"A 44-Year Journey Down the Immunohistochemistry Highway ……"

Richard W. Cartun, PhD, MS

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Rcartun@sbcglobal.net

(860) 490-7633 Cell

"A 44-Year Journey Down the Immunohistochemistry Highway ……"

Richard W. Cartun, PhD, MS

Department of Pathology & Laboratory Medicine

Hartford Hospital

Hartford, CT

(July 1978 - June 2022)


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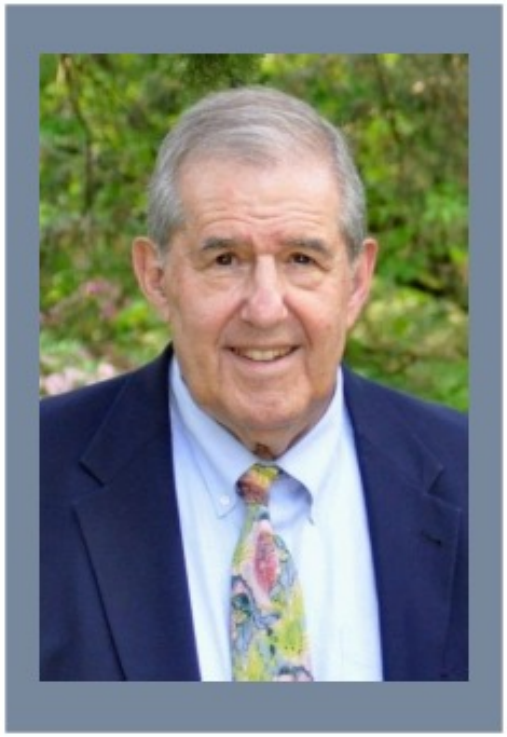

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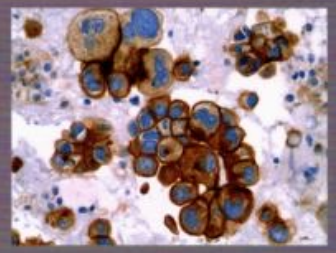
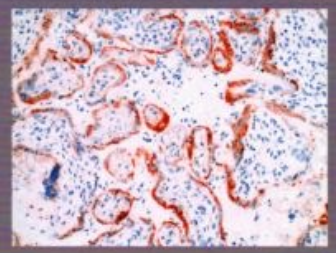
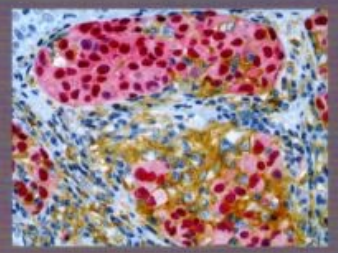


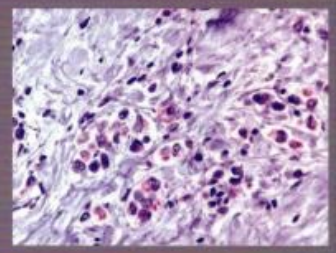




Diagnostic Immunohistochemistry

Theranostic and Genomic Applications

DAVID J. DABBS



The Journal of
Histotechnology
anatomy, histochemistry, microscopy, pathology

Editorial: Immunohistochemistry: New Vistas, New Problems

For the last century, pathologists have relied on examination of formaldehyde-fixed paraffin sections of human tissue stained with hematoxylin and eosin for histopathological diagnoses. Given suitably sectioned and stained material, they have been

generation stains. When pathologists read the disclaimer on all commercial kits, "Not Approved For Diagnostic Use," they may be discouraged from using the new technology. Should a

"Not Approved For Diagnostic Use"

The mucicarmine stain has withstood the test of time because virtually all the neoplasms are mucin secreting adenocarcinomas, and it is pivotal in the diagnosis of stomach cancer. Pathologists rely on this stain as detection of signet ring mucin containing cells is considered pathognomonic for this disease. At this point in time, a classical tinctorial "first generation" special stain still provides useful diagnostic information.

It might not be cost effective, at the present time, for some laboratories to maintain an extensive panel of kits if there are not sufficient immunocases. Despite these limitations, who now would dare refute the need to incorporate antibodies to S-100, prekeratin, carcinoembryonic antigen, or prostatic epithelial antigen into the "special stain" armamentarium of *most* laboratories. The diagnosis of amelanotic melanoma, often difficult if

"March 1986"

News | September 29, 1998

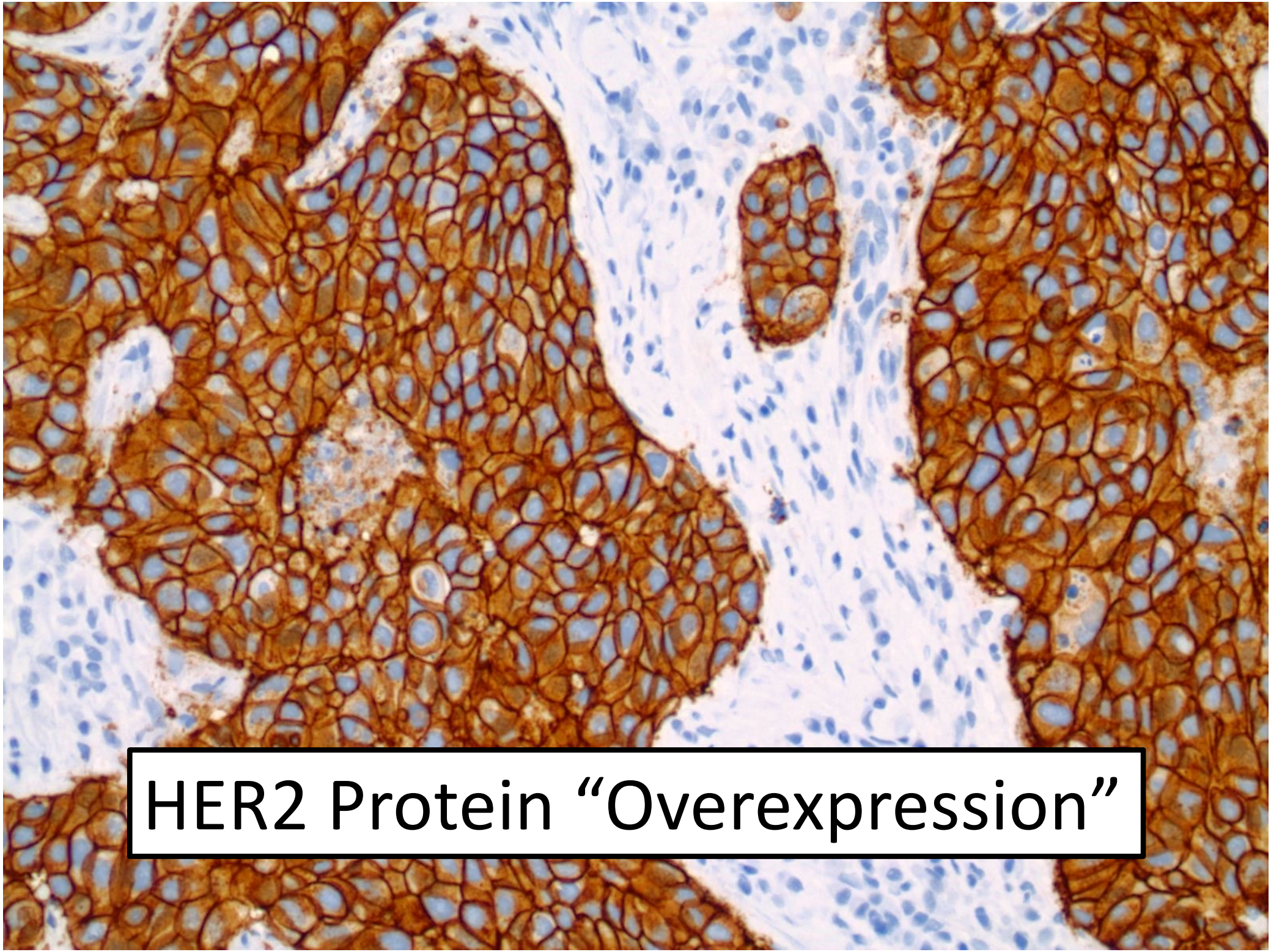
FDA Approves DAKO HercepTest for HER2 Overexpression

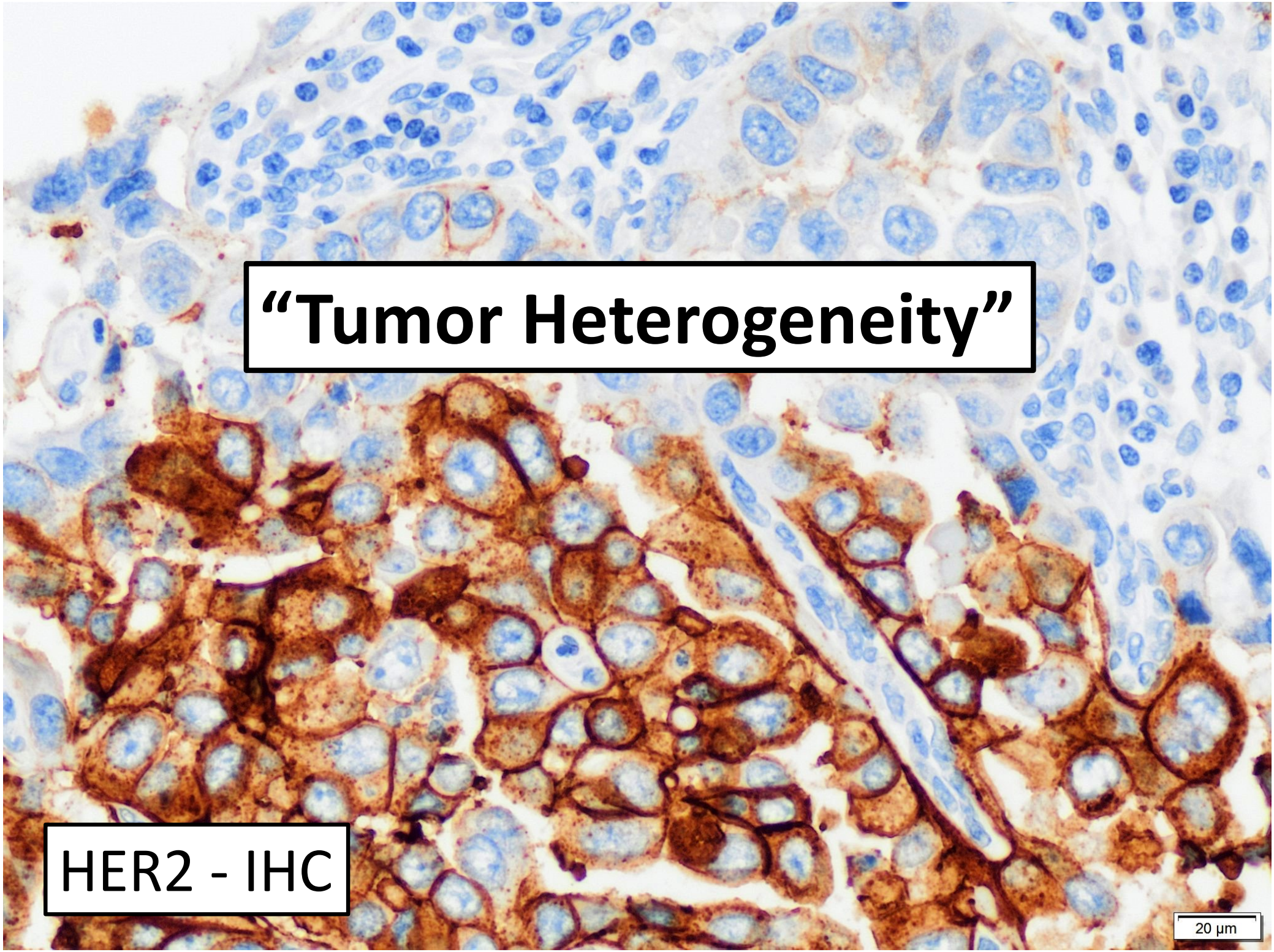
The era of pharmacogenomics is upon us. On September 25, Genentech, Inc. (South San Francisco, CA) received U.S. Food and Drug Administration approval for the first biotech drug for treating metastatic breast cancer. The drug, Herceptin, targets the HER2 gene expressed by about 25% of all breast cancer patients and which is believed to be responsible for the aggressiveness of certain types of breast cancer.

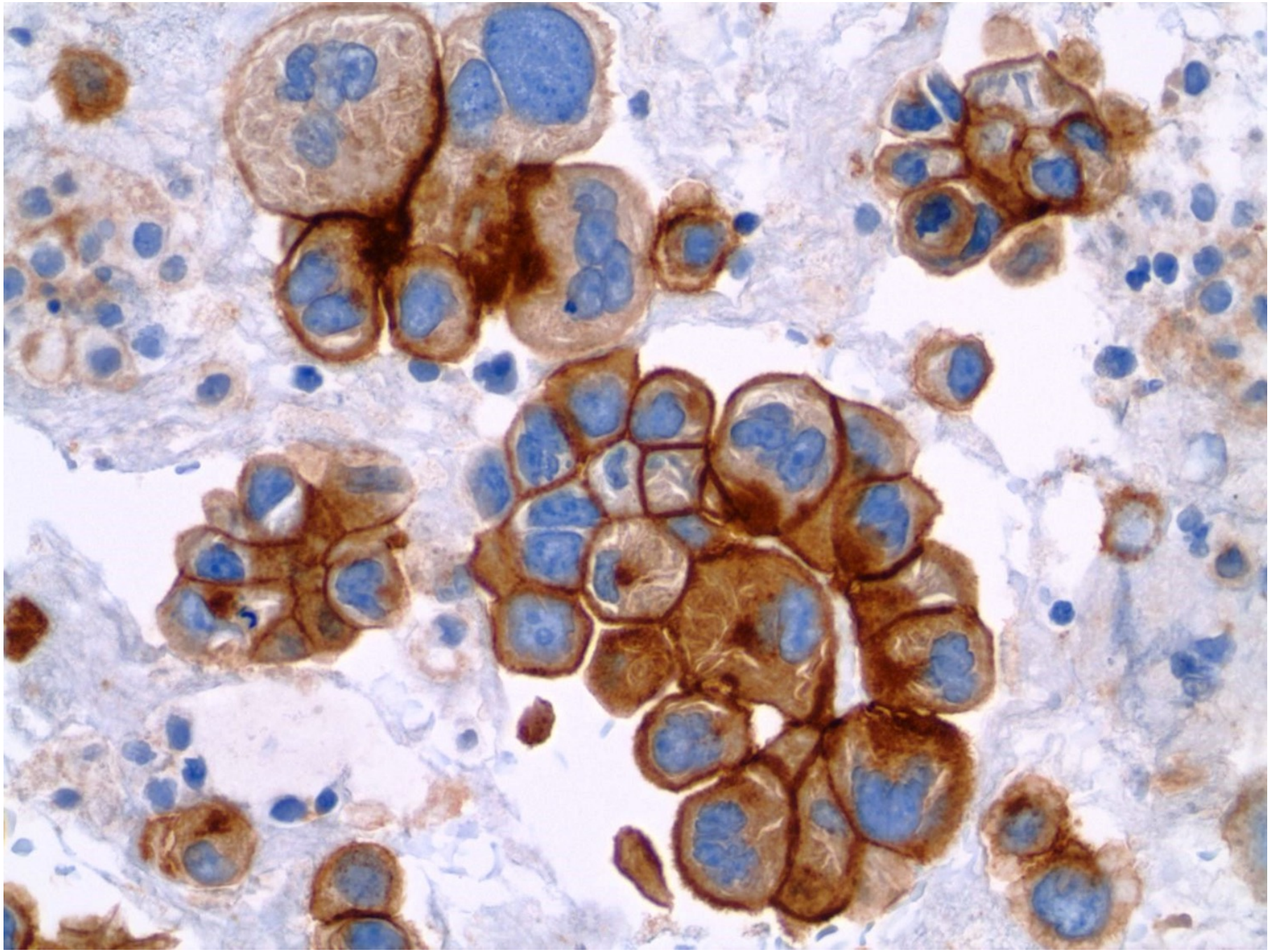
On the same day DAKO Corp. (Carpinteria, CA) received FDA approval for HercepTest, an *in vitro* assay for the HER2 gene. The timing was not a coincidence, since Genentech and DAKO had been collaborating on HercepTest since at least March 1998. Nevertheless, the approval of a gene-targeting drug and an assay for the drug's potential effectiveness on the same day marks the beginning of what should be an exciting trend: the emergence of gene-based therapies and genetic tests that predict the treatments' effectiveness.

HercepTest Kit for HER2 Detection

"Companion Diagnostic"


HER2 Protein “Overexpression”


“Tumor Heterogeneity”
HER2 - IHC
20 µm



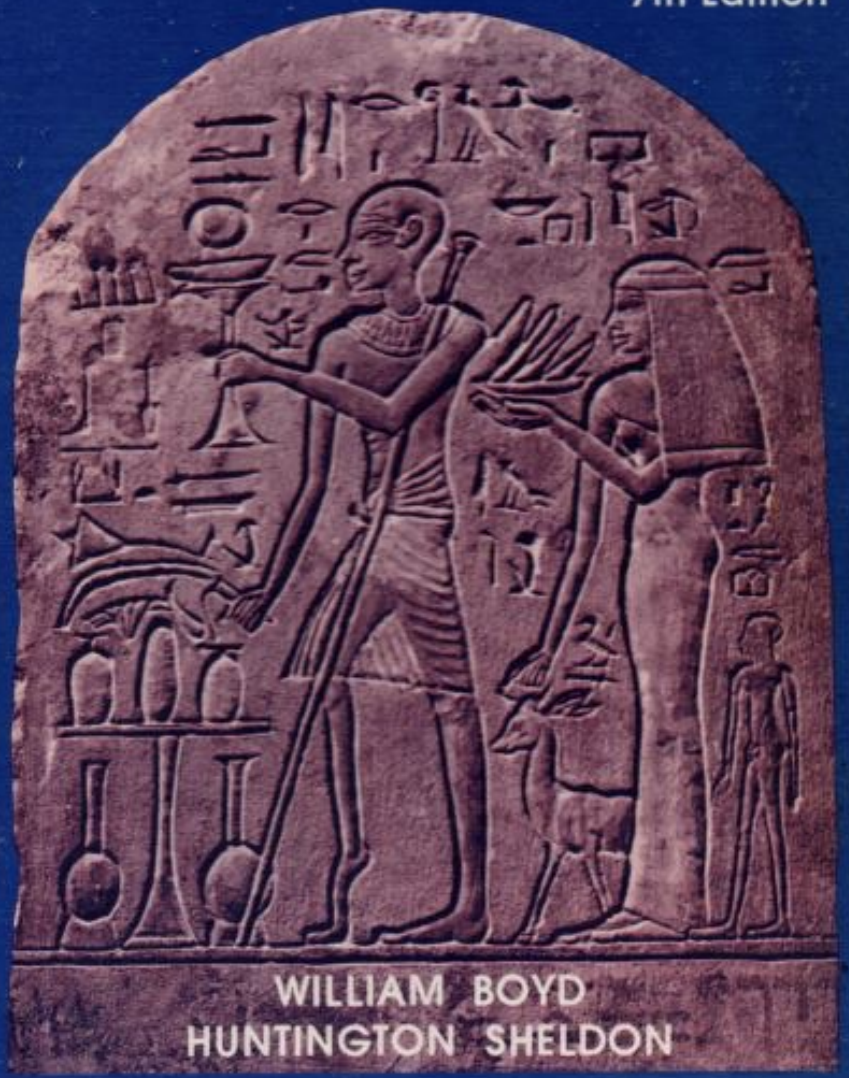
Immunoperoxidase detection of PD-L1

Chapter One

“What do I want to be when I grow up?”

