

# OS AVANÇOS DA PATOLOGIA NO BRASIL

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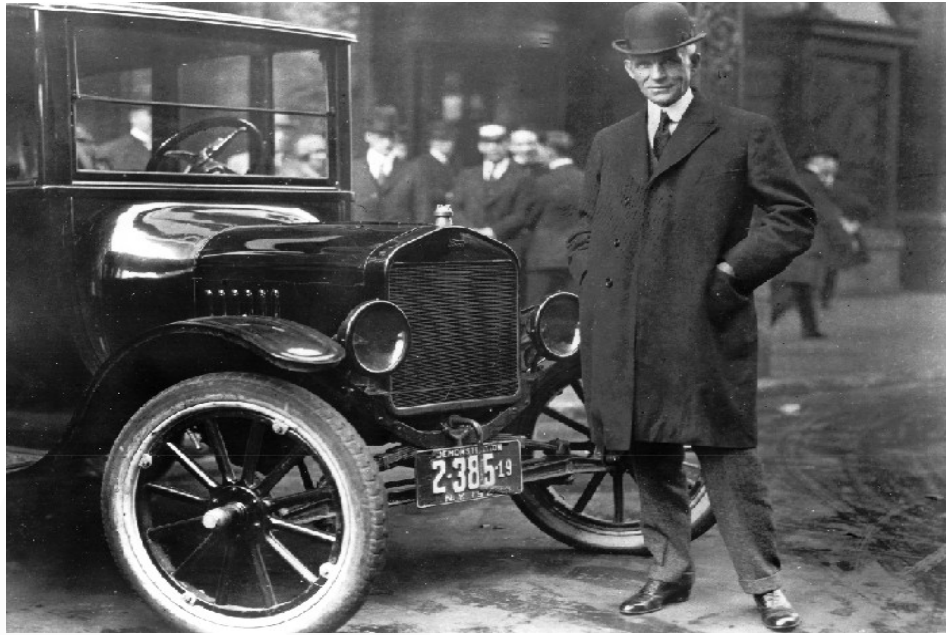






The World is changing





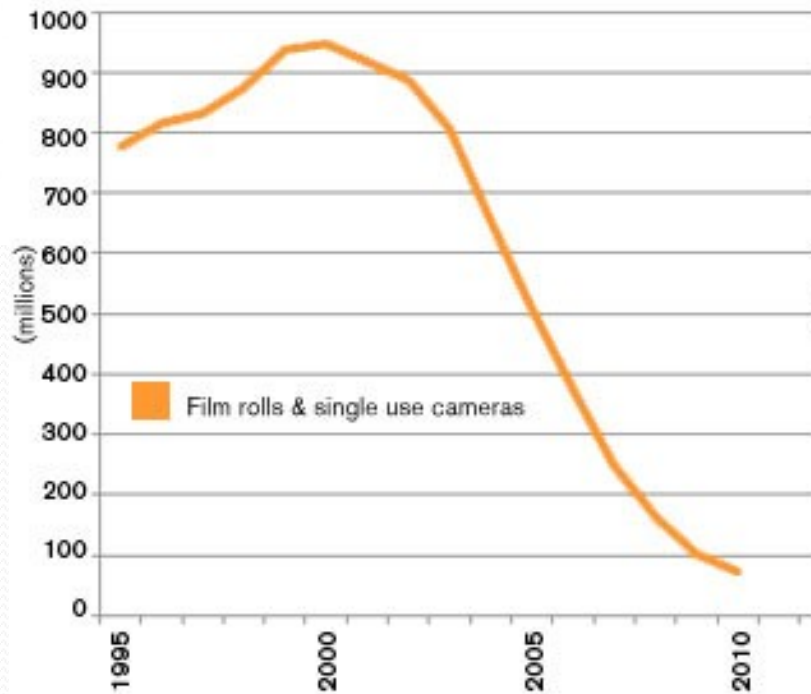




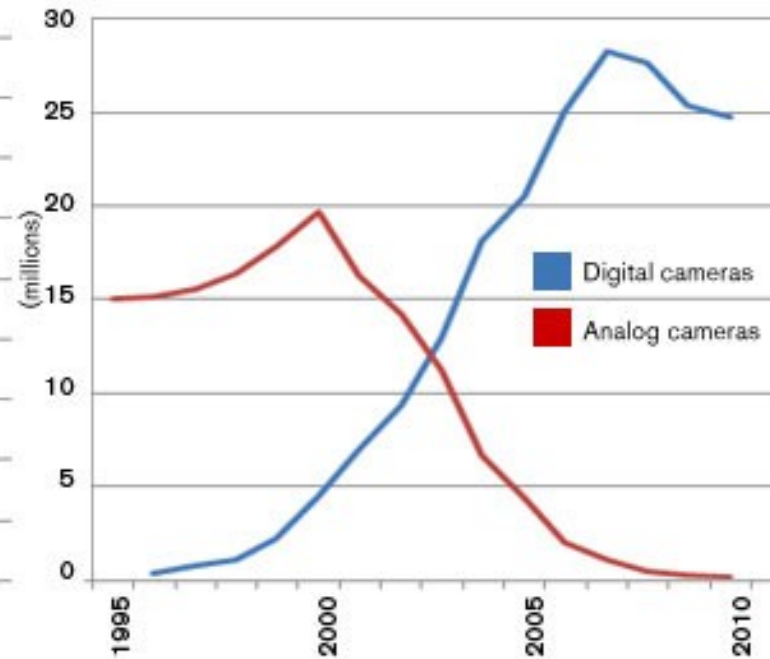
# Inovação Sustentável/Disruptiva

## Decline of Film

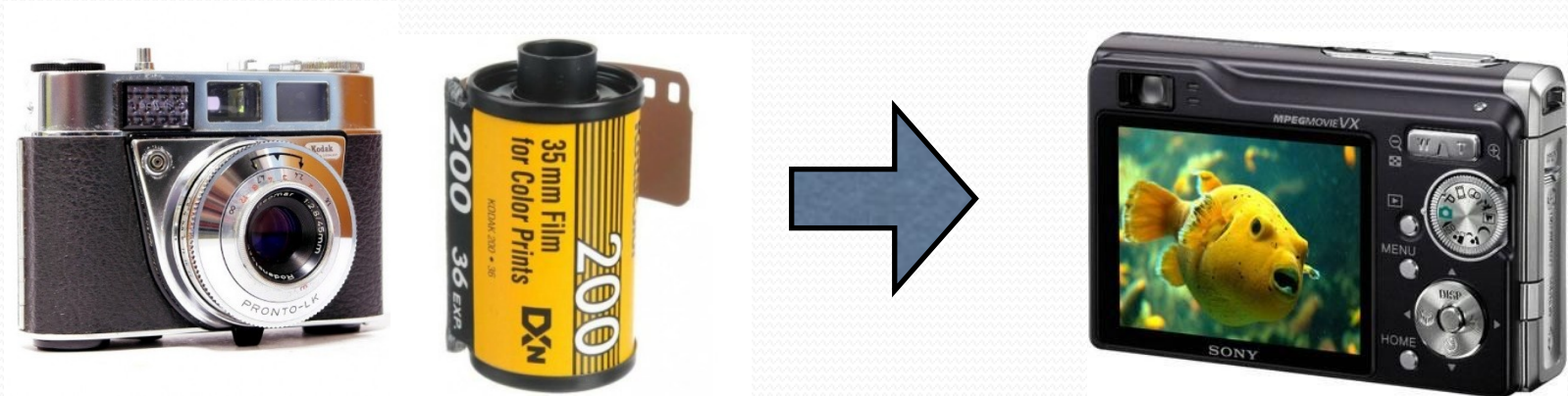
Film rolls sold



Camera sales



# Inovação Disruptiva





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Doctors in every branch of medicine were asked, "What cigarette do you smoke?" The brand named most was Camel!

