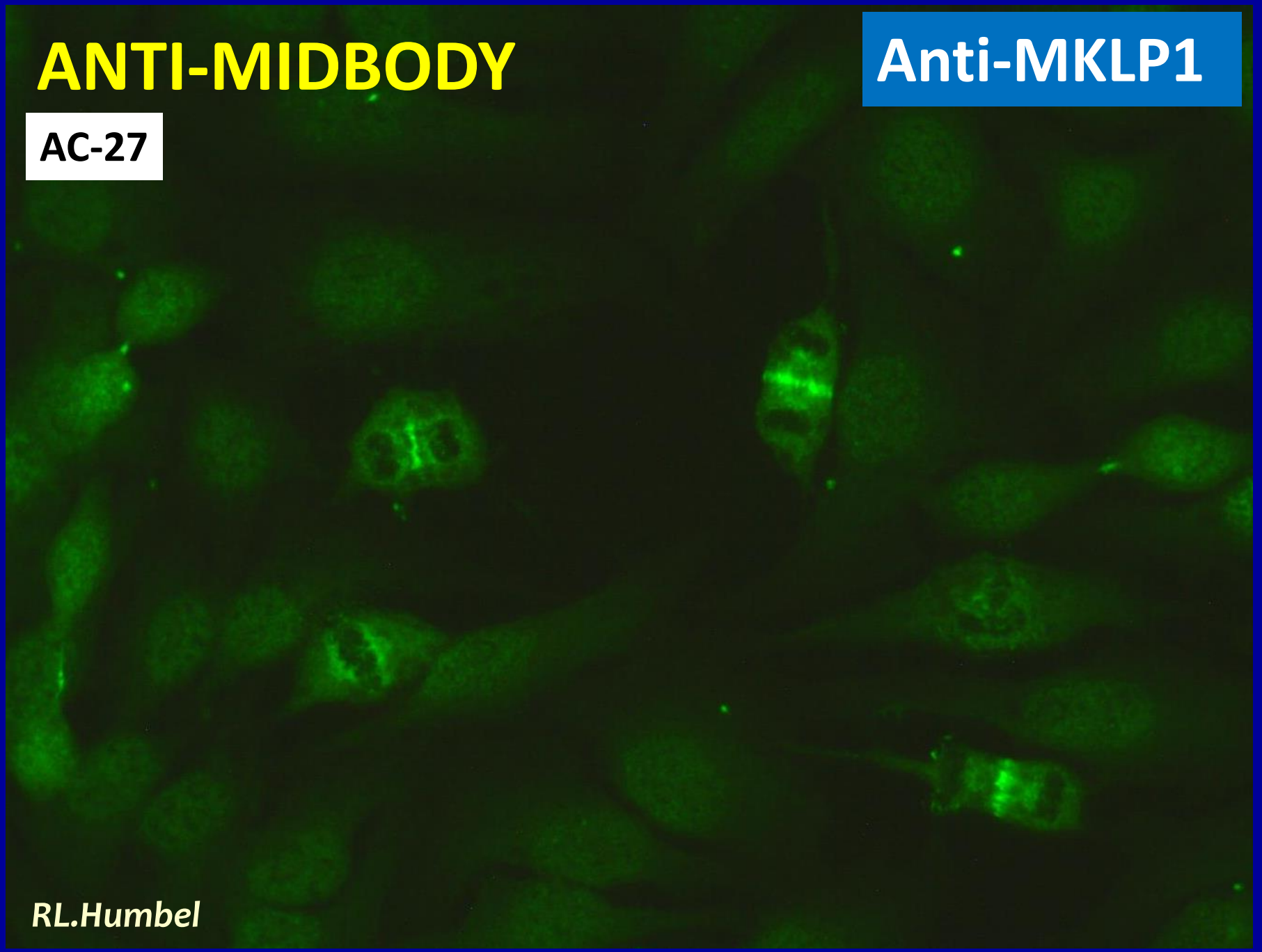


RL.Humbel

ANTI-MIDBODY

AC-27

Anti-MKLP1