AN INTRODUCTION to the STUDY of DISEASE

7th Edition



WILLIAM BOYD
HUNTINGTON SHELDON

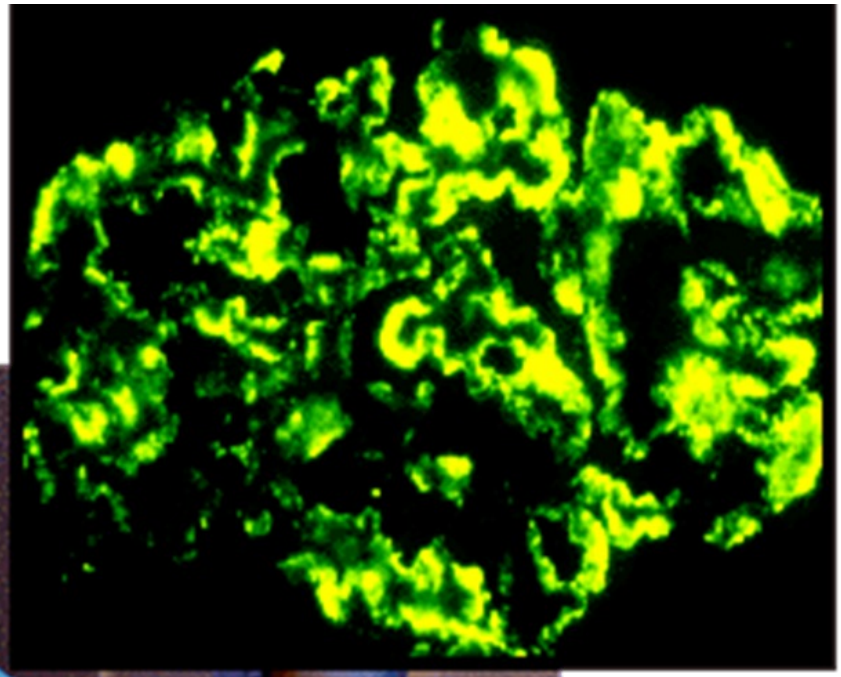

UConn Health Center

Hartford Hospital, Hartford, CT

My Responsibilities in 1978 ……

- Evaluation and triage of renal biopsy tissue in Ultrasound/Radiology
- Immunofluorescence testing performed on frozen tissue sections of kidney
- Identification of estrogen receptor protein in frozen tissue sections using an FITC-conjugated estradiol compound
- Identification of B- and T-cell lymphocytes using sheep erythrocyte rosetting and detection of surface immunoglobulin in cell suspensions

Renal Biopsy - Immunofluorescence

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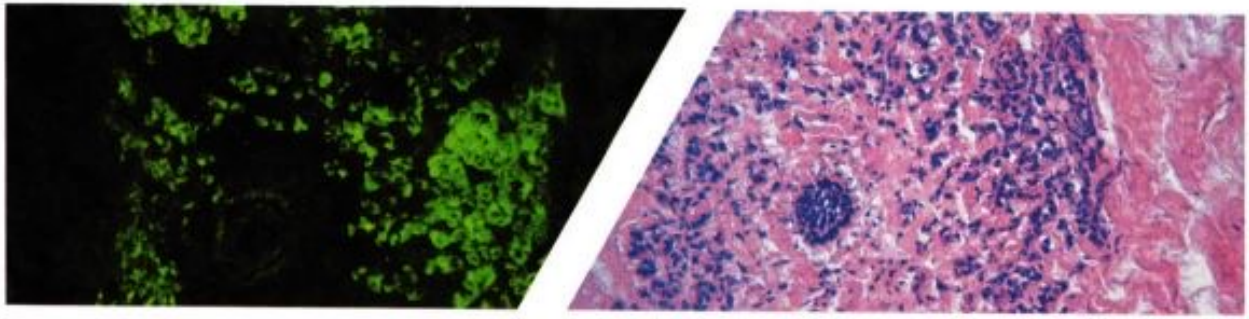
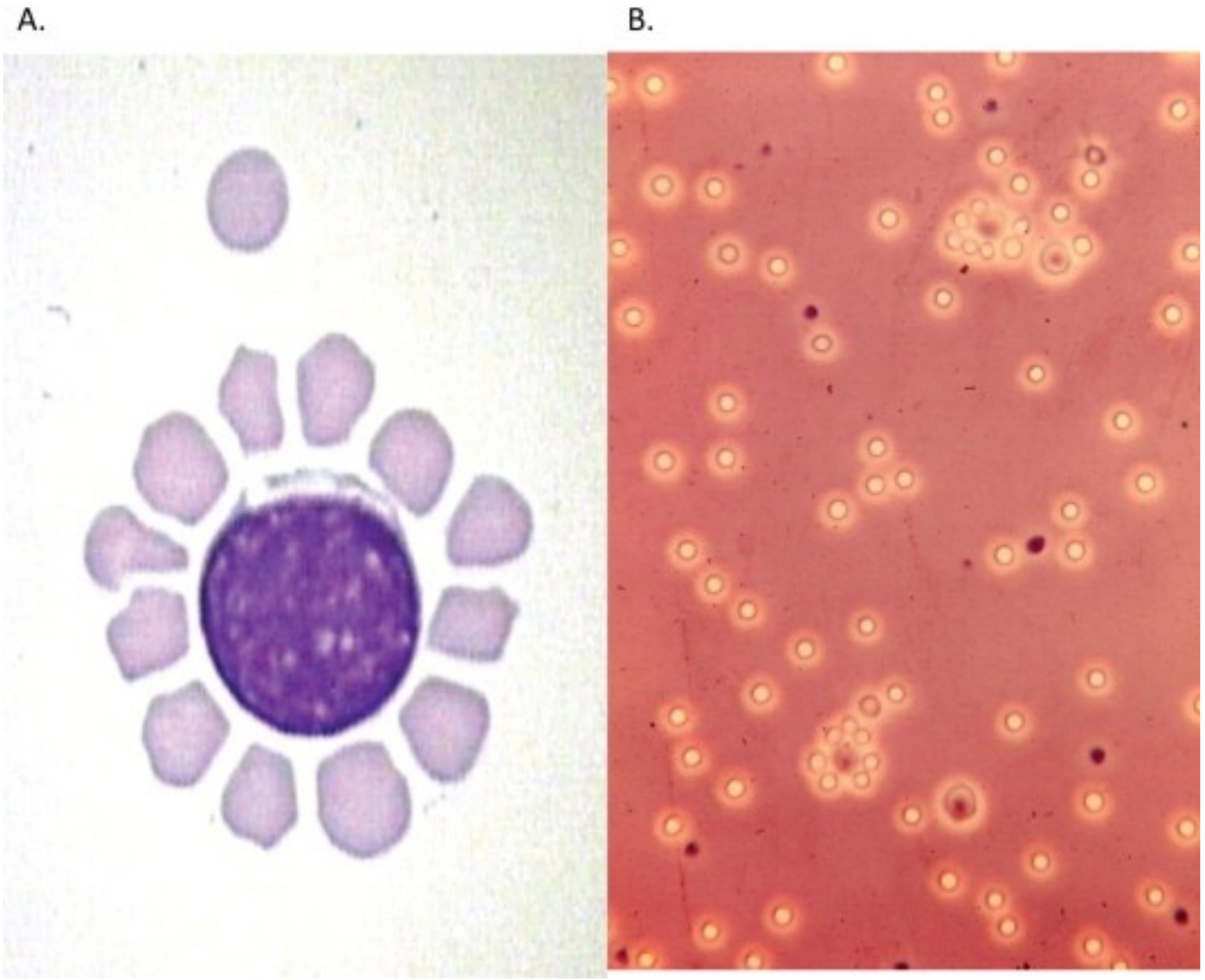


H & E Stained Tissue Sample



T-Cell Lymphocyte Rosette

Chapter Two

“ Immunoperoxidase …….. what’s that?”

Clive R. Taylor, M.D., D.Phil.

J. clin. Path., 1974, **27**, 14-20

The demonstration of plasma cells and other immunoglobulin-containing cells in formalin-fixed, paraffin-embedded tissues using peroxidase-labelled antibody

C. R. TAYLOR AND J. BURNS

From the Department of Pathology, Gibson Laboratories, Radcliffe Infirmary, Oxford

SYNOPSIS A method is described for the demonstration of specific immunoglobulin in plasma cells and other lymphoid cells in sections taken from routine surgical histology specimens which have been formalin fixed and paraffin embedded.

An indirect sandwich technique was employed using specific rabbit antihuman immunoglobulin antisera (anti-K, L, G, A, and M) and a swine antirabbit serum Ig G, conjugated with horseradish peroxidase. The presence of plasma cells was revealed by staining the tissue-bound peroxidase-labelled antibody, having previously stained the endogenous peroxidase a contrasting colour.

It was possible to demonstrate clearly immunoglobulin in the plasma cells of tissues processed and embedded several years previously.

Some of the potential uses of the method are discussed.

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CONTINUING MEDICAL EDUCATION
CERTIFICATE OF PARTICIPATION

NAME: RICHARD CARTUN

COURSE TITLE: IMMUNOPEROXIDASE STAINING TECHNIQUES USED IN DIAGNOSTIC PATHOLOGY

DATE: APRIL 18 - 19, 1980

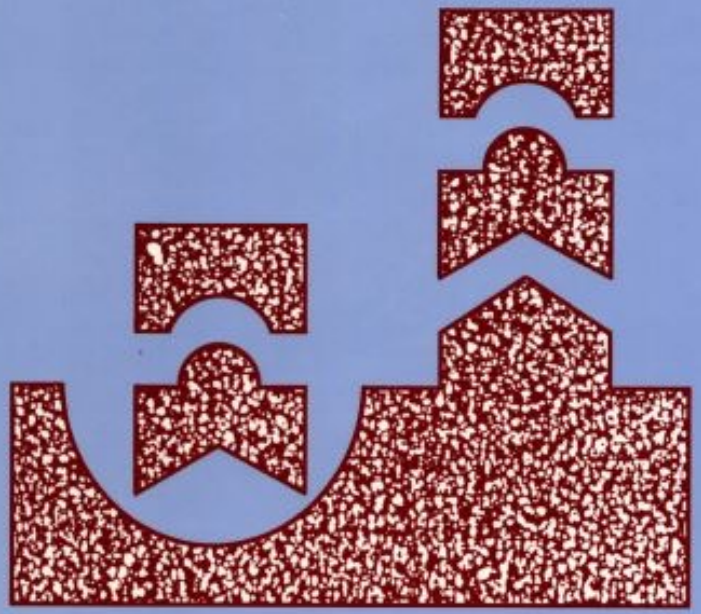
TIME: APRIL 18 - 7:00 - 10:00 P.M.
APRIL 19 - 8:00 - 5:30 P.M.

AMA CATEGORY 1 CREDIT 8

This program has also been approved for 0.8 CEU's Category 3.
PACE program approval number 80025 .

IMMUNOHISTOPATHOLOGY
A Practical Approach to Diagnosis

Jules M. Elias



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2019 - Damien Laudier
2020 - Tony Henwood
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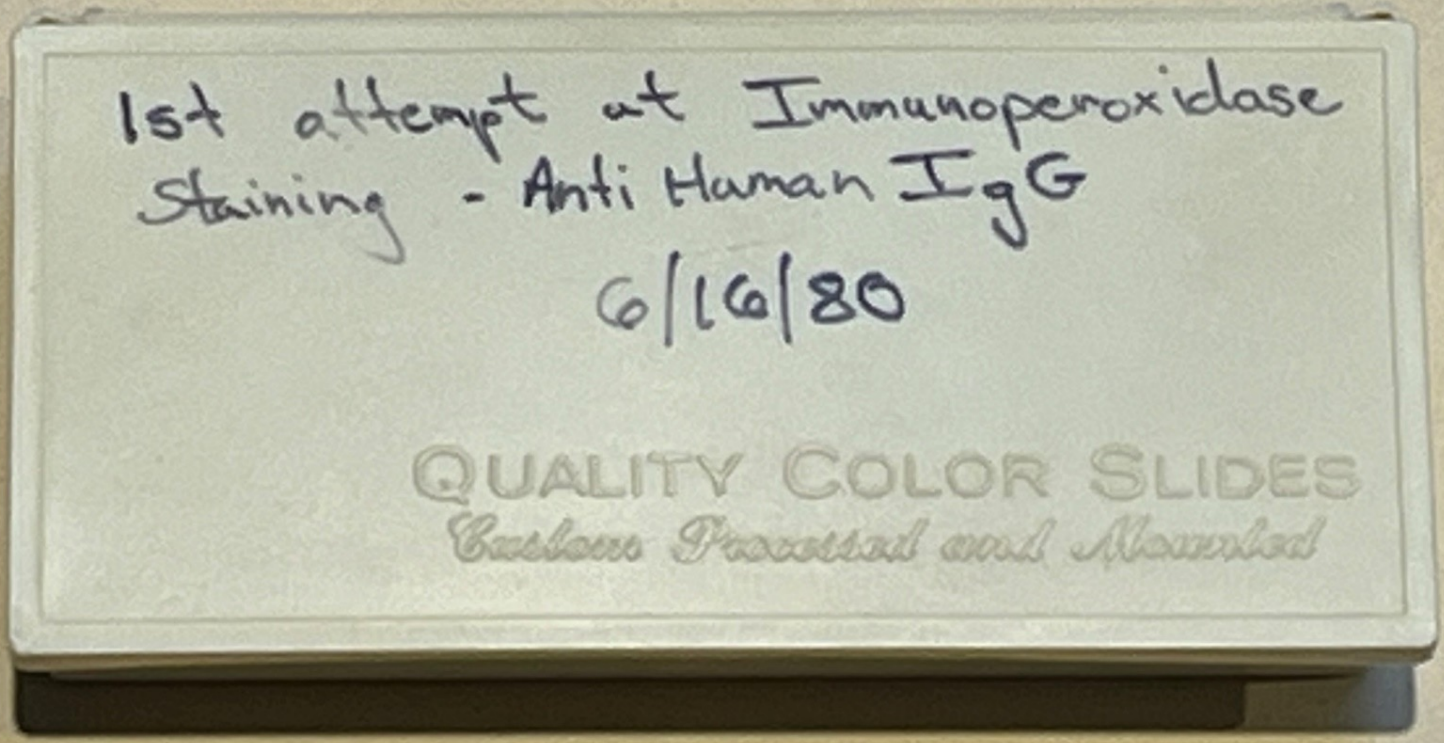
1st attempt at Immunoperoxidase
Staining - Anti Human IgG
6/16/80
QUALITY COLOR SLIDES
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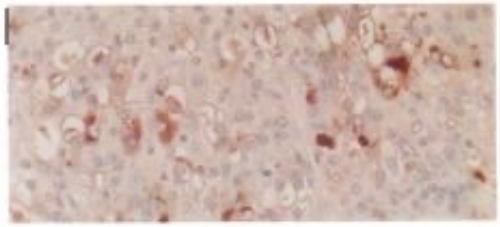
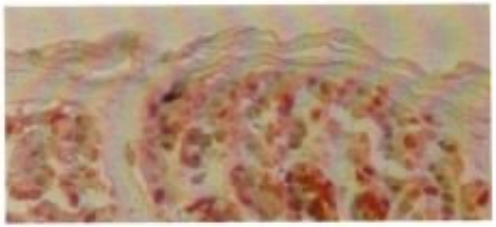
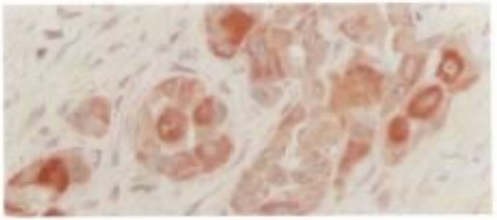


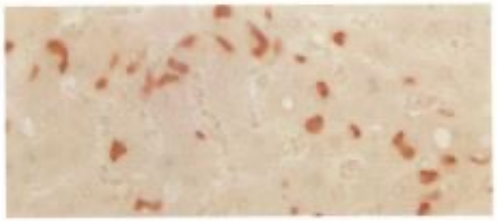
Liver, **AFP**
A liver section from a hepatoblastoma case stains positively for the oncofetal antigen alpha-1-fetoprotein (AFP). A major glycoprotein of the fetus, AFP has been localized in hepatocellular carcinoma and yolk sac tumors of the ovary and testis. AFP is a useful marker for distinguishing these types of tumors from other neoplasms.



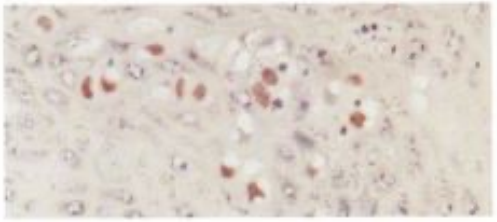
Skin, **S-100**
A skin biopsy from a case of superficial, spreading, malignant melanoma showing intense positive staining for S-100 protein. S-100 is a nervous system associated protein that is a useful marker for the identification of melanocyte derived tumors such as nevi and melanoma, regardless of melanin content. It can also aid in distinguishing poorly differentiated melanoma from tumors of obscure histological origin.