You'll enjoy Camels for the same reason so many doctors prefer them. Camels have mild and delicious, quick after-punch, and a flavor unmatched by any other cigarette. Make this cigarette your favorite. Camels are the only cigarette that will give you more than just a puff. They will give you a smooth, steady smoke. You'll see how enjoyable a cigarette can be!

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enjoy the smooth smoking of fine tobaccos

...smoke **PALL MALL**  
the cigarette whose mildness  
you can measure



## Study This Puff Chart:

PUFF BY PUFF... YOU'RE ALWAYS AHEAD WITH PALL MALL



The further your cigarette filters the smoke through fine tobacco, the milder that smoke becomes. At the first puff, PALL MALL's smoke is filtered further than that of any other leading cigarette.

Again after 3 puffs of each cigarette your own eyes can measure the extra length for extra mildness as the smoke of PALL MALL's traditionally fine tobacco is filtered further. Moreover, after 16 puffs of each cigarette...

...or 17 puffs, Pall Mall's greater length of fine tobacco still travels the smoke further - filters the smoke and makes it mild. Thus Pall Mall gives you a smoothness, mildness and satisfaction no other cigarette offers you.

Wherever you go today, you will see more and more people smoking PALL MALL - the cigarette whose mildness you can measure.

Outstanding  
...and they are mild!



P.S. LET A CARTON OF PALL MALLS SAY "MERRY CHRISTMAS" FOR YOU

# SANTA CLAUS, YOU BASTARD !!!

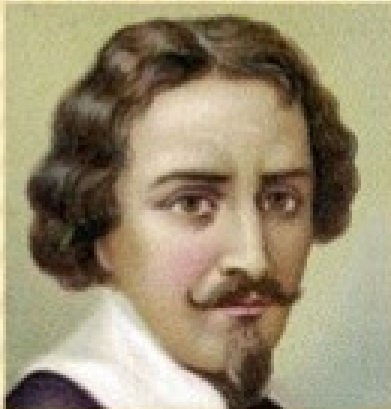
DUE TO HARD ECONOMIC TIMES SANTA IS FORCED  
TO SELL DEADLY CANCER STICKS.

SO AGAIN "SANTA CLAUS, YOU BASTARD. !!!"



# CELL HISTORY

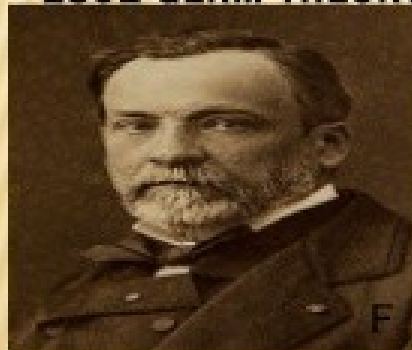
**ZACHARIAS-JANSEN ANTON VON LEEUWENHOEK**



**1665-CELL ROBERT HOOKE MICROGRAPHIA**



**LOUIS PASTEUR  
1861-GERM THEORY**



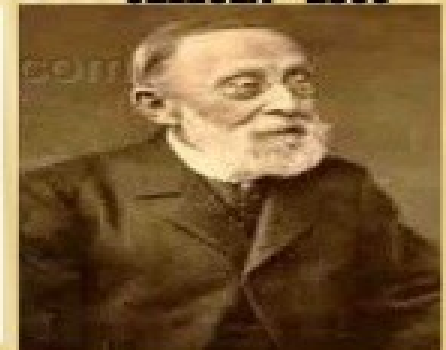
**ROBERT BROWN  
1833-NUCLEUS**



**THEODOR SCHWANN-  
ANIMALS-CELLS-1838**



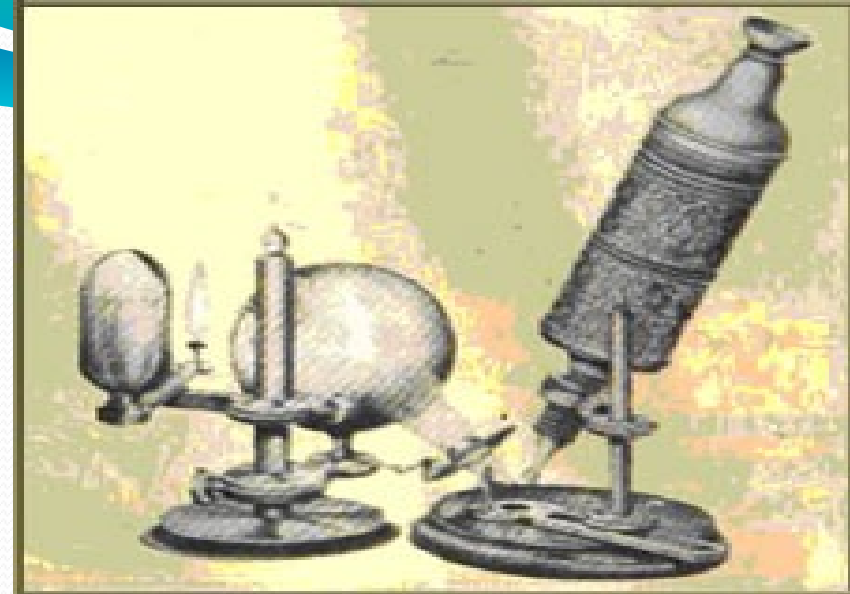
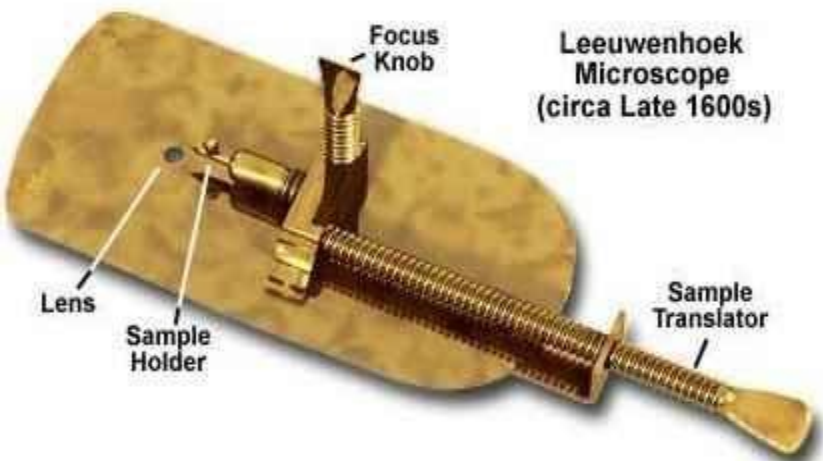
**RUDOLF VIRCHOW  
"OMNIS CELLULA E  
CELLULA" 1858**