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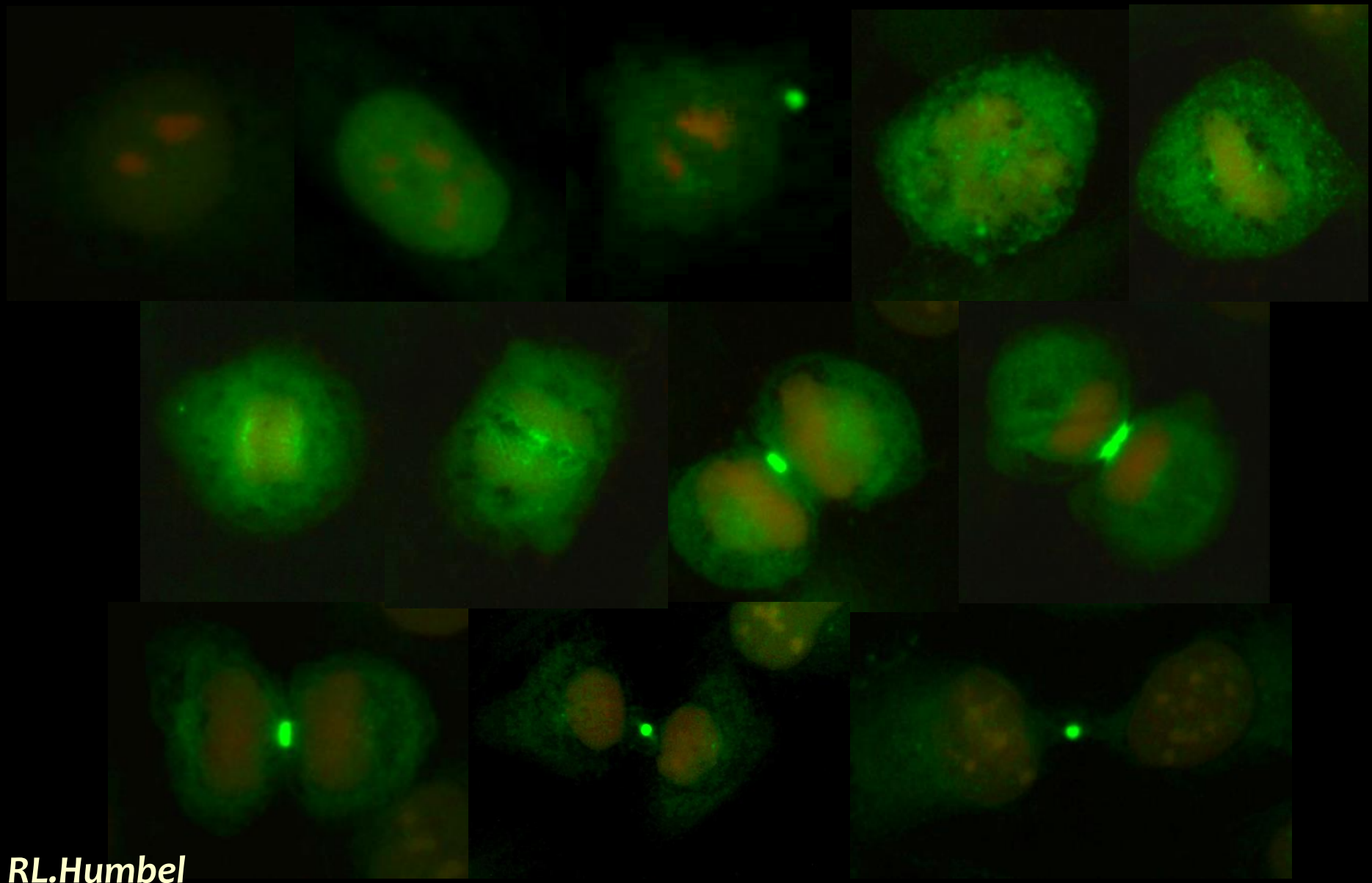
ANTI-MIDBODY : MKLP1



RL.Humbel