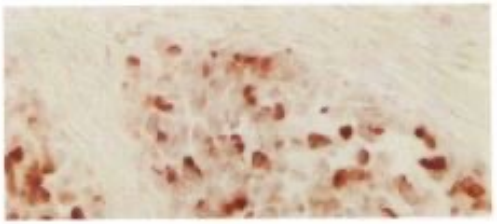
Skin, **Keratin**
A skin biopsy from a patient with well differentiated squamous cell carcinoma shows positive staining for keratin. The variation in stain intensity is due to the difference in the amount of keratin produced by the individual tumor cells. Keratin is a useful marker for establishing the epithelial nature of primary and metastatic tumors, especially for those exhibiting uncharacteristic morphology.



Liver, **HBsAg**
Hepatitis B surface antigen (HBsAg) representing the capsular material of the hepatitis B virus can be localized in the cytoplasm of infected liver cells. HBsAg can be detected in cases of type B viral hepatitis, chronic carriers, cirrhosis, and hepatocellular carcinoma. The results of immunoperoxidase staining are usually more intense and easier to interpret than those achieved by using an orcein stain.



Skin, **Papillomavirus**
Immunoperoxidase staining for papillomavirus shows localization in the nuclei of infected cells present in this skin biopsy. Papillomavirus can be identified in a variety of proliferative squamous lesions as well as in many cases of cervical dysplasia. Some types of papillomavirus have the ability to undergo malignant transformation especially in immunosuppressed patients. Indications of papillomavirus infection have been found in a high percentage of women with cervical neoplasia.



Colon, **PSA**
Positive staining for prostate specific antigen (PSA) identifies this colon tumor as being metastatic from the prostate. All primary and metastatic prostatic carcinomas show positive staining for PSA regardless of their morphological differentiation, while nonprostatic malignancies do not stain. PSA is a useful tool in the identification of tumors of prostatic origin.

gastrin-producing tumors. This study demonstrates the reliability of the immunocytochemical method for the specific identification of cell types in pancreatic islet cell tumors.

Prostatic Specific Antigen: an Immunohistologic Marker for Prostatic Neoplasms

M. NADJI, S. Z. TABEI, A. CASTRO, T. M. CHU, M. C. WANG, AND A. R. MORALES. Department of Pathology, University of Miami, Miami, Florida and Department of Diagnostic Immunology Research and Biochemistry, Roswell Park Memorial Institute, Buffalo, New York.

An antiserum to a human prostate-specific antigen was utilized by immunoperoxidase staining to evaluate its potential value as a diagnostic histologic marker for tumors of prostatic origin. Formalin-fixed paraffin embedded surgical and autopsy material from 122 known cases of primary and metastatic prostatic carcinomas and 78 cases of nonprostatic neoplasms were studied by peroxidase antiperoxidase technique. Antiserum to prostate-specific antigen was produced in rabbit with a partially purified prostatic antigen and was absorbed with normal female serum and human tissue extracts. This antiserum was shown to be specific to human prostate by immunoprecipitation techniques. In normal and hyperplastic glands, positive reactions were observed in the epithelial linings of acini and ducts as well as in secretions and concretions. The epithelia of periurethral glands, seminal vesicles, transitional epithelia of the urinary bladder and prostatic urethra were immunohistologically negative. All primary and metastatic prostatic carcinomas showed positive cytoplasmic staining for prostatic-specific antigen regardless of their morphologic differentiation. None of the nonprostatic malignancies stained positively for prostatic specific antigen.

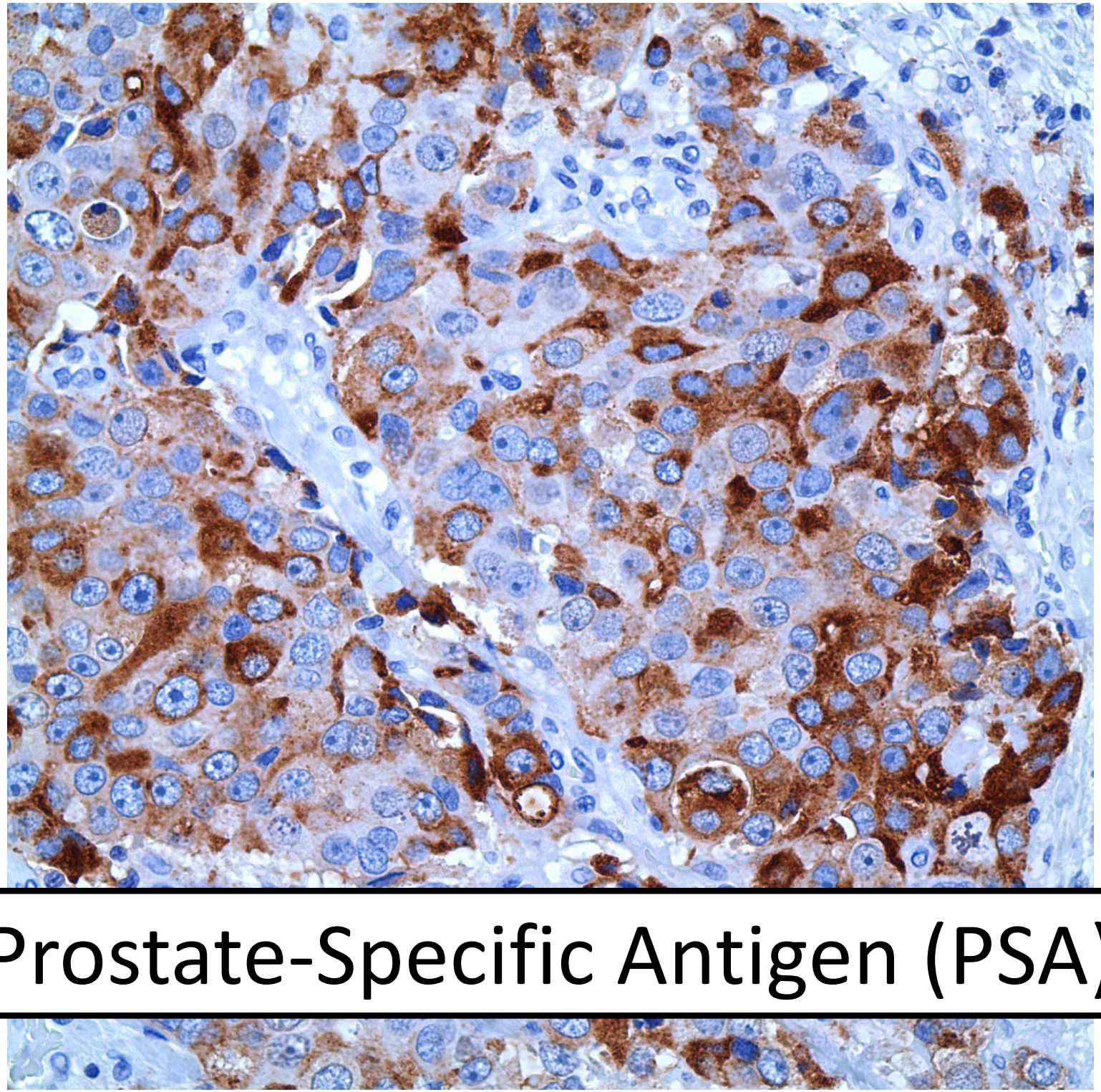
Our data demonstrate the value of prostatic-specific antigen as a potential immunohistologic marker to distinguish between prostatic and nonprostatic neoplasms. Furthermore, this is the first specific immunohistologic marker for benign and malignant prostatic epithelium which does not represent prostatic acid phosphatase.

ganglioma and hemangiopericytoma. Previously established criteria were used to evaluate these 19 clinically malignant tumors. Four of the tumors were less than 6 cm. in maximum diameter. Infiltrating margins were absent in seven cases. Three tumors had fewer than 10 mitotic figures per 50 high power fields. Small cells were observed in only three cases. Epithelioid smooth muscle tumors may present a difficult diagnostic problem, both in relation to their varied histologic appearance and assessment of their malignant potential.

Malignant Lymphoma, Poorly Differentiated Lymphocytic Type, Diffuse: a Tumor of Varying Histologic Subtypes

RICHARD NEIMAN, HUN KIM, RISA MANN, AND HENRY RAPPAPORT. Pathology Panel for Lymphoma Clinical Studies and the Eastern Cooperative Oncology Group.

In the original Rappaport Classification, malignant lymphoma, poorly differentiated lymphocytic type (PDL) represented a heterogeneous group of tumors whose cells were considered less than well differentiated but smaller than the tumor cells of histiocytic lymphoma. The studies of follicular center cell lymphomas by Lukes and Collins and of lymphoblastic lymphoma by Nathwani, Kim, and Rappaport have indicated that DPDL includes at least two distinctive clinicocomorphologic subtypes—tumors composed of cells with cleaved nuclei occurring in older patients, and lymphoblastic tumors occurring primarily in the pediatric age group. In order to better define the morphologic spectrum of DPDL and to assess the relative frequency of its histological subtypes we reviewed 198 cases from the Repository Center for Lymphoma Clinical Studies accessioned from the Eastern Cooperative Oncology Group. A number of cases were eliminated from the study because the material was inadequate for proper subclassification, because the pattern of the lymphoma was indeterminable, or contained residual nodularity. Sixty three cases remained to be subclassified. The largest group, comprising 31 cases, was lymphoblastic lymphoma. Only 14 cases were composed predominantly of cells with cleaved nuclei; 8 were com-



Prostate-Specific Antigen (PSA)

CLINICAL PRACTICE

Caren G. Solomon, M.D., M.P.H., *Editor*

Screening for Prostate Cancer

Paul F. Pinsky, Ph.D., and Howard Parnes, M.D.

This Journal feature begins with a case vignette highlighting a common clinical problem. Evidence supporting various strategies is then presented, followed by a review of formal guidelines, when they exist. The article ends with the authors' clinical recommendations.

A 60-year-old patient asks whether he should undergo screening for prostate cancer and, if he undergoes screening and the results are positive, what his options would be with respect to further diagnostic testing and treatments. How would you respond?

From the Early Detection Branch (P.F.P.) and the Prostate and Urologic Cancer Branch (H.P.), Division of Cancer Prevention, National Cancer Institute, National Institutes of Health, Bethesda, MD. Dr. Pinsky can be contacted at pinskyp@mail.nih.gov or at the Division of Cancer Prevention, 9609 Medical Center Dr., National Cancer Institute, Rockville, MD 20852.

N Engl J Med 2023;388:1405-14.
DOI: 10.1056/NEJMcp2209151

THE CLINICAL PROBLEM

Prostate cancer is currently the most diagnosed cancer (excluding nonmelanoma skin cancer) and the second leading cause of cancer death among U.S. men. Prostate cancer was diagnosed in an estimated 268,500 men in 2022, and approximately 34,500 died of it.[1] The disease occurs primarily in older persons, with the incidence greatest among men in their 70s and mortality highest among men in their 80s. The incidence among non-Hispanic Black men is 1.7 times as high as that among non-Hispanic White men, and mortality is 2.1 times as high; incidence and mortality are lower among Hispanic men and Asian men than among White men and non-Hispanic Black men.[1]

Measurement of prostate-specific antigen (PSA), a protein secreted by both normal and malignant prostate epithelial cells, was approved by the Food and Drug Administration (FDA) in 1986 for use in monitoring patients with known prostate cancer and later (in 1994) as an aid in the detection of prostate cancer in conjunction with digital rectal examination in patients 50 years of age or older.[2,3]

0022-1554/81/0605-0504$02.50
The Journal of Histochemistry and Cytochemistry

Vol. 29, No. 4, pp. 577–580, 1981
Printed in U.S.A.

Brief Reports

Use of Avidin-Biotin-Peroxidase Complex (ABC) in Immunoperoxidase Techniques:

A Comparison between ABC and Unlabeled Antibody (PAP) Procedures

SU-MING HSU, L. RAINE, and H. FANGER

Department of Pathology, Rhode Island Hospital and Division of Biological and Medical Sciences, Brown University, Providence, Rhode Island 02902

Received for publication June 23, 1980 and in revised form November 3, 1980 (BR 80-110)

The use of avidin-biotin interaction in immunoenzymatic techniques provides a simple and sensitive method to localize antigens in formalin-fixed tissues. Among the several staining procedures available, the ABC method, which involves an application of biotin-labeled secondary antibody followed by the addition of avidin-biotin-peroxidase complex, gives a superior result when compared to the unlabeled antibody method. The availability of biotin-binding sites in the complex is created by the incubation of a relative excess of avidin with biotin-labeled peroxidase. During formation of the complex, avidin acts as a bridge between biotin-labeled peroxidase molecules; and biotin-labeled peroxidase molecules, which contain several biotin moieties, serve as a link between the avidin molecules. Consequently, a "lattice" complex containing several peroxidase molecules is likely formed. Binding of this complex to the biotin moieties associated with secondary antibody results in a high staining intensity.

KEY WORDS Avidin; Biotin; Immunoenzymatic techniques.

0022-1554/91/$3.30
The Journal of Histochemistry and Cytochemistry

Vol. 39, No. 6, pp. 741–748, 1991
Printed in U.S.A.

Rapid Communication

Antigen Retrieval in Formalin-fixed, Paraffin-embedded Tissues: An Enhancement Method for Immunohistochemical Staining Based on Microwave Oven Heating of Tissue Sections

SHAN-RONG SHI, MARC E. KEY,[1] and KRISHAN L. KALRA

BioGenex Laboratories, San Ramon, California 94583.

Received for publication January 15, 1991; accepted March 12, 1991 (IC2212).

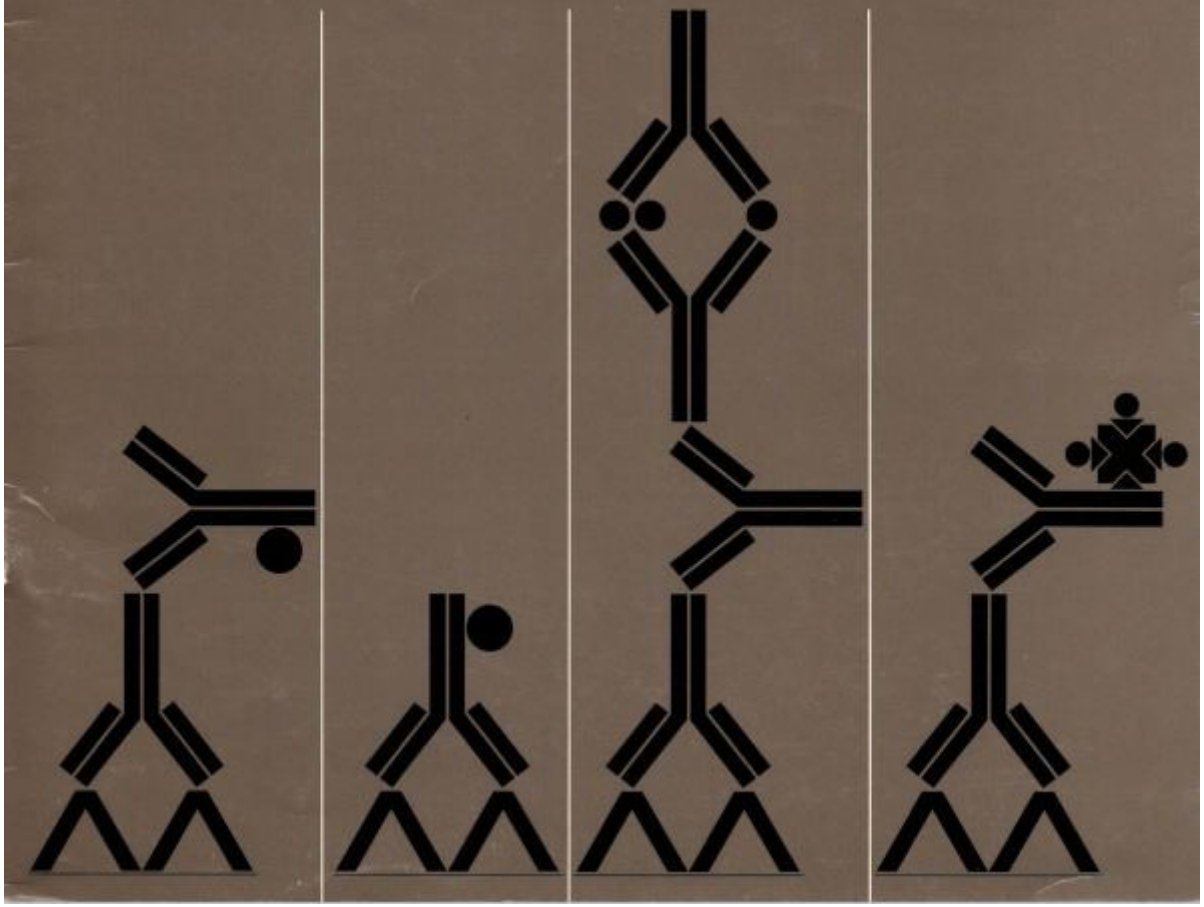
We describe a new approach for retrieval of antigens from formalin-fixed, paraffin-embedded tissues and their subsequent staining by immunohistochemical techniques. This method of antigen retrieval is based on microwave heating of tissue sections attached to microscope slides to temperatures up to 100°C in the presence of metal solutions. Among 52 monoclonal and polyclonal antibodies tested by this method, 39 antibodies demonstrated a significant increase in immunostaining, nine antibodies showed no change, and four antibodies showed reduced immunostaining. In particular, excellent immunostaining results were obtained with a monoclonal antibody to vimentin as well as several different keratin antibodies on routine formalin-fixed tissue sections after pre-treatment of the slides with this method. These results showed that after antigen retrieval: (a) enzyme predigestion of tissues could be omitted; (b) incubation times of primary antibodies could be significantly reduced, or dilutions of primary antibodies could be increased; (c) adequate staining could be achieved in long-term formalin-fixed tissues that failed to stain by conventional methods; and (d) certain antibodies which were typically unreactive with formalin-fixed tissues gave excellent staining. (*J Histochem Cytochem 39:741–748, 1991*)

KEY WORDS: Immunohistochemistry; Antigen retrieval; Formalin-fixed tissue; Paraffin sections; Microwave.