Miescher (1871) : **DNA**

watson and crick : double helix structure of dna

Singer & nicholson in (1972):**Fluid Mosaic model**



# LABORATÓRIOS NO BRASIL

- GRANDE QUANTIDADE DE LABORATÓRIOS DE QUALIDADE NO BRASIL
- GRANDES GRUPOS
- MAIOR QUANTIDADE DE LABORATÓRIOS DE PEQUENO E MÉDIO PORTE
- TODAS AS TÉCNICAS SÃO OFERECIDAS: IMUNO-HISTOQUÍMICA, PATOLOGIA MOLECULAR: RT-PCR, SEQUENCIAMENTO
- POUCAS TÉCNICAS QUE ALGUMAS FARMAS FAZEM: FOUNDATION ONE



# PATOLOGISTAS

- CFM: 3210 PATOLOGISTAS (VIES)
- RESIDENTES 269 DADOS DE 2018 – R1 120, R2 69, R3 77, R4 3
- VAGAS DE RM EM PATOLOGIA 463
- CONCENTRAÇÃO : SUDESTE, SUL, AUMENTANDO NO NORDESTE, CENTRO-OESTE E NORTE COM ALGUMAS CAPITAIS COM NUMERO SUFICIENTE
- MUITAS CIDADES SEM LABORATÓRIOS DE PATOLOGIA

# RESIDÊNCIA MÉDICA

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- GRANDE PARTE DEFASADA
- SEM NECRÓPSIA
- SEM IMUNO-HISTOQUÍMICA (TÉCNICA IMPLEMENTADA NOS USA NA DÉCADA DE 1980)
- PATOLOGIA MOLECULAR NÃO É REALIDADE NA QUASE TOTALIDADE
- GRANDE NÚMERO DE DESISTÊNCIAS





## A Brief Historical Note on Staining by Hematoxylin and Eosin

D. Friday King, M.D., and Laura A.C. King, M.D.

Paraffin-embedded tissue sections are routinely stained with hematoxylin and eosin (H&E) nowadays. Only when clinical history, gross examination, or H&E study suggests it are other "special" stains employed to delineate better features poorly or not at all brought out by hematoxylin and eosin.

Staining is such an integral part of modern microscopy of tissue that it is difficult to think of being without it. The procedure did not become widespread, however, until Joseph von Gerlach popularized the use of carmine in 1858 (1). As so often happens in science, serendipity was at work. For years Gerlach had been experimenting with ammoniacal carmine. One evening, he inadvertently left a section of cerebellum in a very dilute solution. In the morning he found the cellular details to be well stained. He had previously experimented with carmine in too high a concentration (2).

Encouraged by Gerlach's success, microscopists began to experiment with a wide range of natural substances, as well as with newly introduced synthetic aniline dyes. Hematoxylin, the only natural dye still in widespread use, is purified logwood extract. The logwood tree (*Haematoxylon campechianum*), which produces a heavy, red wood of fine texture, is native to Central America and the West Indies. For many years, it was the major cash crop of the Campeche region of Mexico because logwood was used extensively in industry and for coloring wine before the development of synthetic materials (3). Although Quekett (4) had briefly mentioned staining animal tissues with logwood dye, Waldeyer (5) was the first to investigate it for this purpose. Employing a plain aqueous extract, he tried to stain the axis cylinders of neurons, but with little success. Later, in 1865, Bohmer (6) did succeed in staining tissues with hematoxylin by combining it with alum as a mordant. Alum had already

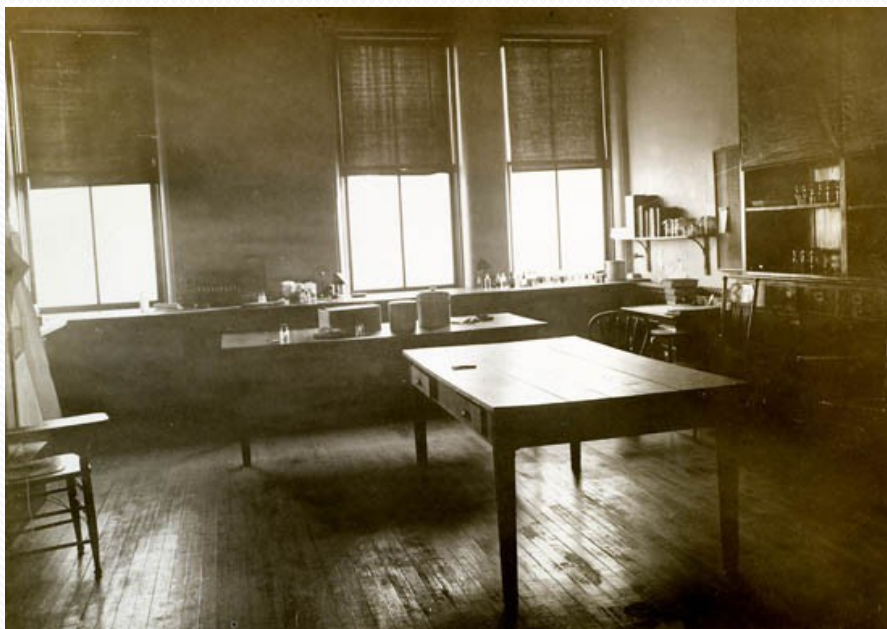
been widely used in the dye industry as a mordant, which Waldeyer had overlooked. Hematoxylin is a glucoside produced by treating an aqueous extract of logwood with ether. When it is oxidized, hematein, the true dye, results.

Eosin, the potassium salt of tetrabromofluorescein, was synthesized by Baeyer (7) and his co-workers in 1871. The name itself is derived from a Greek word meaning "morning red" (8). In 1876 Dreschfeld (9) and Fischer (10) described its usefulness as a tissue stain. A few months later, Busch (11) reported "on the double staining of the ossification border with eosin and hematoxylin." Over a century later, these still remain the most commonly used materials for tissue staining. □

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1. Gerlach, J. von: *Mikroskopische Studien aus dem Gebiet der menschlichen Morphologie*. Erlangen, 1858, 72 pp.
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11. Busch, H.: Ueber die Doppelfärbung des Ossificationsrandes mit Eosin und Haematoxylin. *Arch Physiol* 1878;594-595.

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Ele não trabalha em  
consultório, mas  
você pode marcar  
uma consulta com ele.



Ele está lá,  
nos “bastidores”  
da medicina.

Agora você já sabe quem é  
o responsável pelo laudo do  
seu exame: o médico patologista.

Você pode não conhecê-lo,  
mas ele está nos laboratórios e  
diagnostica o seu problema de saúde.

Este é um ato médico complexo e  
cuidadoso, cujo resultado auxilia  
seu médico, a indicar a melhor  
maneira de tratar o seu problema.



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*Você já ouviu falar?*



Agradecemos aos que, dia após dia, se propõem a otimizar laudos e técnicas de análise, com o objetivo de oferecer o melhor diagnóstico aos pacientes.



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Somos responsáveis por diagnosticar, através da análise de tecidos e órgãos, diferentes condições de saúde da população.