Handbook of Immunoperoxidase Staining Methods

Janice A. Bourne

Immunochemistry Laboratory
DAKO CORPORATION



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DIAGNOSIS

REMARKS

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Chapter Three

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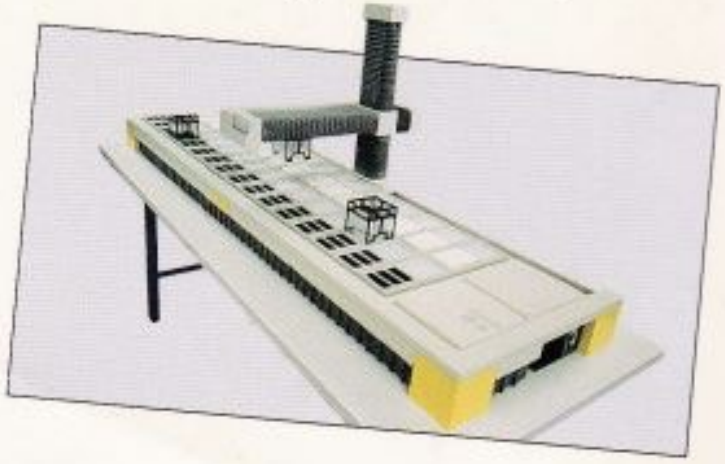
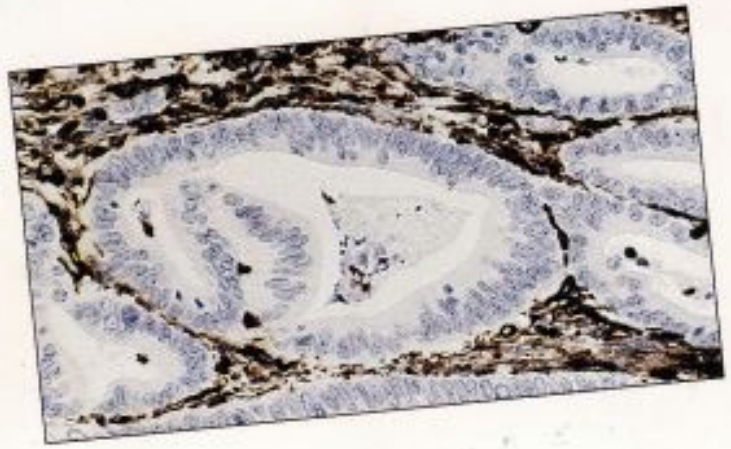
- Runs up to 5 Protocols Simultaneously
- Full On-site Support and Training



Immunodetection of Vimentin

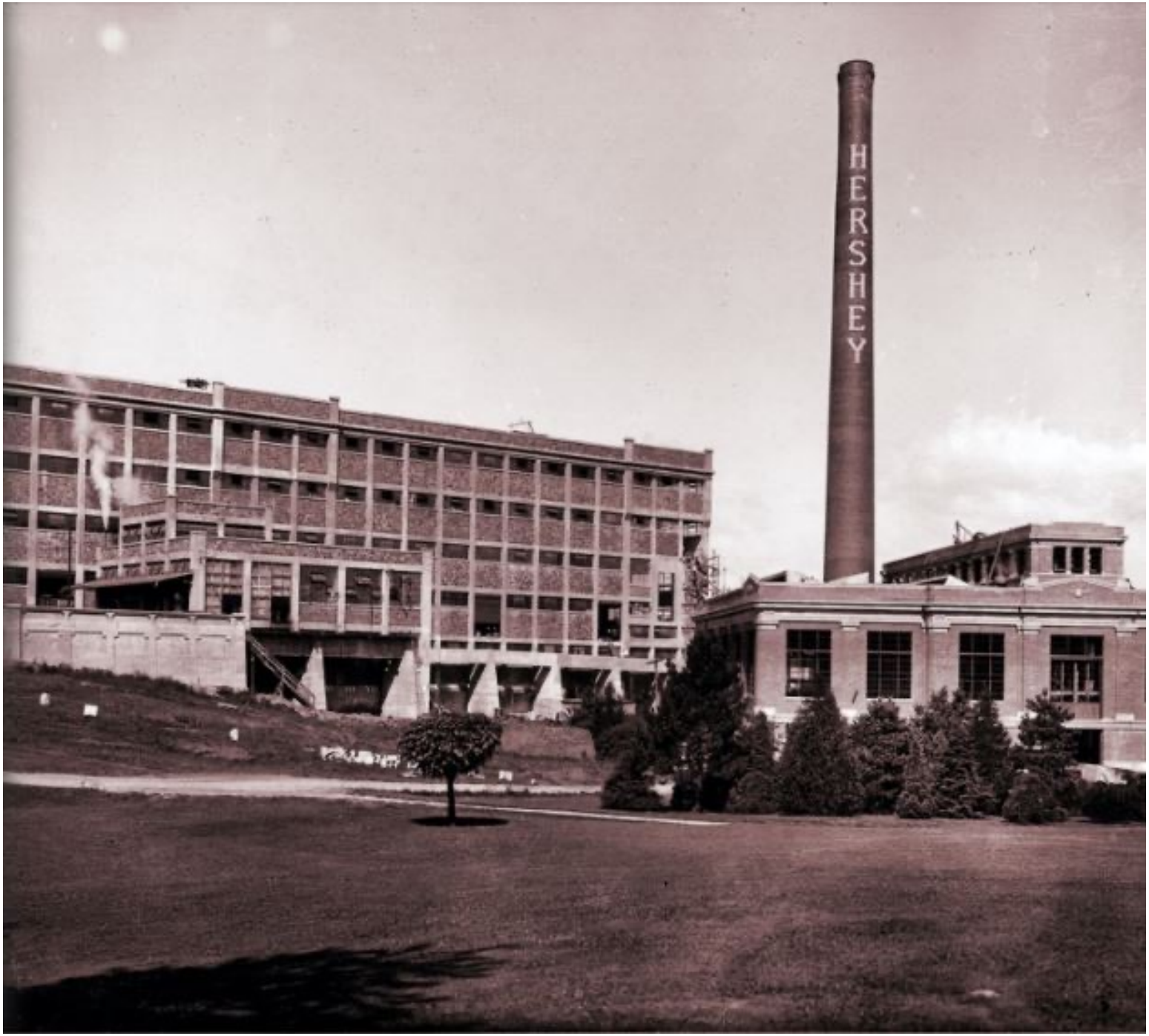
Stain Slide "B" with
anti-Vimentin and
Compare to Slide "A"



Milton S. Hershey Medical Center, Hershey, PA

“Fisher Code-On Stainer”

Courtesy of Nina Rodenroth!

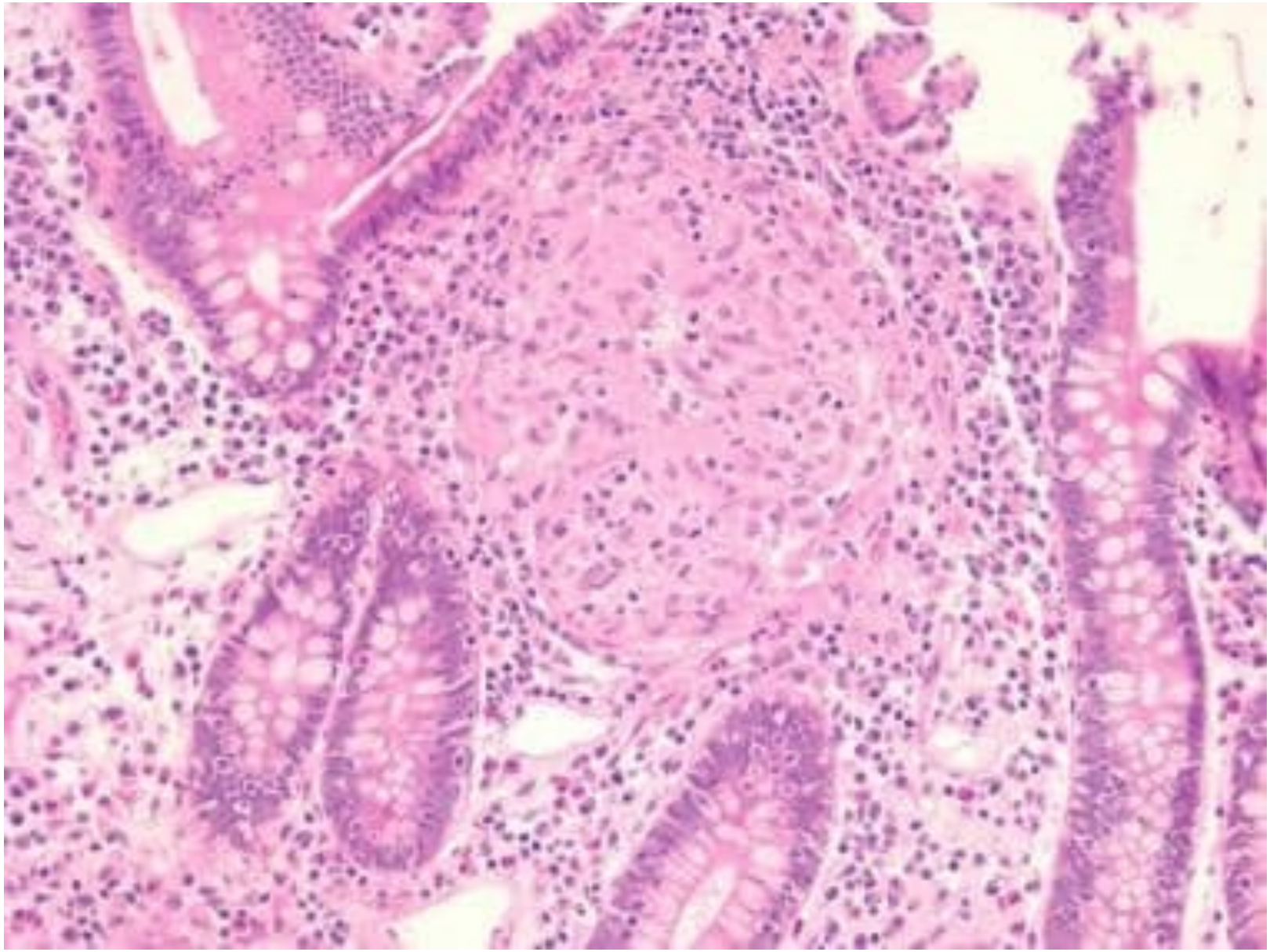

HERSHEY


HERSHEY'S
KISSES
Reese's
PEANUT BUTTER CUPS

“Your immunoperoxidase slides are ready ……”

Milton S. Hershey Medical Center, Hershey, PA

Herbert Van Kruiningen, DVM, PhD, MD
Pathobiology Department
University of Connecticut, Storrs, CT



Crohn’s Disease - Granuloma

Chapter 5

“Infectious Diseases”

› Mod Pathol. 1993 Mar;6(2):212-9.

An immunocytochemical search for infectious agents in Crohn's disease

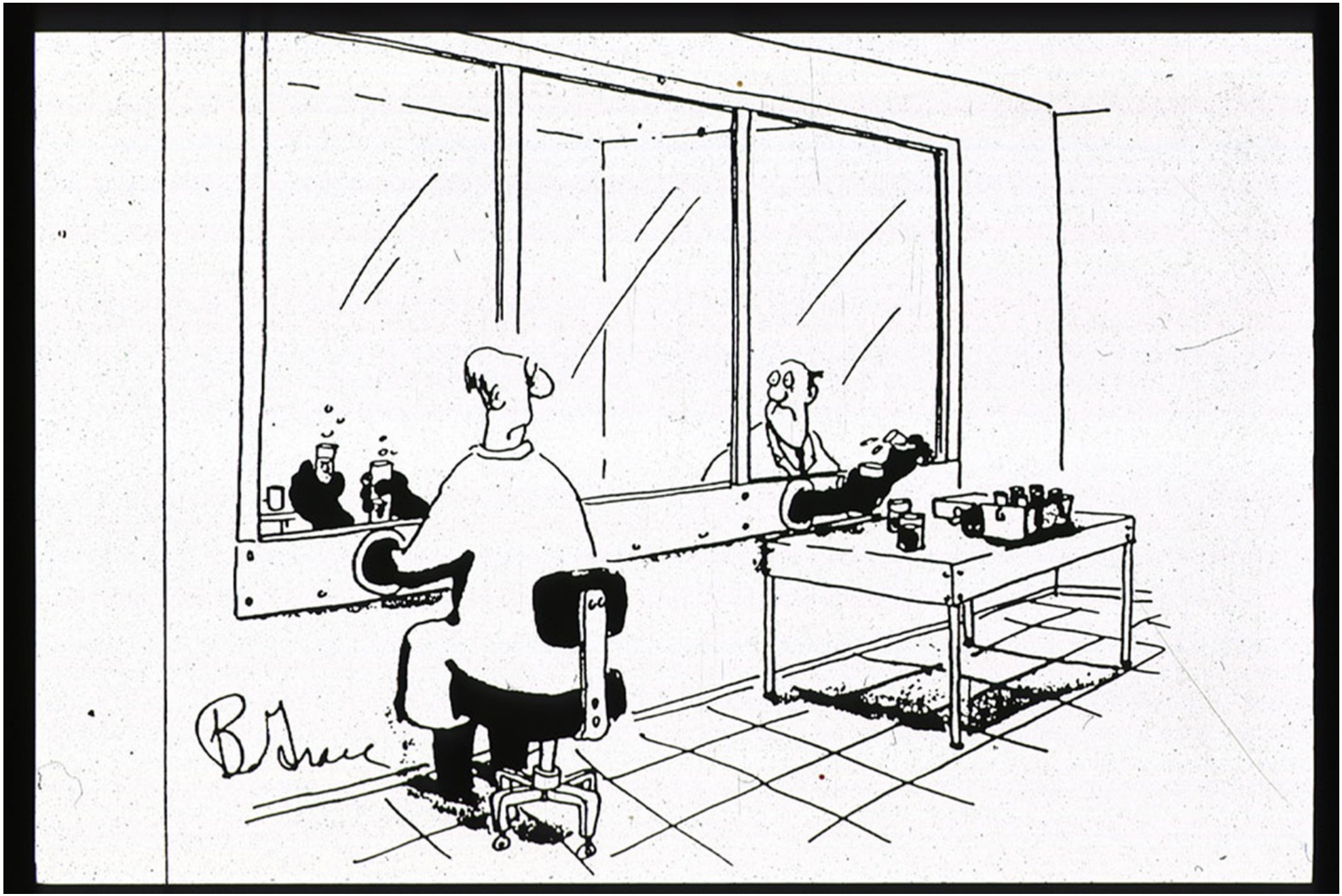
R W Cartun [1], H J Van Kruiningen, C A Pedersen, M M Berman

Affiliations + expand

PMID: 8483893

Abstract

Microorganisms have long been suspected of causing Crohn's disease (CD); however, an etiologic agent has yet to be identified. Few studies have employed immunocytochemistry (ICC) to examine tissue from patients with CD for microbial antigens. We investigated 36 formalin-fixed tissues from 16 patients with CD with ICC. No evidence of adenovirus, Borrelia, Brucella, BVDV, Campylobacter, Campylobacter-like organisms, Chlamydia, coronavirus, CMV, EBV, Legionella, mycobacteria, Pseudomonas, rotavirus, Salmonella, Shigella, staphylococci, Toxoplasma gondii, Treponema, or Yersinia was found. ICC identified E. coli and streptococcal antigens in 11 (69%) and 10 (63%) of the 16 cases studied, respectively. Escherichia coli immunoreactivity was located in ulcers, within the lamina propria, and along fissures. Streptococcal immunolabeling occurred within mucosal epithelial cells, in the lamina propria, in ulcers, along fissures, in granulomatous inflammation including multinucleate giant cells, and in lymph nodes. These results suggest that some of the granulomas in CD may result from immunologic processing of bacterial antigens following their penetration through a compromised mucosa. E. coli and streptococcal antigens may contribute to the pathogenesis of CD.



CASE REVIEW

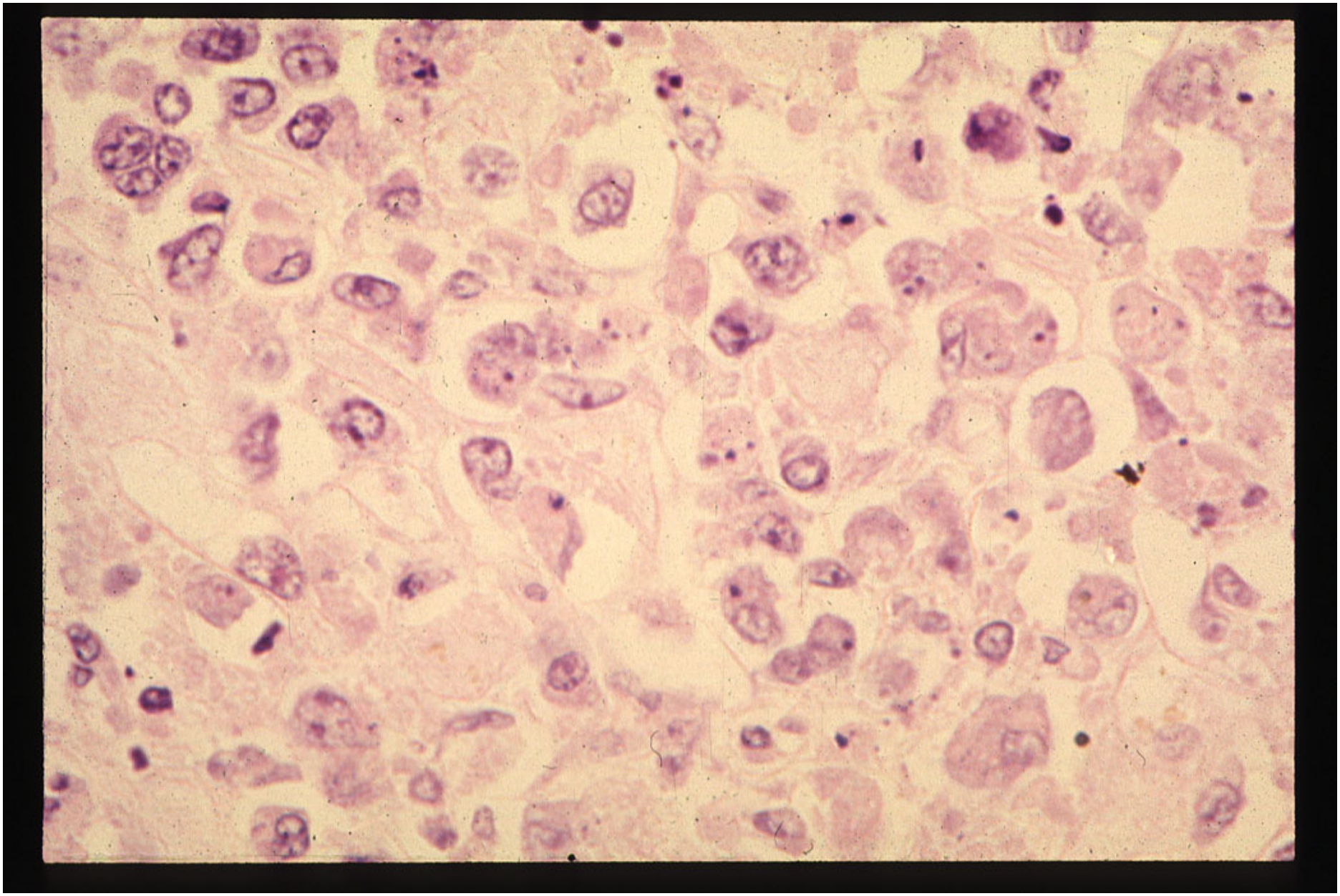
Use of Immunohistochemistry in the Surgical Pathology Laboratory for the Diagnosis of Infectious Diseases

Richard W. Cartun, PhD

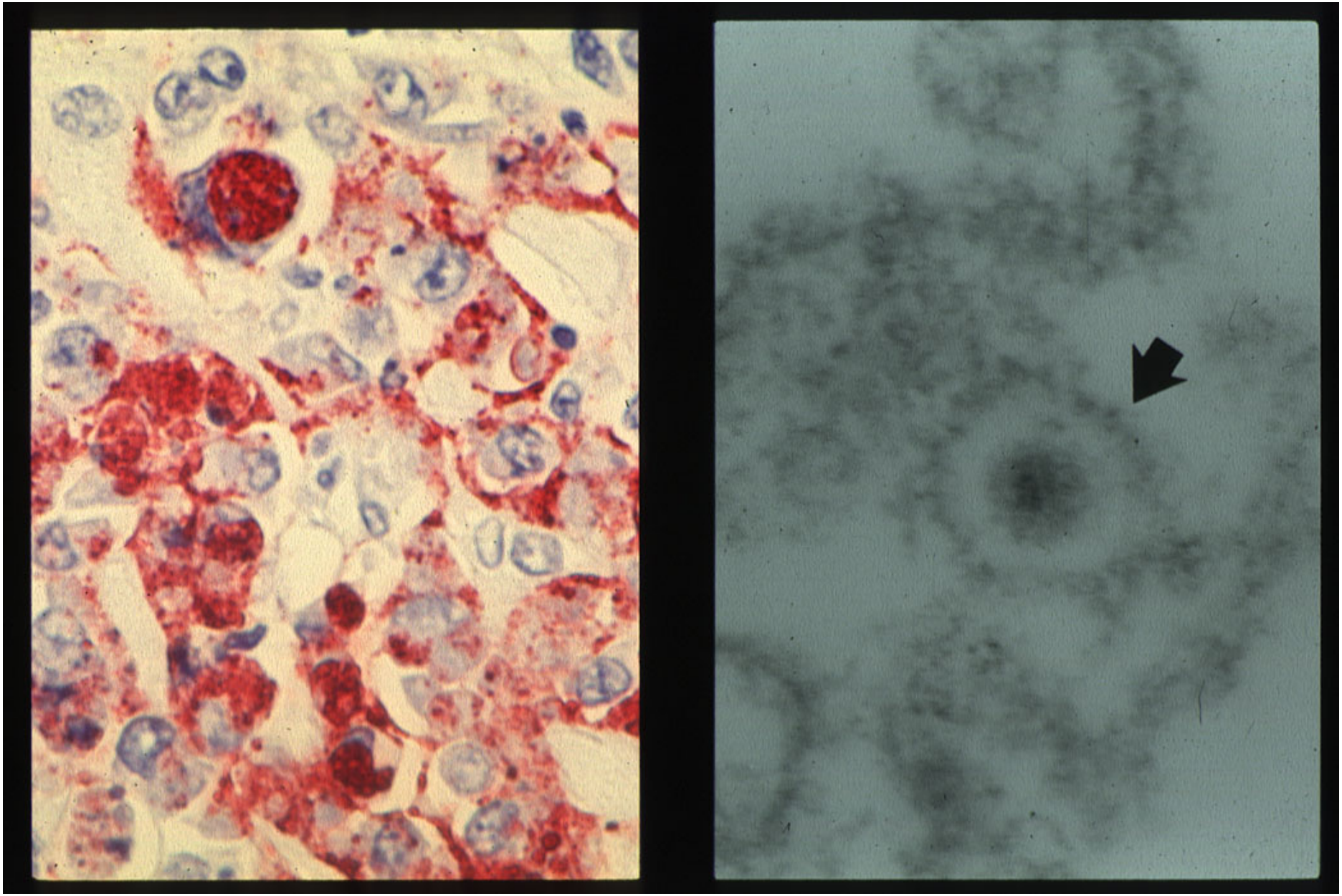
The use of immunohistochemistry (IHC) has revolutionized the practice of surgical pathology during the past twenty years. Although significant advances have occurred in the identification and classification of solid tumors, the immunophenotyping of malignant lymphoma and leukemia, and the identification of hormone receptors and other prognostic markers, the area of infectious disease diagnosis remains somewhat overlooked, certainly underused. However, as awareness of the advantages of using IHC for this application grows, and as new commercially available antibodies make their way to the IHC laboratory, surgical pathologists will have a powerful tool to diagnose and investigate infectious agents in tissue specimens as laboratory medicine enters the twenty-first century.

pneumococcal antigens in pneumococcus-infected tissue more than 50 years ago.[3] In addition, chapters on the identification of hepatitis B viral antigens and the detection of human papillomaviruses appeared in two of the first monographs written on IHC in the early 1980's.[4,5] More recently the use of IHC for infectious disease diagnosis has been detailed in several review articles and books.[6–8] The intent of this article is to provide the pathologist with an overview of the use of IHC for infectious disease diagnosis, not to discuss specific applications which are covered elsewhere.[6–8]

Case Report



Autopsy Heart - H&E



HIV p24 Protein - IP Electron Microscopy

Cat Scratch Disease

R. A. Griesemer, D.V.M., Ph.D., and
L. G. Wolfe, D.V.M., Ph.D.

SUMMARY

Cat scratch disease is a self-limiting, infectious disease of man characterized by regional lymphadenopathy following the scratch or bite of a cat. The disease is widespread and affects thousands of people annually. The risk is greatest for children and veterinarians.

The etiologic agent, presumably a virus, is unknown. The role of the cat in the transmission of the disease is also unknown, although cats appear to serve merely as vectors. There is no evidence to incriminate any of the known feline viruses as etiologic agents.

CAT SCRATCH DISEASE (cat scratch fever, nonbacterial regional lymphadenitis, benign inoculation lymphoreticulosis) is an infectious disease of man of unknown etiology. It has been the subject of some 600 articles and several reviews.[3,15,24,30,31] Typically, a cat scratch or bite is followed in about 7 to 12 days (range 3 to 61 days) by a papule at the site of injury that sometimes develops into a pustule. Several weeks later (range 1 to 12 weeks) the regional lymph node or nodes enlarge, become painful, and may suppurate. The swollen lymph nodes regress spontaneously in 2 to 6 weeks, although persistent lymphadenopathy infrequently occurs.

Constitutional signs are usually mild, occur in about 15% of patients. The most frequently reported is an oculoglandular syndrome, and less frequent reports deal with thrombocytopenic purpura,[4] erythema nodosum,[28] encephalitis,[1,23] osteolysis,[6] pneumonitis,[16] pharyngeal angina,[14] and mesenteric adenitis.[33] The finding of atypical clinical forms and lesions in a variety of organ systems raises the question whether cat scratch disease is a single entity.[31] It would not be surprising if some patients had concurrent but unrelated illnesses during the clinical course of cat scratch disease.

Microscopic examination of skin lesions[10] or enlarged nodes[34] reveals pyogranulomatous inflammation which must

› Science. 1983 Sep 30;221(4618):1403-5. doi: 10.1126/science.6612349.

Cat scratch disease: a bacterial infection

D J Wear, A M Margileth, T L Hadfield, G W Fischer, C J Schlagel, F M King

PMID: 6612349 DOI: 10.1126/science.6612349

Abstract

Histopathologic examination of lymph nodes from 39 patients with clinical and pathological criteria for cat scratch disease revealed delicate pleomorphic Gram-negative bacilli in 34 of the 39 nodes. They were within the walls of capillaries in or near areas of follicular hyperplasia and within microabscesses. They were best seen with the Warthin-Starry silver impregnation stain. Organisms in lymph node sections exposed to convalescent serum from three patients and to immunoperoxidase stained equally well with all three samples. The organisms did not react with hyperimmune sera to Legionella pneumophila nor to several species of Rickettsia. These bacilli appear to be the causative agents of cat scratch disease.

February 2001
REV 020501

What's New

CD117

CD117 c-kit recognizes a protein of 145kDa identified as CD117/p145^{kit}. This antibody cocktail recognizes the extracellular domain and is expressed by a variety of normal and abnormal cell types. In normal cells, the CD117 antibody has been shown to label breast epithelium, germ cells, melanocytes, stem cells and mast cells. In abnormal cells, it has been shown to label testicular germ cells, endometrial carcinomas, papillary and follicular thyroid carcinomas, small cell carcinomas, melanomas and ovarian epithelial carcinomas. It has also been shown to be an effective marker for mast cell disorders, gastrointestinal stromal tumors and immunotyping of blasts in human bone marrow.

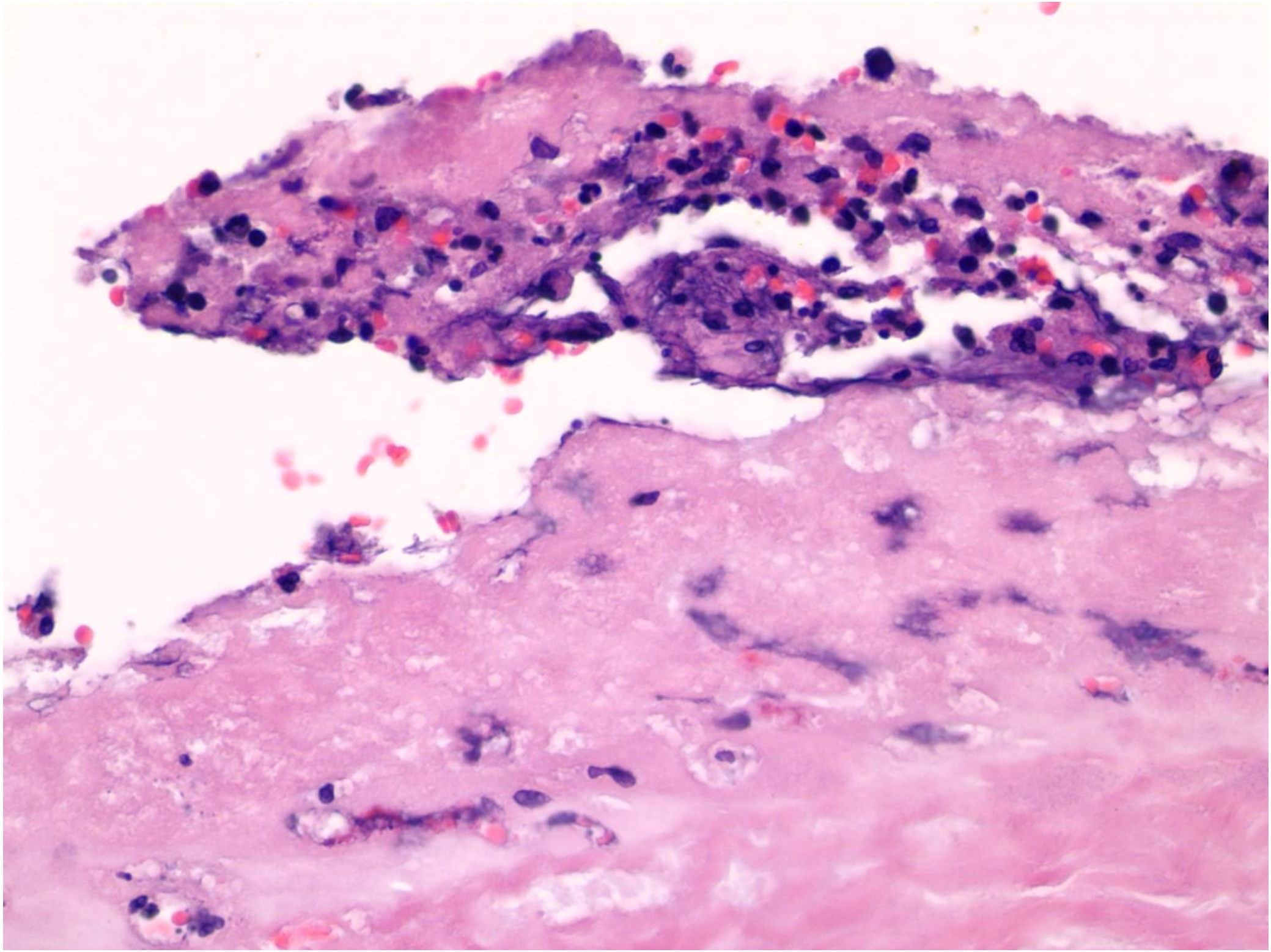
Cat Scratch Fever

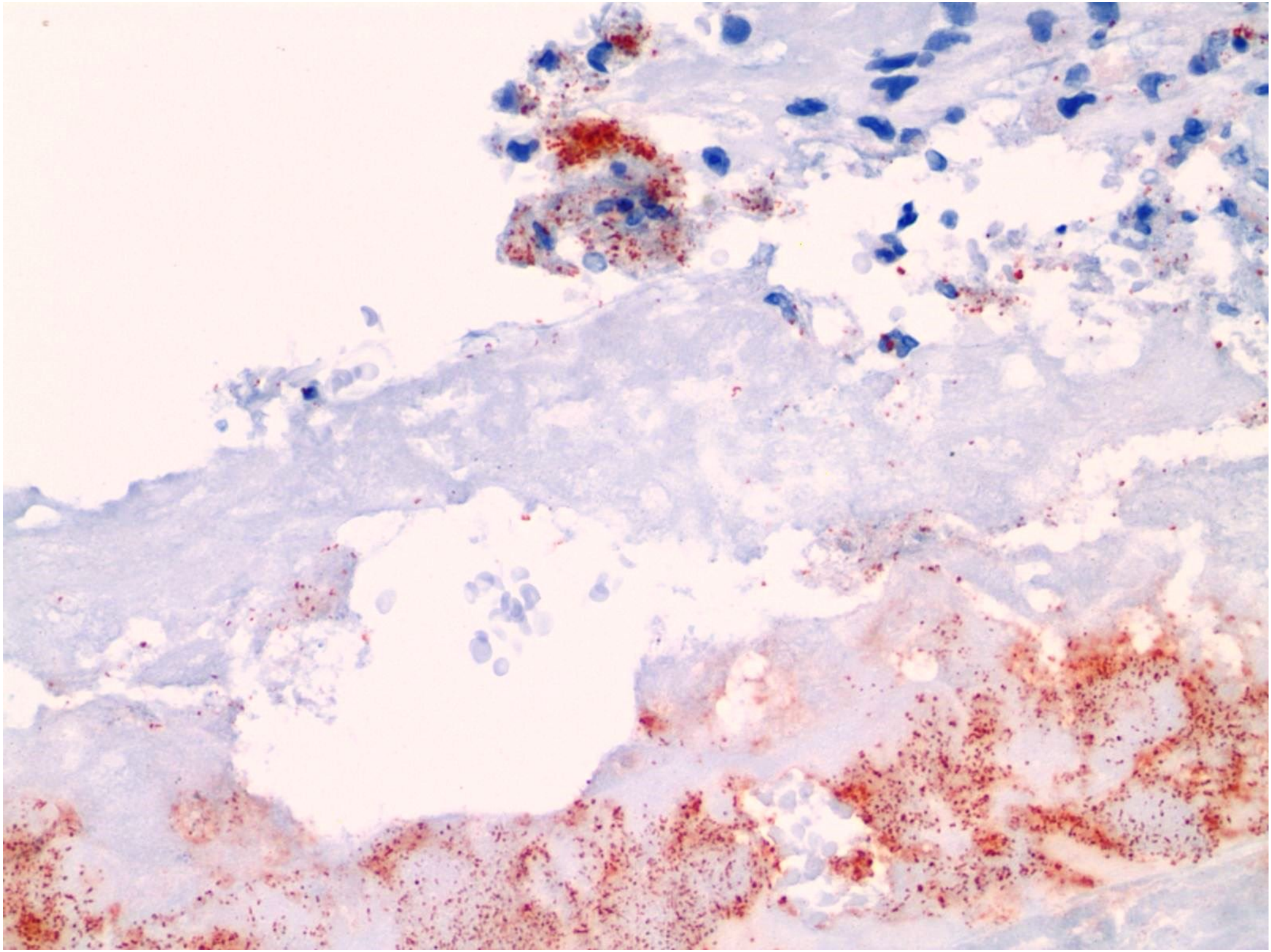
The causative bacterial agent of cat scratch disease has been identified as bartonella henselae. In the past, complicated silver stains and/or PCR were used to identify and confirm this agent. This monoclonal antibody identifies bartonella henselae in formalin-fixed paraffin-embedded tissues. Cross-reactivity tests were performed on (12) Bartonella henselae strains, (11) Bartonellaquintana strains, (2) Bartonella bacilliformis strains, (1) B. elizabethae, (1) B. grahamii, (1) B. taylorii, (1) B. doshiae, and (1) B. vinsonii strains. Reactivity was only obtained with Bartonella henselae. All B. henselae strains were found positive with this MAb. We have also tested spirochete, helicobacter pylori and tuberculosis and all were also found negative.