O Médico Patologista desenvolve laudos que auxiliam médicos de diferentes especialidades, na **identificação de doenças** e na decisão pela **intervenção** mais adequada.

movimento  
**médicos**patologistas  
rio grande do sul

XXVII CONGRESSO BRASILEIRO DE POPULOGIA / XXVII CONGRESO DE LA SOCIEDAD LATINOAMERICANA DE POPULOGIA  
UMA PUBLICAÇÃO DA SOCIEDADE BRASILEIRA DE POPULOGIA / REVISTA DE POBLO

Muñoz, 2011, p. 4, sobre los grupos transnacionales y la integración económica en América: los multinationals corporates (los Grupos Transnacionales de Corporación) y los Conglomerados de la Sociedad Latinoamericana del Pacífico, se refieren a EFTSA, el grupo de países de América Latina, desde 1974 a 1975 de acuerdo, en lugar de la integración económica, incluso luego y incluso la zona de integración económica de América Latina, también denominada de zona económica, involucrando a América Latina y el Caribe, y de una forma más restringida, pero, como distintas áreas económicas o actividades económicas de los países que, finalmente, involucran, al menos, a América Latina y el Caribe, como por ejemplo:

[illegible]

**Abstract** Pedagogues in charge of student support practice a complex & significant role.

Universidade Brasileira de Rio  
de Janeiro, apresentando resultados  
de algumas pesquisas (1999-2000).  
Página 2

**Phylogeny 30.31**

**Phages attached to bacteria**  
 when the phage attaches to a  
 specific molecule called a  
**phage tail**

# PACQ - SBP

- Programa de Acreditação
- O Programa de Acreditação está baseado em **requisitos críticos (RC)** e **requisitos importantes (RI)**, que buscam verificar o cumprimento de um rol de processos e procedimentos imprescindíveis à segurança dos pacientes e dos colaboradores.
- Serão verificados a Compliance, a rastreabilidade das amostras, adequação de instalações, manutenção de equipamentos e procedimentos internos de segurança, o sistema da qualidade do laboratório como um todo, incluindo os requisitos de gestões da qualidade, procedimentos e processos laboratoriais, recursos humanos e gestão administrativa.
- Entende-se que uma instituição só poderá prover **exames de qualidade** e com **segurança** se estiver em pleno funcionamento, dentro dos **parâmetros técnicos, legislativos** e com **saúde financeira** para patrocinar todos os requerimentos necessários para sua sustentabilidade.
- É imperativo que a instituição tenha incentivo a buscar **melhoria contínua da qualidade**, como um caminho de sentido único e sem volta.

# O Desenvolvimento da Patologia

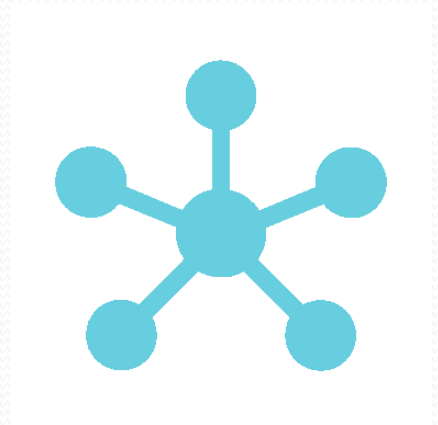



A mudança do foco do objeto



# O QUE O CLINICO/CIRURGIÃO ESPERA DO PATOLOGISTA

- **Tamanho e extensão local do tumor**
- **Situação das margens de ressecção**
- **Tipo histológico**
- **Grau de malignidade**
- **Embolização vascular: venosa e linfática**
- **Infiltração de filetes nervosos**
- **Expressão imuno-histoquímica (Ex: ER, RP, p53, etc.)**
- **PERFIL IMUNO-HISTOQUÍMICO (CD20, CD3, CD30, CD246, PD-L1, ROS1, ALK, ETC)**
- **PATOLOGIA MOLECULAR (EGFR, HER-2 BRAF)**
- **PESQUISA DE VIRUS (HPV, CLAMIDIA)**
- **PERFIL MOLECULAR - SEQUENCIAMENTO**





Com a utilização de sofisticadas técnicas,  
a patologia deixou de ser um método  
apenas artesanal e tornou-se uma  
especialidade cada vez mais essencial  
para diagnósticos e tratamentos eficazes





# **DESAFIO**

DO PATOLOGISTA

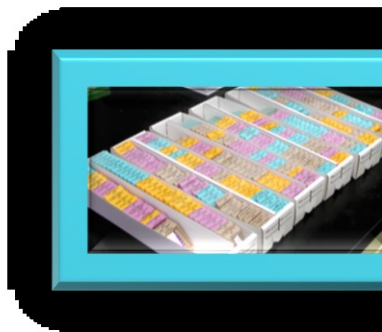
**MENOS  
MATERIAL**

**MAIS  
INFORMAÇÕES  
PARA TESTES**

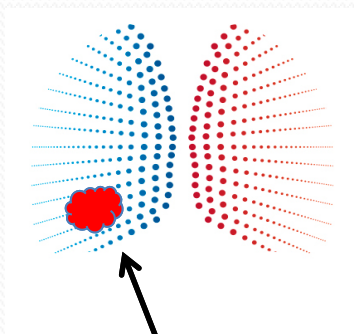




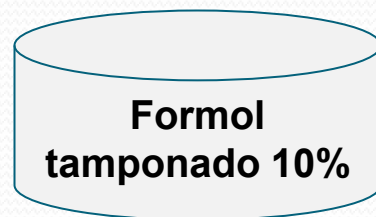




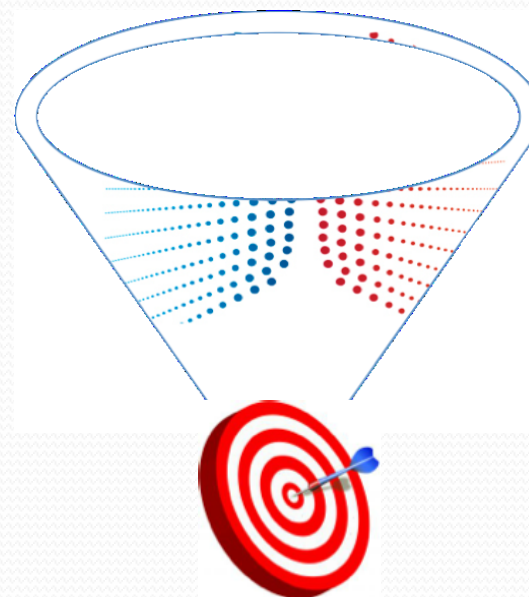
**O BLOCO DE PARAFINA  
VALE OURO!!!**



Biópsia



Material bem  
caracterizado e  
preservado



# A Review of Preanalytical Factors Affecting Molecular, Protein, and Morphological Analysis of Formalin-Fixed, Paraffin-Embedded (FFPE) Tissue

## How Well Do You Know Your FFPE Specimen?

B. Paige Bass, PhD; Kelly B. Engel, PhD; Sarah R. Greytak, PhD; Helen M. Moore, PhD

• **Context.**—Formalin fixation and paraffin embedding is a timeless, cost-efficient, and widely adopted method of preserving human tissue biospecimens that has resulted in a substantial reservoir of formalin-fixed, paraffin-embedded blocks that represent both the pathology and preanalytical handling of the biospecimen. This reservoir of specimens is increasingly being used for DNA, RNA, and proteomic analyses.

**Objective.**—To evaluate the impact of preanalytical factors associated with the formalin fixation and paraffin embedding process on downstream morphological and molecular endpoints.