Cryptococcus Neoformans

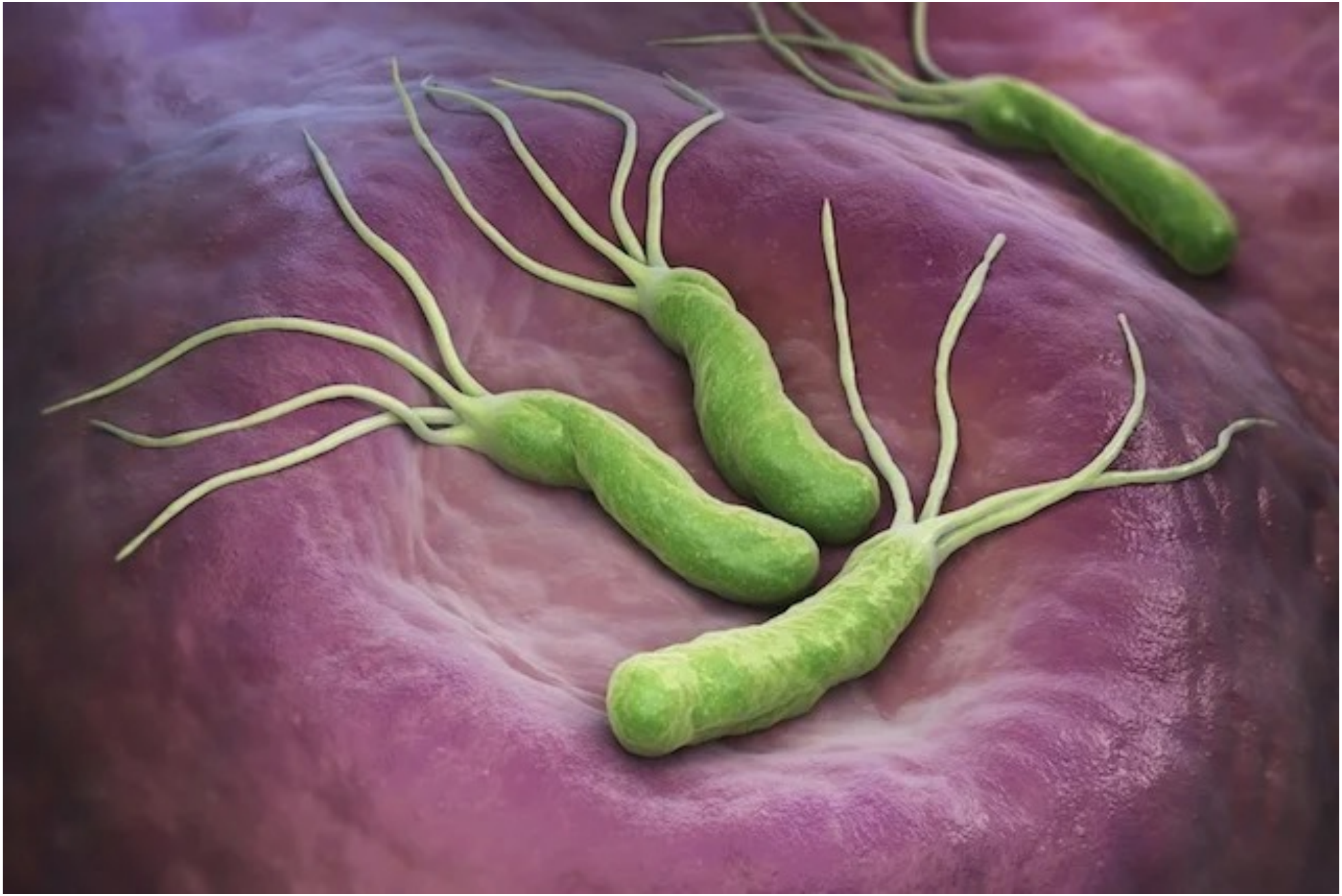
Cryptococcus neoformans, an encapsulated yeast, is a causative agent in pulmonary infections. It has been associated with meningitis and can be fatal in immune-suppressed patients. This antibody reacts with cryptococcus neoformans in formalin-fixed tissues. In the past, silver stains and Southgate Mucicarmine were used to identify this agent. This antibody has been shown to cross-react with C. albidus and Torulopsis glabrata, but not Candida albicans.


SOUNDS OF THE SEVENTIES • GUITAR POWER
1. Cat Scratch Fever Ted Nugent
2. Saturday Night Special Lynyrd Skynyrd
3. Let It Ride Bachman-Turner Overdrive
4. Lady Love Robin Trower
5. Paranoid Black Sabbath
6. Statesboro Blues The Allman Brothers Band
7. Ready for Love Bad Company
8. Locomotive Breath Jethro Tull
9. Under My Wheels Alice Cooper
10. Why Does Love Got to Be So Sad Derek and the Dominos
11. Ride with Me Steppenwolf
12. Blues Power Eric Clapton
13. Black Night Deep Purple
14. Train, Train Blackfoot
15. Juke Box Music The Kinks
16. Rocky Mountain Way Joe Walsh
17. Blue Morning, Blue Day Foreigner
18. Beggars Day Crazy Horse
SOUNDS OF THE SEVENTIES
GUITAR POWER
SEE PROGRAM NOTES INSIDE
TIME LIFE MUSIC
WARNER SPECIAL PRODUCTS

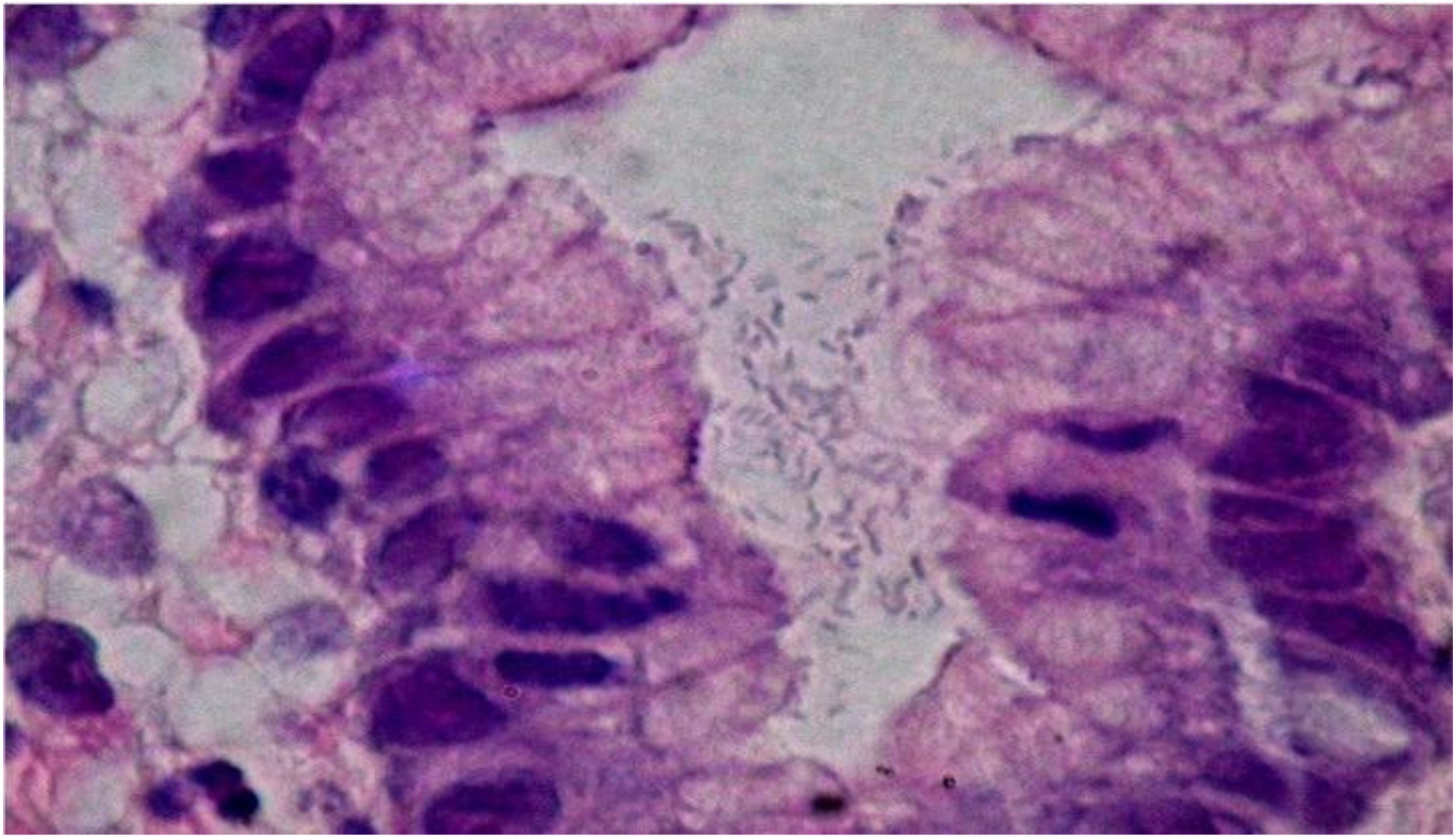




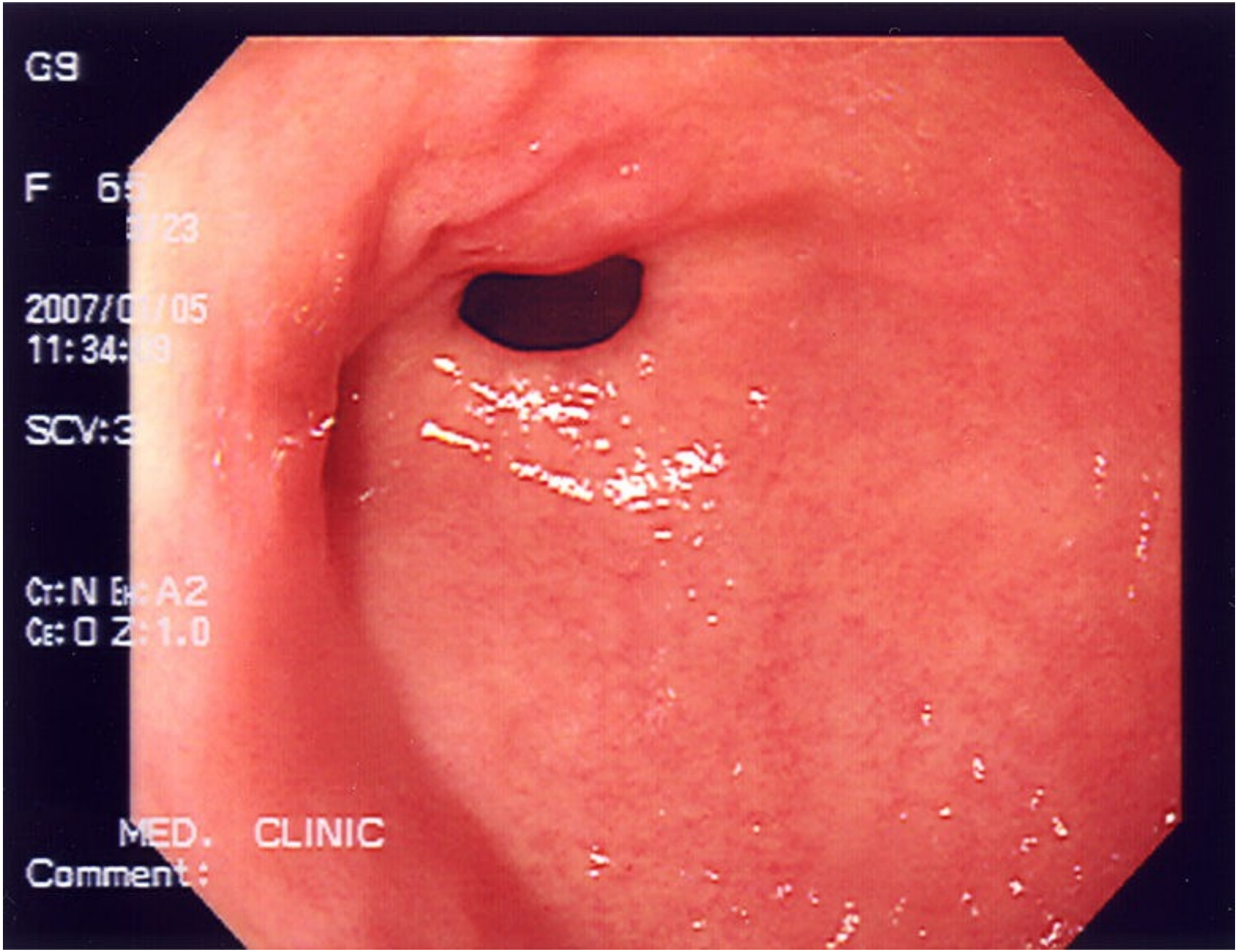
Barry J. Marshall
and
J. Robin Warren



Helicobacter pylori

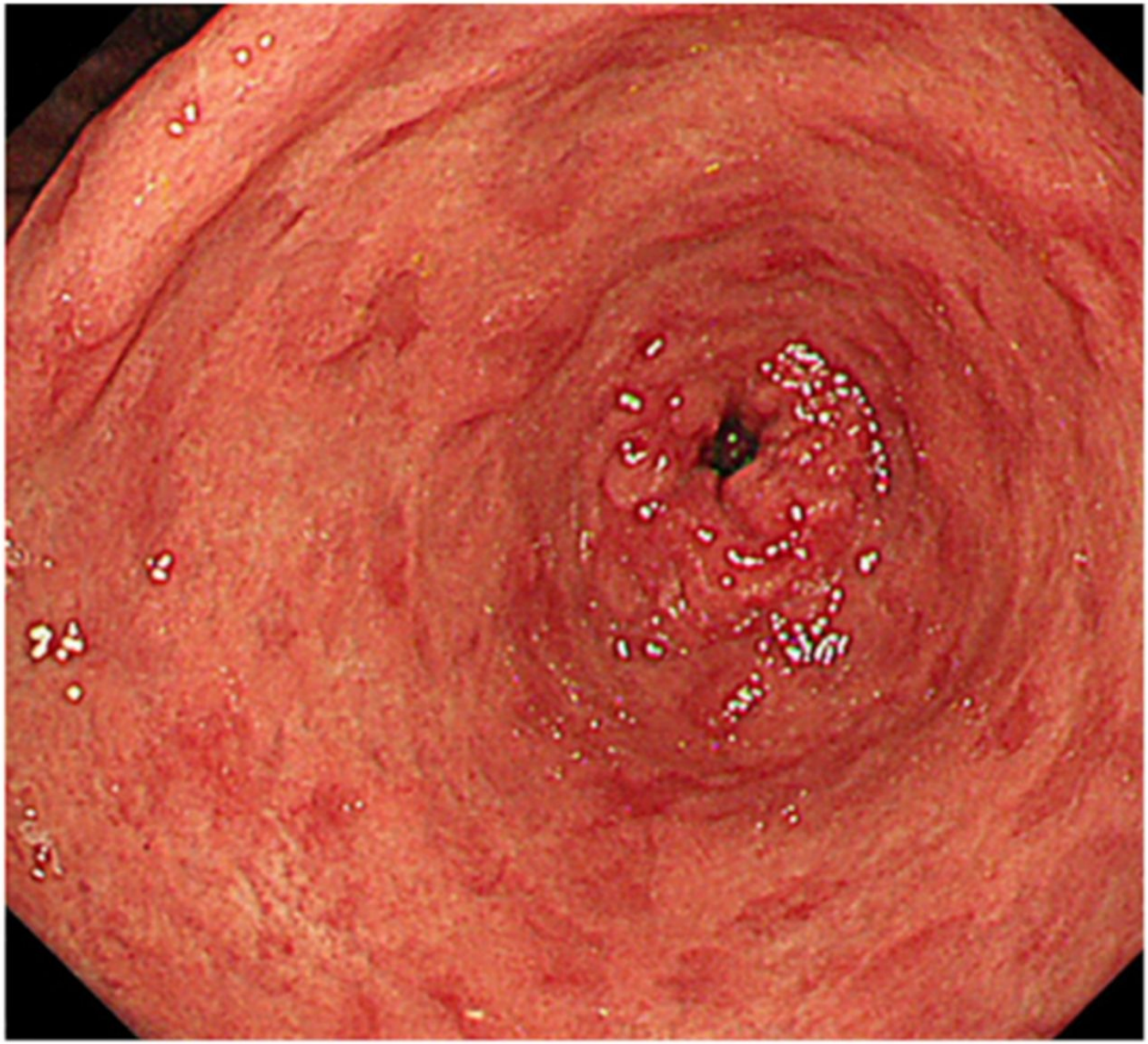


Helicobacter pylori - H&E

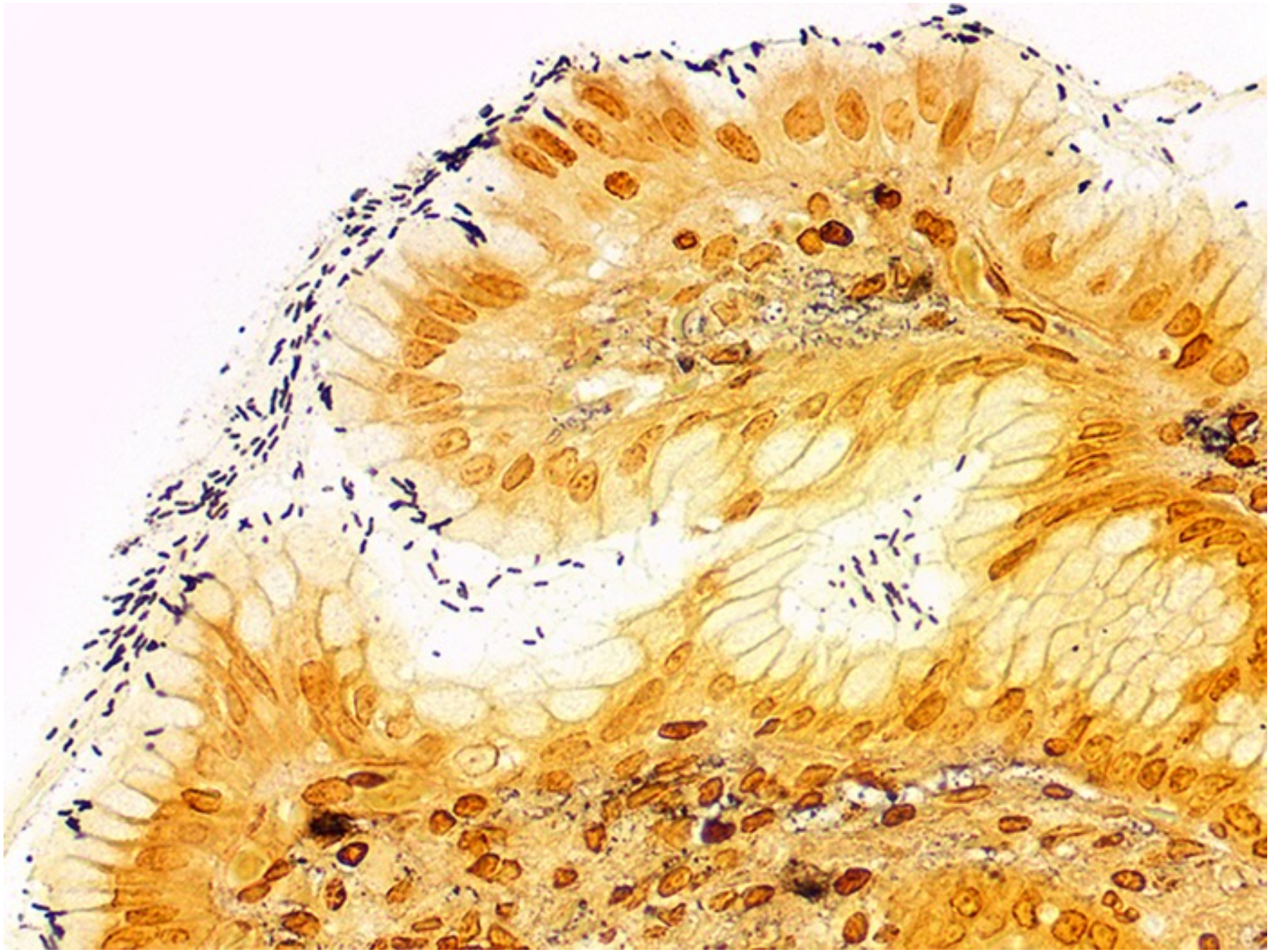


Endoscopy of Stomach - Normal

Culture of *H. pylori*



Endoscopy of Stomach - Gastritis



H. pylori - Silver Histochemical Stain

3 October 2005

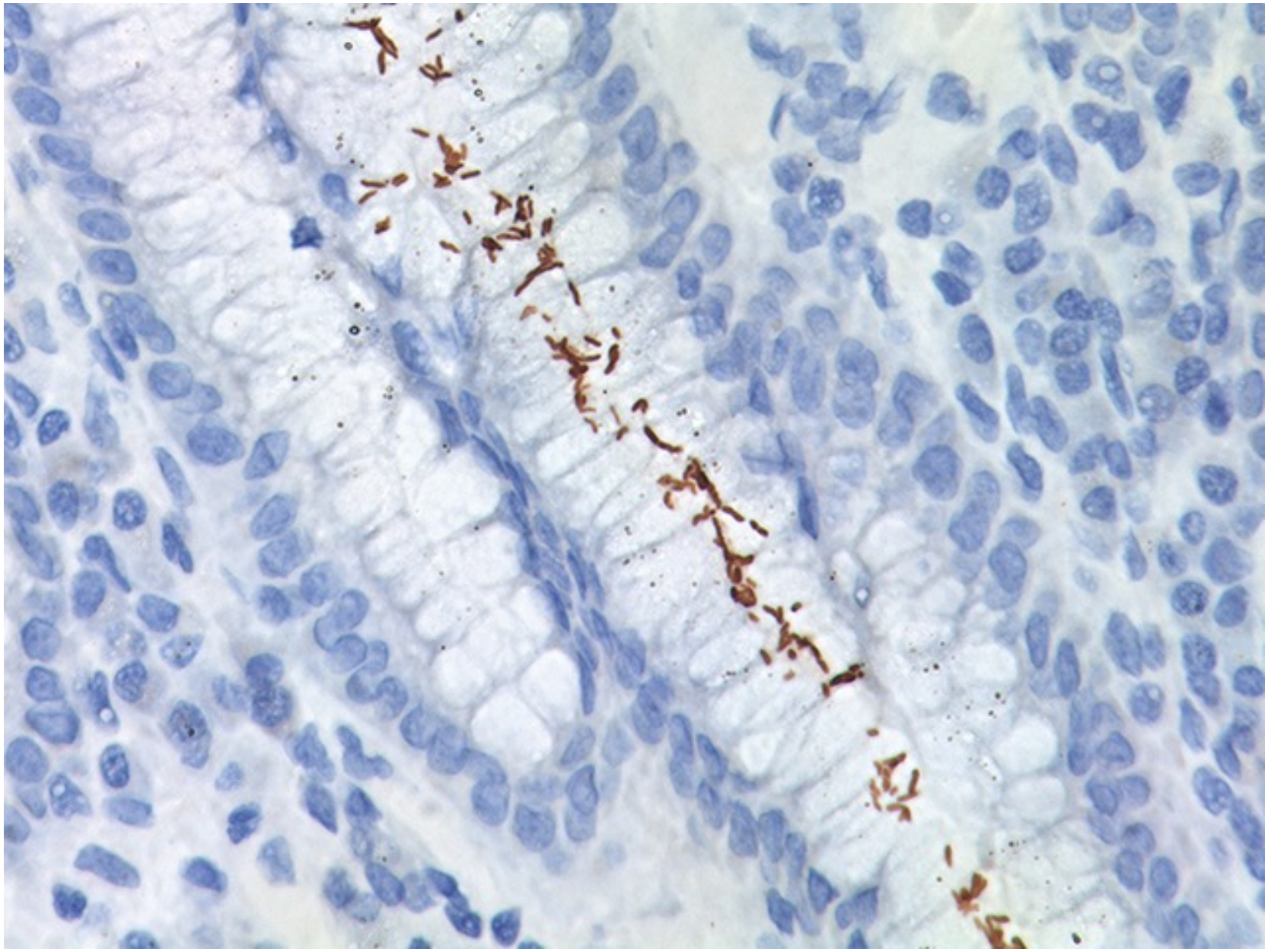
The Nobel Assembly at Karolinska Institutet has today decided to award
The Nobel Prize in Physiology or Medicine for 2005 jointly to

Barry J. Marshall and **J. Robin Warren**

for their discovery of **"the bacterium *Helicobacter pylori* and its role in gastritis and peptic ulcer disease"**

Summary

This year's Nobel Laureates in Physiology or Medicine made the remarkable and unexpected discovery that inflammation in the stomach (gastritis) as well as ulceration of the stomach or duodenum (peptic ulcer disease) is the result of an infection of the stomach caused by the bacterium *Helicobacter pylori*.



H. pylori - Immunoperoxidase

› Mod Pathol. 1991 Jul;4(4):498-502.

Immunocytochemical identification of Helicobacter pylori in formalin-fixed gastric biopsies

R W Cartun [1], G A Kryzmowski, C A Pedersen, S G Morin, H J Van Kruiningen, M M Berman

Affiliations + expand

PMID: 1924280

Abstract

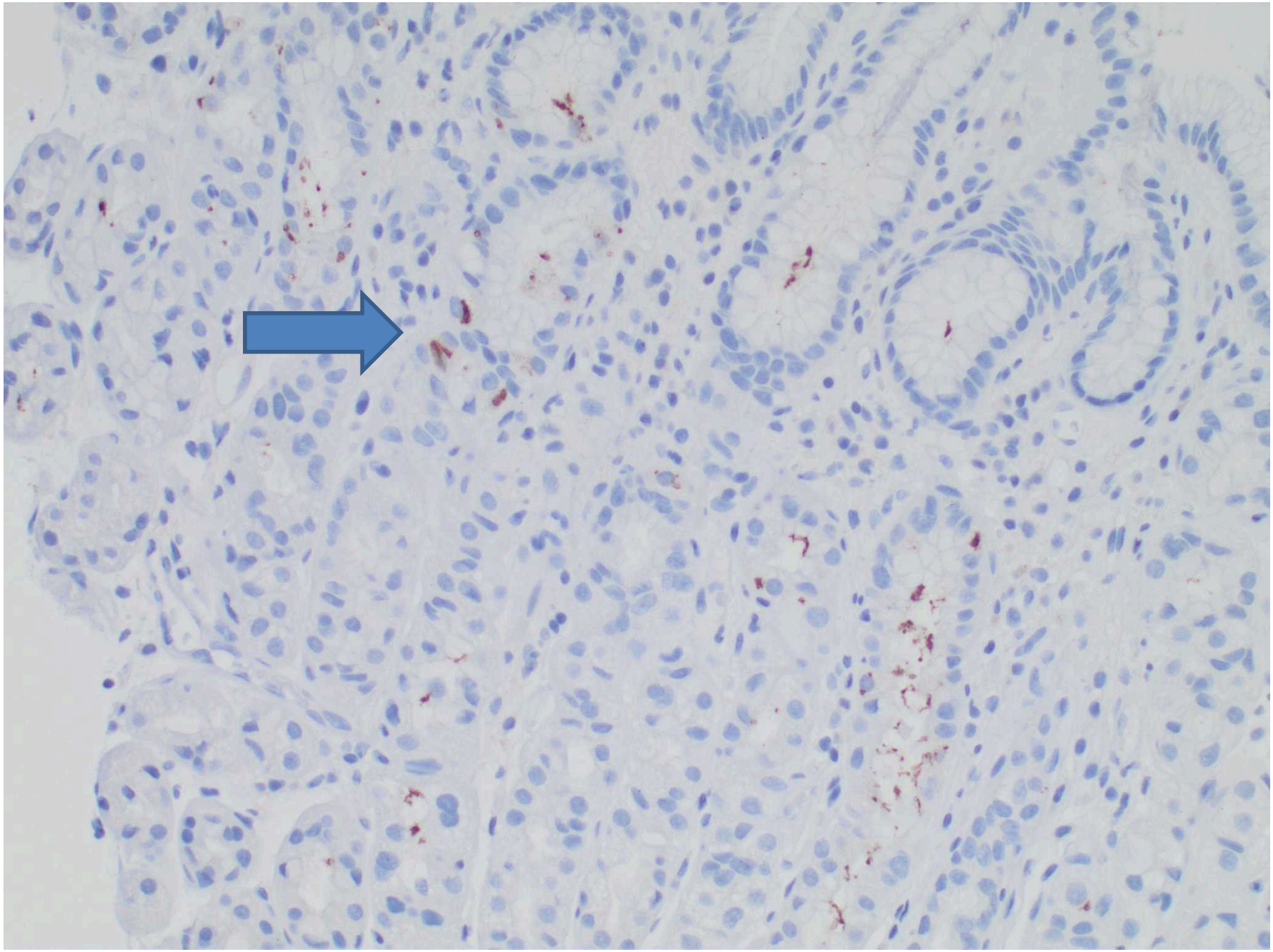
H&E and special histochemical stains are used by most laboratories to identify Helicobacter pylori (H. pylori) in gastric biopsy specimens. However, background staining can complicate recognition of H. pylori and small numbers of organisms may be overlooked. Additionally, histochemical stains do not distinguish H. pylori from other spiral organisms. We investigated two commercially available monoclonal antibodies, one directed against Campylobacter coli and C. jejuni (MAB002) and the other against a Campylobacter species flagellar antigen (MAB001), to evaluate potential use in immunocytochemical examinations of fixed tissues. MAB002 reacted with C. jejuni but not H. pylori organisms. MAB001 labeled C. jejuni as well as H. pylori and, therefore, was used to study 220 gastric biopsies from patients undergoing endoscopy. Acute and/or chronic gastritis was present in 60.5% (133/220) of the biopsies examined. MAB001 positivity was identified in 62.4% (83/133) of the tissues with gastritis. Only 2 of 87 (2.3%) specimens without gastritis demonstrated MAB001 labeling. The resulting immunoreactivity was easily identified, allowing specimens to be screened quickly and accurately. No labeling was seen with the non-Helicobacter/Campylobacter bacteria or normal tissues evaluated in this investigation. MAB001 can be used to identify H. pylori in histologically processed tissue and will assist pathologists, clinicians, and researchers studying the distribution and pathogenicity of this organism in humans and animals.

Original Article

Is the Sanctuary Where *Helicobacter pylori* Avoids Antibacterial Treatment Intracellular?

L. ENGSTRAND, MD,[1] DY GRAHAM, MD,[3,4,7] A. SCHEYNIUS, MD,[2] R.M. GENTA, MD,[4–6] AND FAK EL-ZAATARI, PhD[3,4]

The sanctuary site where *Helicobacter pylori* evades antimicrobial therapy is unknown, but considerable data exist about an intracellular location for *H pylori*. Ten *H pylori*–infected volunteers received standard triple antimicrobial therapy for 2 weeks. Gastric mucosal biopsy specimens were obtained with jumbo forceps on therapy days 0, 3, 14, and 42. Hematoxylin-eosin staining was used for classification of gastritis and the Genta stain for the visualization of *H pylori*. Immunohistochemical staining was used to detect HLA-DR antigens, human heat shock protein (HSP60), and the bacterial HSP60 antigen. Bacterial HSP60 was expressed on the mucosal surface and within epithelial cells. No such expression of human HSP60 was found, which supports a bacterial origin for the intracellular HSP60. Coexpression of bacterial HSP60 and HLA-DR was always observed, indicating an ongoing local immune response. Infection was cleared on day 14, but when examined 4 weeks after completion of therapy, Genta staining indicated that only five volunteers remained free of *H pylori*. However, results of immunohistochemical staining were negative at this time for only two volunteers. Disappearance of intracellular expression of bacterial HSP60 remained after therapy and correlated with the intensity of chronic inflammatory cell infiltration. These data are consistent with the intracellular localization of *H pylori* having a role in inflammation and as a protective strategy against extracellular antibacterial activity. (Key words: *Helicobacter pylori;* Antimicrobial therapy; Intracellular compartment; Gastritis; Carcinogen) Am J Clin Pathol 1997;108:504–509.



H. Pylori - Immunoperoxidase

Chapter 6

“Antibodies”


Monoclonal Anti-Cytokera
MAK-6 T.M.
KA-4 Anti-Cytokeratin Types 14
UCD/PR-10.11 Anti-Cytokeratin
Contents: 0.5ml. (lyophilized)
Storage: lyophilized 2-8°C
FOR RESEARCH USE
not for use in diagnostic
Triton Biosciences
TEIN ANTISERUM,
YOPHILIZED
th 0.5 mL of water. Store
0.1% sodium azide or at
aliquots.
CH PURPOSES ONLY
Rec 2-1-91
IN VITRO TE
ANTI pS2™
ANTICORPS MONOCLONAL
PH./LIOFIL. + 3 ml H2O
Date d'exp./Exp. date
Verf.-D./Data di scad.
Fecha de cad.:
JUL. 92
CIS bio International
Anti-Leu-M1 (CD15)
STORE AT 2° TO 8°C.
research use only. Not for
in human diagnostic or
therapeutic procedures.
Becton Dickinson Immunocytometry
Systems, Mtn. View, CA 94043
23-1365-00
CHEMICON
TEMECULA, CA 92390
(714) 676-0080
ANTI-ER
LOT 8588
1.28 MG/M
dako corp.
MAb
ktail-CK22
04/9
T Cell, CD43
(DAKO-CD43)
Supernatant, 15mM NaN3
Store at 4-8°C or -20°C
Made by DAK
Monoclonal mouse
to human
Monoclonal
Progest
Store at 2-8°C
3ml antibody in PBS with
carrier protein and
preservative. Use only with
Super Sensitive Kit.

Hartford Hospital

January 13, 1987

Jeffrey Schlom, Ph.D.
Chief, Laboratory of Tumor
Immunology and Biology
Building 10, Room 8B07
National Cancer Institute
National Institutes of Health
Bethesda, MD 20892

Dear Dr. Schlom,

I read with great interest your recent article in Laboratory Investigation, "ras Gene Alterations and Enhanced Levels of ras p21 Expression in a Sprectrum of Benign and Malignant Human Mammary Tissues".

Would it be possible to receive a sample of the RAP-5 monoclonal antibody so that we can experiment with it here at Hartford Hospital? If so, please bill our hospital's Federal Express account number, 0061-1145-9, to cover shipping costs. Also, if possible, could you please reference our P.O. No., 43575, on the Federal Express form.

Thank you for your generosity.

Sincerely,

Richard W. Cartun
Immunopathology Divison

(203) 524-3535

RWC/rc/0364v

Federal Express Account No.

P.O. No.

Hartford Hospital

PATHOLOGY and LABORATORY MEDICINE

January 26, 1990

Roger Warnke, M.D.
Department of Pathology
Stanford University of Medical Center
Stanford, CA 94305

Dear Dr. Warnke:

I recently read your article, **"An Epitope of the Transferrin Receptor is Exposed on the Cell Surface of High-Grade but not Low-Grade Human Lymphomas"**, which appeared in the December issue of *Blood*.

Have you had any success in labeling formalin-fixed, paraffin-embedded tissues with your "Trump" antibody? Is the antibody going to be commercially available? If not, would it be possible to receive a small sample?

Best regards,

Richard

Richard W. Cartun
Immunopathology Laboratory

J Clin Pathol 1984;**37**:975–983

Monoclonal antibody to cytokeratin for use in routine histopathology

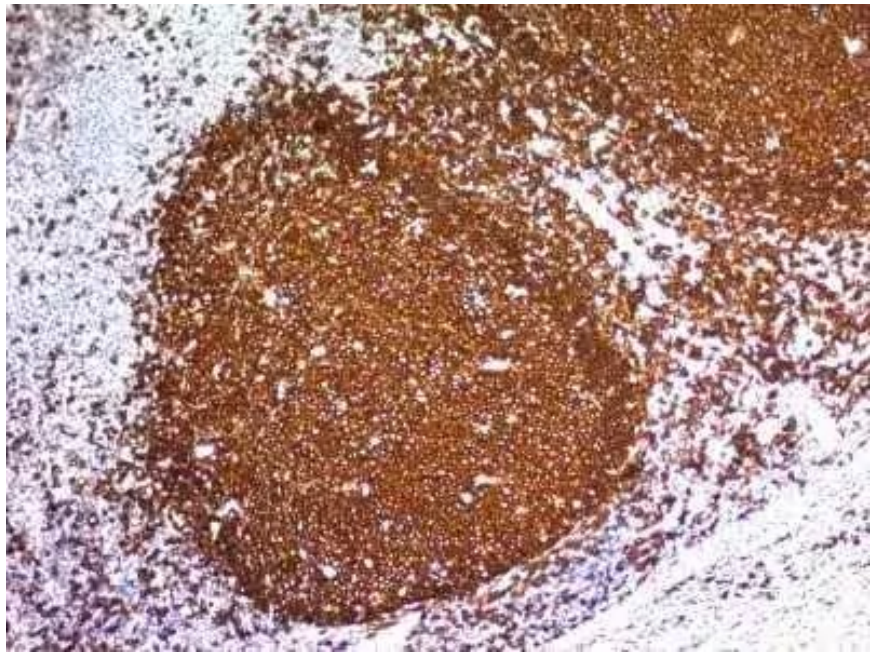
CA MAKIN, LG BOBROW, WF BODMER

From the Imperial Cancer Fund, Lincoln's Inn Fields, London WC2A 3PX, and the Department of Histopathology, University College Hospital Medical School, London WC1E 6JJ

SUMMARY CAM 5.2 is a murine monoclonal antibody, raised against the colon carcinoma cell line HT29, which recognises lower molecular weight intracellular cytokeratin proteins within secretory epithelia. Extensive indirect immunohistochemical studies have confirmed that this antibody stains formalin fixed (and freshly frozen) normal and malignant human tissue in a consistent manner. Reliable staining of conventionally processed pathological tissues provides more accurate identification and staging of human malignant epithelial diseases.