**Data Sources.**—We surveyed the existing literature using the National Cancer Institute's Biospecimen Research Database for published reports investigating the

potential influence of preanalytical factors associated with the formalin fixation and paraffin embedding process on DNA, RNA, protein, and morphological endpoints.

**Conclusions.**—Based on the literature evidence, the molecular, proteomic, and morphological endpoints can be altered in formalin-fixed, paraffin-embedded specimens by suboptimal processing conditions. While the direction and magnitude of effects associated with a given preanalytical factor were dependent on the analyte (DNA, RNA, protein, and morphology) and analytical platform, acceptable conditions are highlighted, and a summary of conditions that could preclude analysis is provided.

(*Arch Pathol Lab Med.* 2014;138:1520–1530; doi:10.5858/arpa.2013-0691-RA)

Formalin fixation and paraffin embedding are part of a globally applied method of tissue preservation; however, they also represent a multistage process that is far from standardized. A recent review article<sup>1</sup> published by our office identified 15 preanalytical factors associated with formalin fixation and paraffin embedding tissue processing that have documented effects on immunohistochemistry (IHC) efficacy and many more that were unaddressed or under-addressed in the scientific literature. While technological advancements afford the molecular analysis of formalin-fixed, paraffin-embedded (FFPE) biospecimens, efforts have achieved varying levels of success, which may be a result of differences in FFPE processing regimens or extraction

techniques. In the present review, we summarize reported effects of FFPE processing factors on molecular and morphological endpoints, explore differences between analytes, and underscore evidence-based and analyte-specific recommendations for specific preanalytical factors when possible. It is our aim that this review will serve as a resource both for the evaluation of archival FFPE specimens and as a guideline for the collection of new FFPE specimens. Although additional sources of preanalytical variability, including extraction methods, antigen retrieval techniques,

Also see p. 1426.

and patient-related factors, may be capable of influencing analytical endpoints, the scope of the present review was limited to evidence available for FFPE fixation and processing factors.

### MATERIALS AND METHODS

Potential sources of preanalytical variability associated with the procurement, fixation, processing, and storage of human FFPE tissue biospecimens were identified based on the experience of the authors, data contributed to the Biospecimen Research Network (<http://biospecimens.cancer.gov/researchnetwork>), and literature evidence and are summarized in Table 1. Targeted surveys were conducted for each preanalytical factor. Relevant peer-reviewed, primary research articles that used human FFPE tissue biospecimens

Preanalytical Factors Affecting FFPE Tissue—Bass et al

Accepted for publication January 8, 2014.

From the Kelly Government Solutions Program, Kelly Services, Rockville (Drs Bass and Greytak), and the Biorepositories and Biospecimen Research Branch, Cancer Diagnosis Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda (Dr Moore), Maryland; and the Preferred Solutions Group, Arlington, Virginia (Dr Engel).

The authors have no relevant financial interest in the products or companies described in this article.

Reprints: Helen M. Moore, PhD, Biorepositories and Biospecimen Research Branch, Cancer Diagnosis Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, 9609 Medical Center Dr, Room 3W422, Mail Stop Code 9728, Bethesda, MD 20892 (e-mail: [moorehe@mail.nih.gov](mailto:moorehe@mail.nih.gov)).

1520 Arch Pathol Lab Med—Vol 138, November 2014

<b>Preanalytical Factor</b>	<b>DNA</b>	<b>RNA</b>	<b>Protein</b>	<b>Morphology</b>
Postmortem interval	<48 h for FISH; <sup>7</sup> ≤4 d for PCR <sup>6</sup>	<4 h <sup>55</sup>	Evidence was insufficient	Evidence was not available
Cold ischemia	<1 h for FISH; <sup>5</sup> ≤24 h for PCR <sup>11</sup>	<12 h <sup>56</sup>	<12 h <sup>5,80-83</sup>	<6 h <sup>91,139-141</sup>
Warm ischemia time	Evidence was insufficient	Evidence was not available	Evidence was not available	Evidence was not available
Specimen size	3–10 mm <sup>3</sup> (Ref. 10)	Evidence was not available	1.2–3.5 mm <sup>3</sup> (Ref. 84)	Evidence was not available
Prefixation handling	Evidence was not available	Evidence was not available	Thresholds are antigen specific <sup>85-87</sup>	Thresholds are tissue and method specific <sup>81,85,142,143</sup>
Decalcification	EDTA <sup>11-14</sup>	Ultrasound or EDTA <sup>14,57-59</sup>	Thresholds are tissue and antigen specific <sup>87-89</sup>	Ultrasound; EDTA; nitric, formic, or acetic acid; DECAL <sup>b</sup> ; Cal-Ex <sup>c</sup> ; D-calcifier <sup>d</sup> ; Plank-Rychlo, Ebner's, or Jenkin's solution <sup>14,89,144,145</sup>
Tissue to fixative ratio	Evidence was not available	Evidence was not available	1:1 to 1:20 ratio <sup>90</sup>	Evidence was not available
Fixative buffer	NBF <sup>10,16-23</sup>	NBF <sup>60</sup>	NBF <sup>66,87,90,93</sup>	Evidence was not available
Fixative delivery method	Immersion, microwave-accelerated, <sup>36-38</sup> or ultrasound-accelerated <sup>39,40</sup>	Immersion, microwave-accelerated, <sup>38</sup> or ultrasound-accelerated <sup>39,40,61</sup>	Immersion, <sup>36,37,40,111</sup> perfusion, <sup>37,108</sup> injection, <sup>107</sup> heat-accelerated, <sup>109</sup> microwave-accelerated, <sup>37,110</sup> or ultrasound-accelerated <sup>39,61</sup>	Immersion, <sup>40,110,111,151</sup> perfusion, <sup>37,108,151,152</sup> microwave-accelerated, <sup>110,111,147</sup> or ultrasound-accelerated <sup>39,40,61</sup>
Fixative concentration	Evidence was not available	Evidence was not available	10% or 15% NBF <sup>91,92</sup>	4% formaldehyde for immersion fixation, <sup>113</sup> 0.5%–1% NBF for microwave-accelerated fixation <sup>147</sup>
Fixation duration <sup>a</sup>	<72 h <sup>6,7,14,18,21,24-30</sup>	8–48 h <sup>57,60-66</sup>	6–24 h <sup>61,66,87,90,91,95-104</sup>	<1 y <sup>11,100,148,149</sup>
Fixation temperature	4°C or ambient <sup>22,25,14,15</sup>	4°C or ambient <sup>25,35,70,71</sup>	4°C or ambient <sup>93,105,106</sup>	Evidence was not available
Dehydration reagent and conditions	Evidence was not available	Evidence was not available	5–10 h at 37–45°C, <sup>90,112,115</sup> 10 h at –20°C, <sup>93</sup> or 10–11 h at 4°C <sup>93,114</sup>	Evidence was insufficient
Clearing reagent and conditions	Evidence was not available	Evidence was not available	30 min to 4 h at 45°C <sup>90</sup> or 4°C <sup>114</sup>	Evidence was insufficient
Paraffin embedding reagent and conditions	Pure paraffin <sup>19</sup>	Evidence was not available	Evidence was insufficient	Evidence was insufficient
Duration of paraffin block storage	≤5 y <sup>3,43,44</sup>	≤1 y <sup>41,59,60,71-78</sup>	≤25 y for IHC, <sup>117-122,124</sup> <10 y for platforms requiring protein extraction <sup>84,103,104,121,126</sup>	Evidence was insufficient
FFPE block size or section thickness	Whole sections preferable to cores <sup>10,52</sup> or isolated nuclei <sup>7</sup>	Evidence was not available	2–4 μm <sup>113</sup>	2–3 μm <sup>160</sup>
Type of slide or adhesive	Evidence was not available	Evidence was not available	Evidence was insufficient	Evidence was insufficient
Slide drying duration and temperature	Evidence was not available	Evidence was not available	24 h at ambient, <sup>93</sup> overnight at 37°C, <sup>90</sup> 16–24 h at 58–68°C <sup>131</sup>	Evidence was not available
Tissue section storage	Insufficient evidence	<3 mo at ambient <sup>79</sup>	<1 wk <sup>118,130,132,135,137</sup>	Evidence was not available

Abbreviations: EDTA, ethylenediaminetetraacetic acid; FFPE, formalin-fixed, paraffin-embedded; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NBF, neutral buffered formalin; PCR, polymerase chain reaction.