“L26 (CD20) mAb”

- Received sample from DAKO (1986)
- Produced by Dr. Ishii in Japan
- Reactive with B-lymphocytes in FFPE tissues
- Optimal dilution 1:400,000



American Journal of Pathology, Vol. 129, No. 3, December 1987

RAPID COMMUNICATION

Utilization of Monoclonal Antibody L26 in the Identification and Confirmation of B-Cell Lymphomas

A Sensitive and Specific Marker Applicable to Formalin- and B5-Fixed, Paraffin-Embedded Tissues

RICHARD W. CARTUN, MS
F. BRUCE COLES, DO,
and WILLIAM T. PASTUSZAK, MD

From the Divisions of Surgical Pathology and Hematopathology, Department of Pathology, Hartford Hospital, Hartford, Connecticut

Immunophenotypic analysis of paraffin-embedded tissues of lymphoproliferative disorders has been facilitated by recent developments of monoclonal antibodies that react with epitopes that survive histologic processing. Leukocyte common antigen (LCA) antitive disorders and a variety of normal and neoplastic nonlymphoid tissues. When applied to sections of benign lymphoid tissue, the L26 antibody labeled germinal center cells, mantle zone and scattered interfollicular lymphocytes, but not histiocytes or plasma cells.

Thomas Hodgkin (April 17, 1798 - April 5, 1866)

› Mod Pathol. 1988 Jul;1(4):274-8.

Hodgkin's disease, lymphocyte-predominant type: immunoreactivity with B-cell antibodies

F B Coles [1], R W Cartun, W T Pastuszak

Affiliations + expand

PMID: 3266337

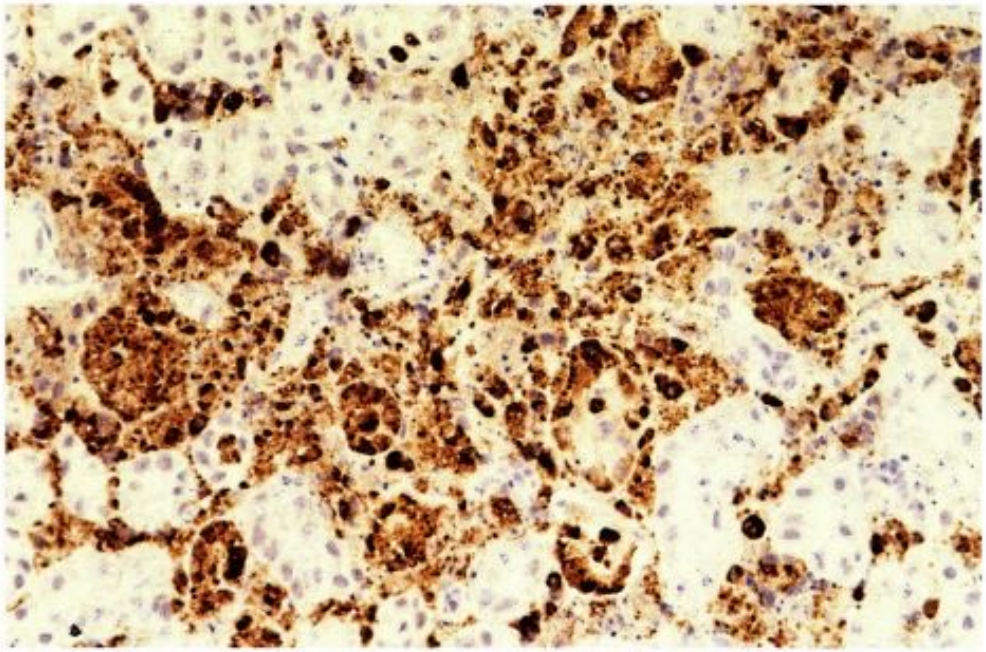
Abstract

Utilizing the monoclonal antibodies L26 (a new antibody possessing immunoreactivity with B-lymphocytes in paraffin-embedded tissue), LN1, LN2, and Leu-M1, 44 cases of Hodgkin's disease (HD) were examined for the presence of immunoreactivity in Reed-Sternberg (R-S) cells by the avidin-biotin-peroxidase complex (ABC) technique. In 16 cases of lymphocyte-predominant Hodgkin's disease (LPHD), the L&H variants of R-S cells exhibited a different pattern of staining compared to R-S cells in other histologic types (total, 28 cases: 11, mixed cellularity; 8, nodular sclerosing; 6, lymphocyte depleted; 3, unclassified). L&H variants in LPHD were immunoreactive for L26 and LN1 in 15 and 14 cases, respectively, whereas R-S cells in the remaining types were negative or rarely positive (3, L26; 2, LN1). Leu-M1 was strongly positive in 27 of 28 cases of non-LPHD versus only 4 of 16 in LPHD. LN2 was reactive in virtually all cases (43 of 44). These findings suggest the possibility that the R-S cells of LPHD are derived from a different lineage than R-S cells in other histologic types of HD or that the latter have somehow lost the ability to express the antigens defined by L26 and LN1. Finally, based on immunologic and morphologic findings in this study, the similarities seen between the nodular and diffuse subtypes of LPHD are felt to favor a close relationship between the two subtypes.

Volume 25 • Number 4 • December 2002

The Journal of Histotechnology

anatomy, histochemistry, microscopy, molecular biology, pathology



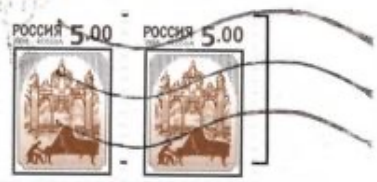
Special Issue: Diagnostic Immunohistochemistry

Guest Editor: Richard W. Cartun, Ph.D.

In this issue:

PAR AVION = ABUA

DR. A.V. PINEVICH
Laboratory of Microbiology
Biological Institute
of St. Petersburg University
Oranienbaumskoye sch. 2
Stary Peterhof
St. Petersburg, 198504 Russia



США

DR. R. W. CARTUN
HARTFORD HOSP, DEPT PATHOL & LAB MED, ANAT PATHOL DIV, 80 SEYMOUR ST,
HARTFORD, CT, 06102, USA

Dear Dr. Cartun

I would greatly appreciate receiving a reprint [s] of your article Editorial - Immunohistochemistry - 25 years & still going strong!

Published in: Journal of Histotechnology (2002) 25 vol. issue 4 page [s] 181-184

Thank you for your courtesy

Yours truly

DR. A.V. PINEVICH
Laboratory of Microbiology
Biological Institute
of St. Petersburg University
Oranienbaumskoye sch. 2
Stary Peterhof

St. Petersburg, 198504 Russia

Norman Institute of Pathology
CHRISTIAN MEDICAL COLLEGE HOSPITAL,
VELLORE 632004. S. INDIA

Dear Dr. Cartun

I shall be much obliged, if you would send me a reprint of your article Immunohistochemistry of Malignant Lymphoma

appearing in J. Histotech, 1992; 15:3:199

Thanking you,

Yours sincerely,

H Krishnaswami

Hemalatha Krishnaswami, MD

Cut and use stub for address

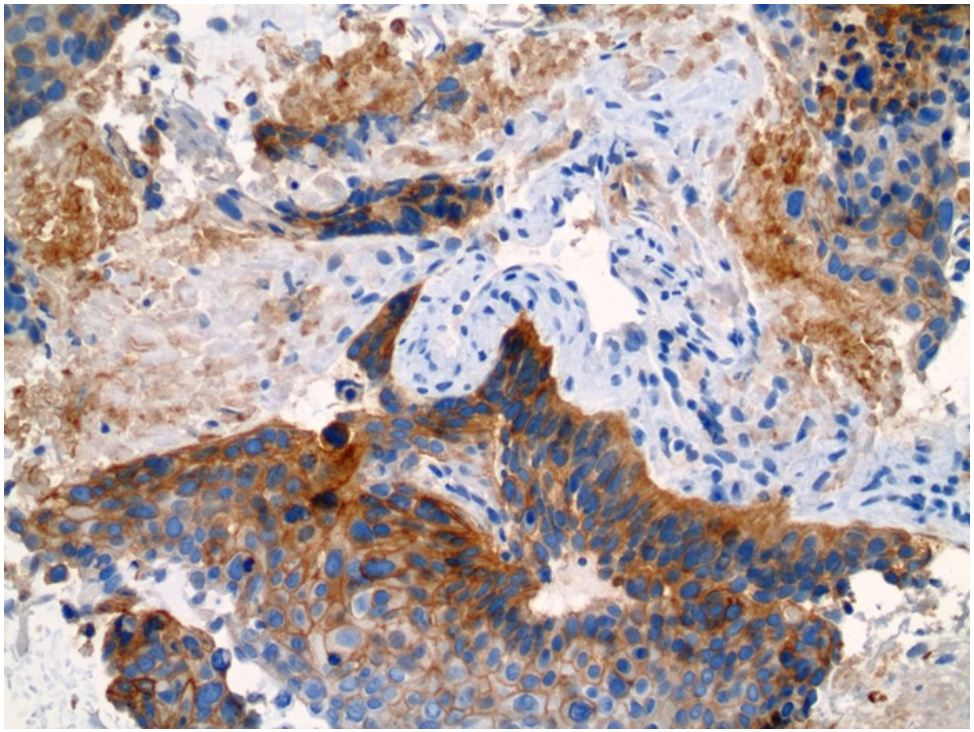
Dr. Hemalatha Krishnas

Norman Institute of Pathology
CHRISTIAN MEDICAL COLLEGE H
RE 632004

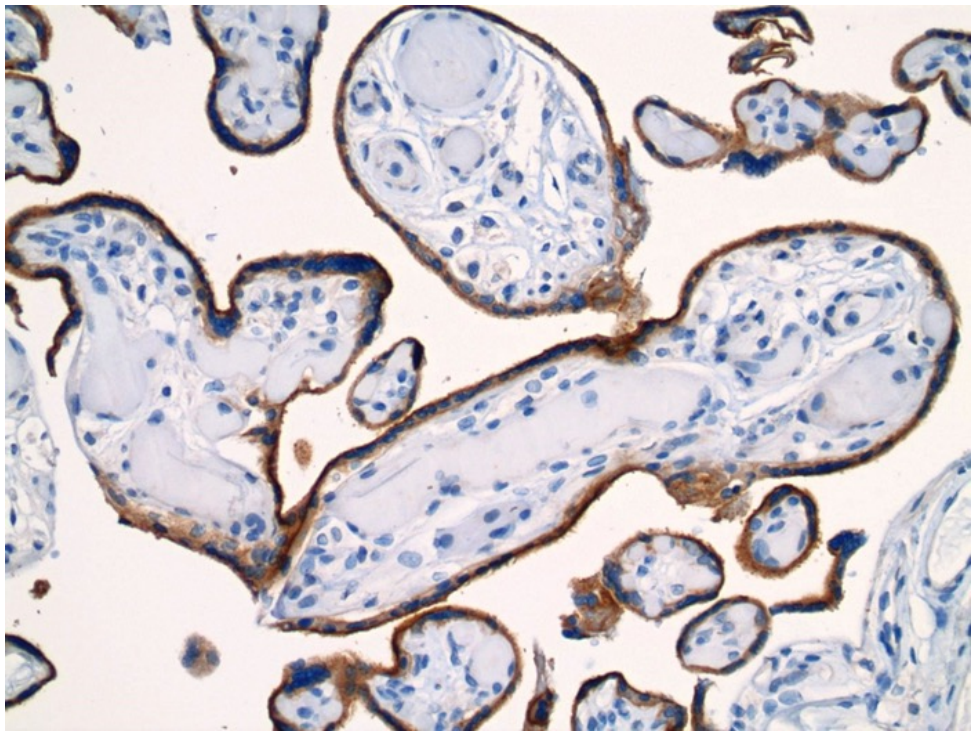
Chapter 7

“Controls”

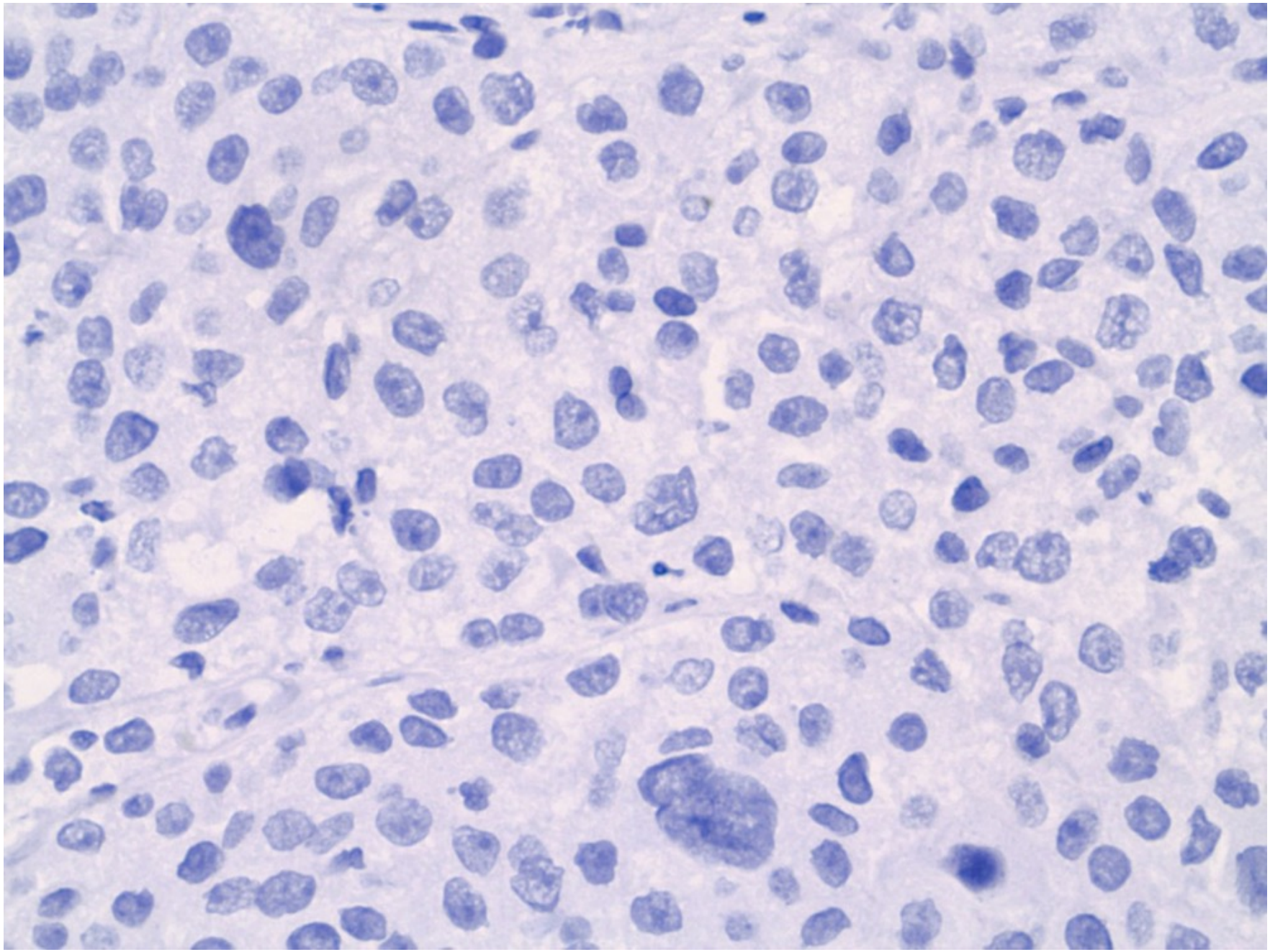
Lung Adenocarcinoma



PD-L1 IP



On-Slide (+) Control?



“Negative Reagent Control”

NSH IHC Forum

- 2007 - Denver
- 2008 - Pittsburgh
- 2009 - Indianapolis
- 2010 - Ft. Lauderdale
- 2011 - Denver
- 2012 - Windsor Locks (where is that?)
- 2013 - Bethesda
- 2014 - Las Vegas
- 2015 - Nashville
- 2016 - Webinar
- 2017, 2018, and 2019 - St. Louis

From: James Dvorak (s) <jdvorak@cap.org>
Subject: RE: Negative Control Slides
To: "richard cartun" <rcartun@sbcglobal.net>
Date: Monday, November 14, 2011, 8:20 AM

Rich,

As I expected, the IHC Committee welcomed the revision to ANP.22570. The next step will be to get the approval of the Checklist Committee. I will be submitting my checklist revisions to them this week for discussion and approval at their January meeting. Again, nothing is certain at this point, but I believe there is good momentum for this change. As you and others I've mentioned this to have indicated, this will be a major change to the negative control requirement and may merit notification via eAlert once approved for the 2012 edition of the checklists.

Regards,

James Dvorak, MT(ASCP)
Senior Technical Specialist, Laboratory Accreditation Program

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ANP.22570 **QC - Antibodies** **Phase II**

For laboratories that use biotin-based detection systems, appropriate negative controls are used.

NOTE: Negative controls must assess the presence of nonspecific staining in patient tissue as well as the specificity of each antibody with the exception listed below. Results of controls must be documented, either in internal laboratory records, or in the patient report. A statement in the report such as, "All controls show appropriate reactivity" is sufficient.

Immunohistochemical tests using polymer-based detection systems (biotin-free) are sufficiently free of background reactivity to obviate the need for a negative reagent control and such controls may be omitted at the discretion of the laboratory director.

For laboratories using older biotin-based detection systems, it is important to use a negative reagent control to assess nonspecific or aberrant staining in patient tissue related to the antigen retrieval conditions and/or detection system used. A separate section of patient tissue is processed using the same reagent and epitope retrieval protocol as the patient test slide, except that the primary antibody is omitted, and replaced by any one of the following:

- *An unrelated antibody of the same isotype as the primary antibody (for monoclonal primary antibodies)*
- *An unrelated antibody from the same animal species as the primary antibody (for polyclonal primary antibodies)*
- *The negative control reagent included in the staining kit*
- *The diluent/buffer solution in which the primary antibody is diluted*

"CAP Anatomic Pathology Checklist"

“It’s been an absolutely wonderful journey down the immunohistochemistry highway ……”

“……. and the journey and the road haven’t ended yet!!!”

Tips for Success …….

- Don’t be afraid to fail.
- Don’t be afraid to try something new …… welcome change.
- Always pay attention to detail.
- Have passion for what you do.
- Always treat others the way you want to be treated.

Thank you everyone!