<sup>a</sup> Acceptable thresholds for fixation duration were based on biospecimens that were fixed in 10% NBF via immersion at ambient temperature.

<sup>b</sup> DECAL (Decal Chemical Corp, Pomona, New York).

<sup>c</sup> Cal-Ex (Fisher Scientific Co, Fair Lawn, New Jersey).

<sup>d</sup> D-calcifier (Lerner Laboratories, Pittsburg, Pennsylvania).



**Table 2. Acceptable Thresholds for Preanalytical Factors Based on Literature Evidence for Specific Analytes**

Preanalytical Factor	DNA	RNA	Protein	Morphology
Postmortem interval	<48 h for FISH, <sup>7</sup> ≤4 d for PCR <sup>8</sup>	<4 h <sup>53</sup>	Evidence was insufficient	Evidence was not available
Cold ischemia	<1 h for FISH, <sup>5</sup> ≤24 h for PCR <sup>31</sup>	<12 h <sup>56</sup>	<12 h <sup>5,80-83</sup>	<6 h <sup>91,139-141</sup>
Warm ischemia time	Evidence was insufficient	Evidence was not available	Evidence was not available	Evidence was not available
Specimen size	3–10 mm <sup>3</sup> (Ref. 10)	Evidence was not available	1.2–3.5 mm <sup>3</sup> (Ref. 84)	Evidence was not available
Prefixation handling	Evidence was not available	Evidence was not available	Thresholds are antigen specific <sup>85-87</sup>	Thresholds are tissue and method specific <sup>81,85,142,143</sup>
Decalcification	EDTA <sup>11-14</sup>	Ultrasound or EDTA <sup>14,57-59</sup>	Thresholds are tissue and antigen specific <sup>87-89</sup>	Ultrasound; EDTA; nitric, formic, or acetic acid; DECAL <sup>b</sup> ; Cal-Ex <sup>c</sup> ; D-calcifier <sup>d</sup> ; Plank-Rychlo, Ebner's, or Jenkin's solution <sup>14,89,144,145</sup>
Tissue to fixative ratio	Evidence was not available	Evidence was not available	1:1 to 1:20 ratio <sup>90</sup>	Evidence was not available
Fixative buffer	NBF <sup>10,16-23</sup>	NBF <sup>60</sup>	NBF <sup>86,87,90,93</sup>	Evidence was not available
Fixative delivery method	Immersion, microwave-accelerated, <sup>36-38</sup> or ultrasound-accelerated <sup>139,40</sup>	Immersion, microwave-accelerated, <sup>38</sup> or ultrasound-accelerated <sup>39,40,61</sup>	Immersion, <sup>36,37,40,111</sup> perfusion, <sup>37,108</sup> injection, <sup>107</sup> heat-accelerated, <sup>109</sup> microwave-accelerated, <sup>37,110</sup> or ultrasound-accelerated <sup>39,61</sup>	Immersion, <sup>40,110,111,151</sup> perfusion, <sup>37,108,151,152</sup> microwave-accelerated, <sup>110,111,147</sup> or ultrasound-accelerated <sup>39,40,61</sup>
Fixative concentration	Evidence was not available	Evidence was not available	10% or 15% NBF <sup>91,92</sup>	4% formaldehyde for immersion fixation, <sup>113</sup> 0.5%–1% NBF for

Fixation duration <sup>a</sup>	<72 h <sup>6,7,14,18,21,24-30</sup>	8-48 h <sup>57,60-66</sup>	6-24 h <sup>61,86,87,90,91,95-104</sup>	microwave-accelerated fixation <sup>147</sup> <1 y <sup>31,100,148,149</sup>
Fixation temperature	4°C or ambient <sup>22,25,34,35</sup>	4°C or ambient <sup>25,35,70,71</sup>	4°C or ambient <sup>93,105,106</sup>	Evidence was not available
Dehydration reagent and conditions	Evidence was not available	Evidence was not available	5-10 h At 37-45°C, <sup>90,112,115</sup> 10 h at -20°C, <sup>93</sup> or 10-11 h at 4°C <sup>93,114</sup>	Evidence was insufficient
Clearing reagent and conditions	Evidence was not available	Evidence was not available	30 min to 4 h at 45°C <sup>90</sup> or 4°C <sup>114</sup>	Evidence was insufficient
Paraffin embedding reagent and conditions	Pure paraffin <sup>19</sup>	Evidence was not available	Evidence was insufficient	Evidence was insufficient
Duration of paraffin block storage	≤5 y <sup>3,43,44</sup>	≤1 y <sup>41,59,60,73-78</sup>	≤25 y for IHC, <sup>117-122,124</sup> <10 y for platforms requiring protein extraction <sup>84,103,104,121,126</sup>	Evidence was insufficient
FFPE block size or section thickness	Whole sections preferable to cores <sup>10,52</sup> or isolated nuclei <sup>7</sup>	Evidence was not available	2-4 μm <sup>113</sup>	2-3 μm <sup>160</sup>
Type of slide or adhesive	Evidence was not available	Evidence was not available	Evidence was insufficient	Evidence was insufficient
Slide drying duration and temperature	Evidence was not available	Evidence was not available	24 h at ambient, <sup>93</sup> overnight at 37°C, <sup>90</sup> 16-24 h at 58-68°C <sup>131</sup>	Evidence was not available
Tissue section storage	Insufficient evidence	<3 mo at ambient <sup>79</sup>	<1 wk <sup>118,130,132,135,137</sup>	Evidence was not available

Abbreviations: EDTA, ethylenediaminetetraacetic acid; FFPE, formalin-fixed, paraffin-embedded; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NBF, neutral buffered formalin; PCR, polymerase chain reaction.

<sup>a</sup> Acceptable thresholds for fixation duration were based on biospecimens that were fixed in 10% NBF via immersion at ambient temperature.

<sup>b</sup> DECAL (Decal Chemical Corp, Pomona, New York).

<sup>c</sup> Cal-Ex (Fisher Scientific Co, Fair Lawn, New Jersey).

<sup>d</sup> D-calcifier (Lerner Laboratories, Pittsburg, Pennsylvania).





# FORMALINA

## TAMPONADA 10%

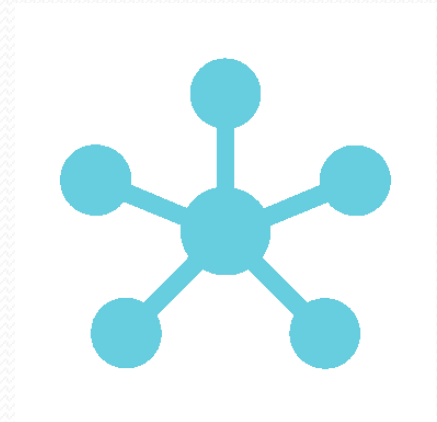
- Formalina neutra tamponada 10% (pH: 6,8-7,4):
- Formaldeído comercial.....100mL
- Água destilada.....900mL
- Fosfato de sódio monobásico.....4g
- Fosfato de sódio dibásico .....6,5g

- Referência: Caputo, L.F.G., Gitirana, L.B., Manso, P.P.A. Conceitos e métodos para a formação de profissionais em laboratórios de saúde. Capítulo 3: Técnicas histológicas. Manual da Fiocruz, volume2. 2005

# FIXAÇÃO

## EFEITOS SOBRE O TECIDO

- – Endurecimento.
- – Solidificação.
- – Diferenciação óptica.
- – Efeitos de coloração.
- – Perda discreta da amostra.
- – Retração da amostra.



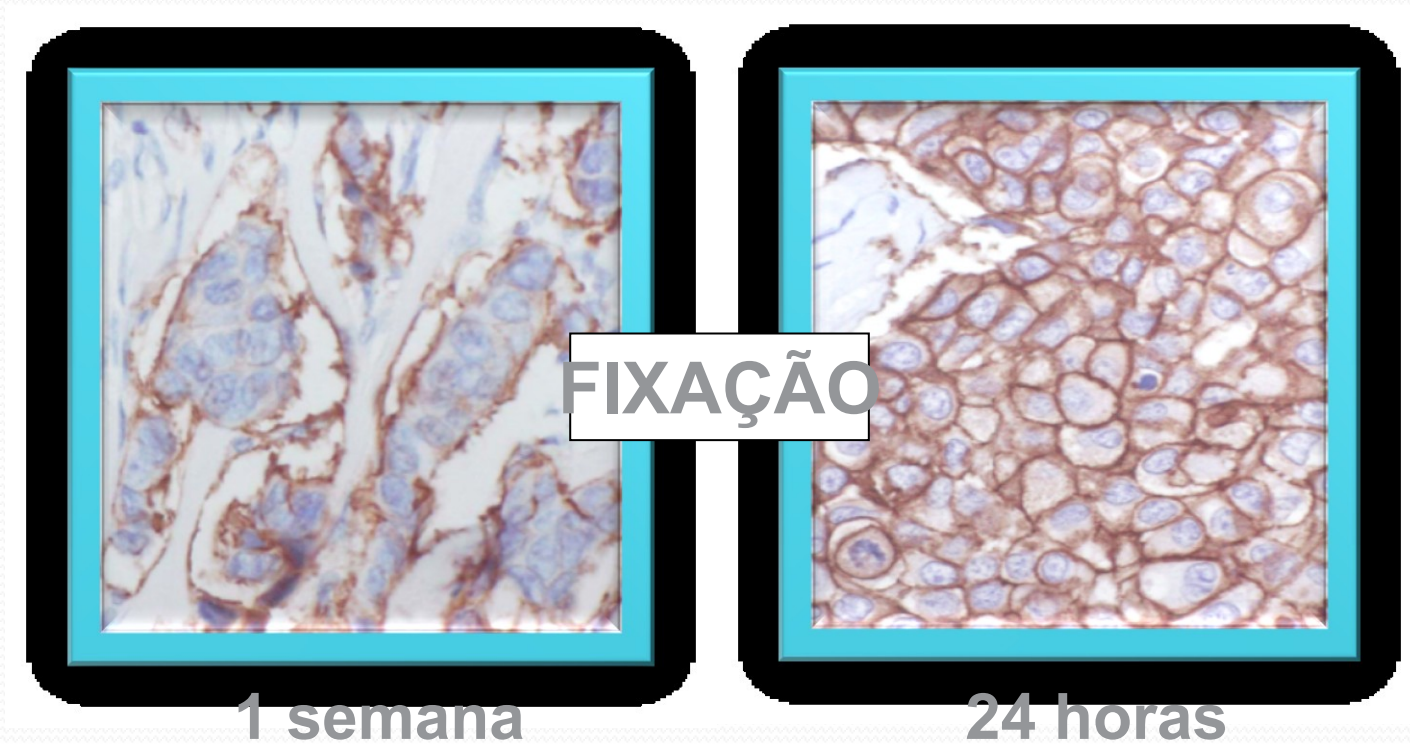
# FIXAÇÃO

## O QUE PODE AFETAR

- Temperatura.
- pH.
- Volume.
- Tempo.
- Pressão.
- Superfície.
- Concentração.
- Vasos capilares e fibras musculares

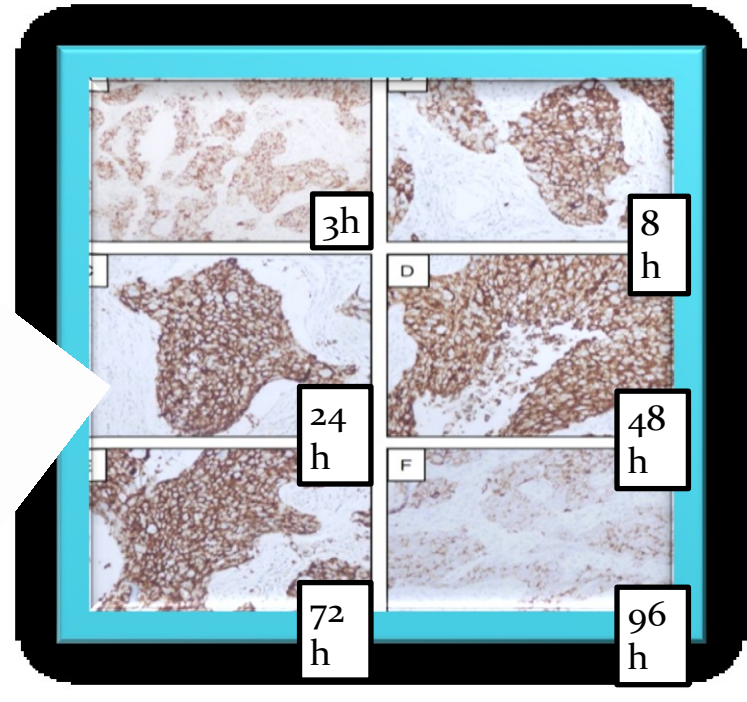


Qual a diferença entre as duas IHQ?

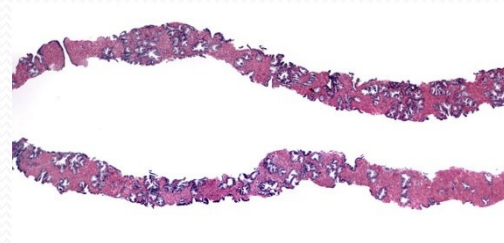
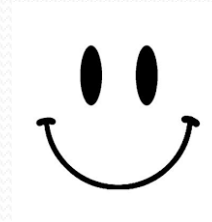
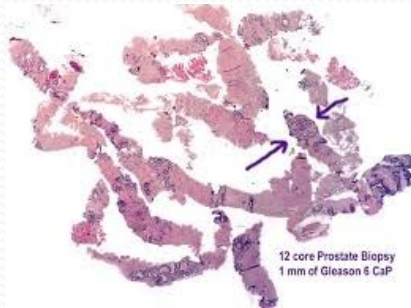


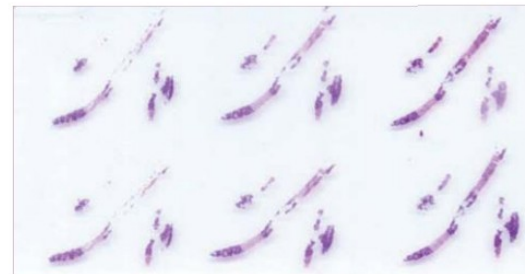
## Assessment of the PD-L1 status by immunohistochemistry: challenges and perspectives for therapeutic strategies in lung cancer patients

Marius Ilie<sup>1,2,3,4</sup> · Véronique Hofman<sup>1,2,3,4</sup> · Manfred Dietel<sup>5,6</sup> · Jean-Charles Soria<sup>1</sup>  
Paul Hofman<sup>1,2,3,4</sup>



# Cuidados pré-analíticos





3 a 9



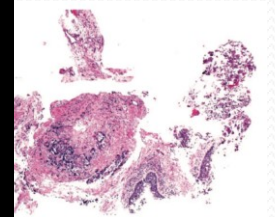
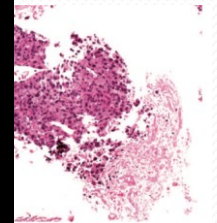
- **100 consecutive biopsies**
- . 3.4 fragments per case
- . 48% of cases had tumor in all
- . Median of 33,4% of the fragments had tumor

0;5: 448–452)

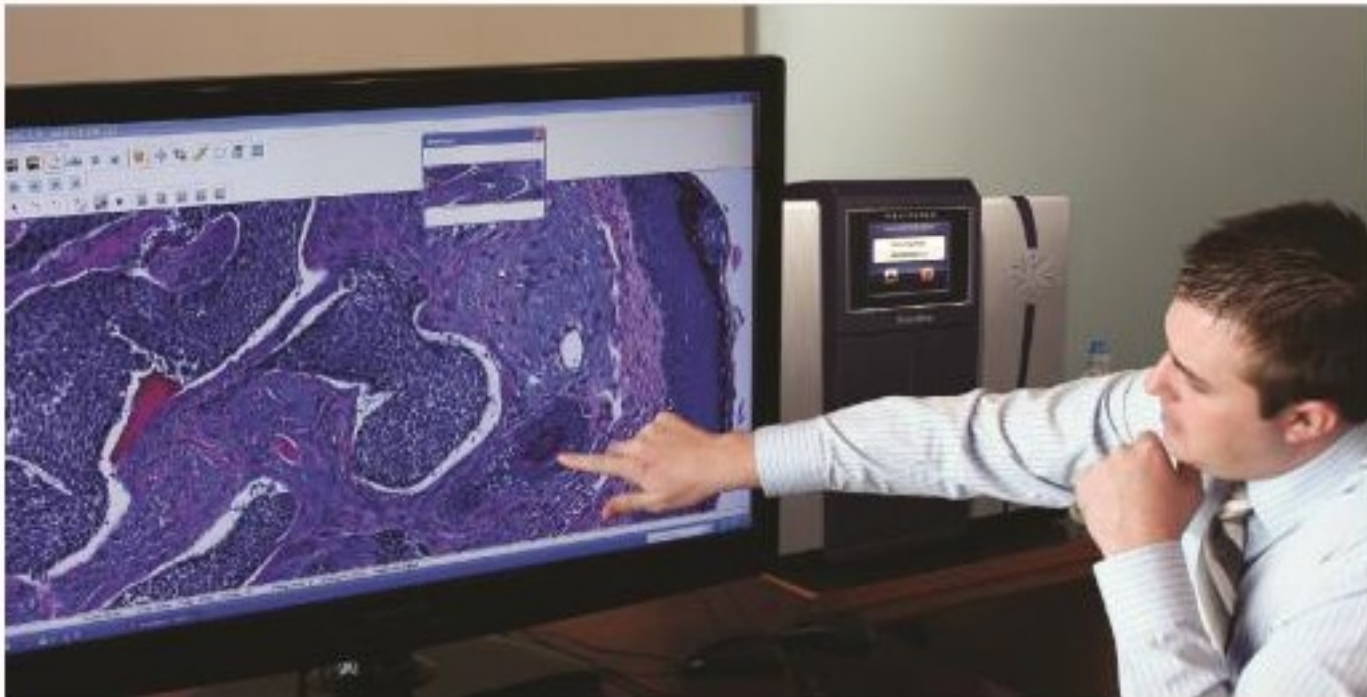
ARTICLE

Tumor in Bronchial Biopsy  
Specimens

MB, ChB,\* Salmah Bakar, MD, MPath,\*  
MD, PhD,† Marianne C. Nicolson, MD,‡  
FRCPath\*

















# Patologia Molecular

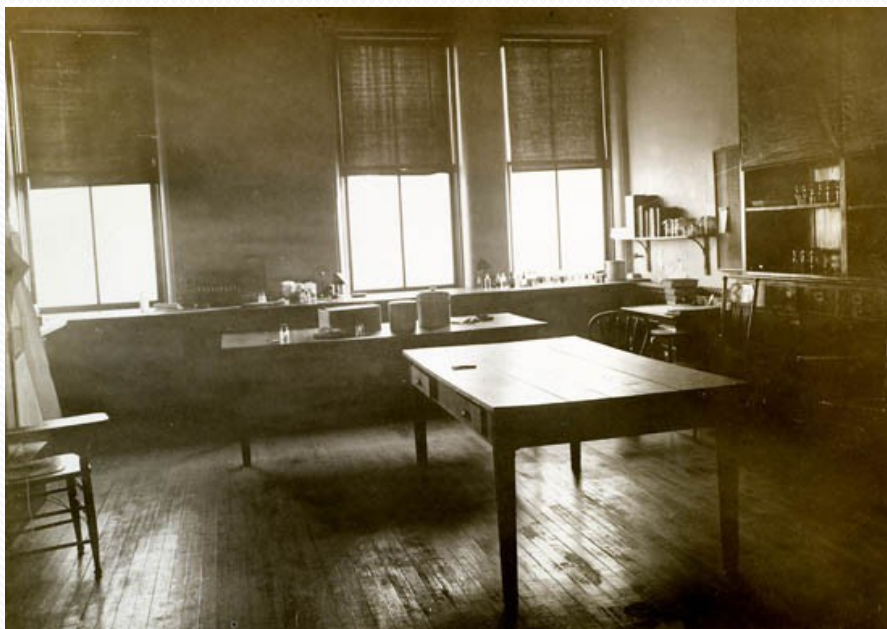


Designed for medical students in the late 19th Century, this Wenham-style binocular microscope, designed and built by Henry Crouch typifies advancements of the period.

- Imuno-histoquímica
- Hibridação *in situ*
- FISH



- cDNA microarray
- Blots
- PCRs
- Sequenciamentos









**OBRIGADO**