免疫组化的质量控制

Quality Control of Immunohistochemistry

昆明医科大学第一附属医院

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2021年5月

附件 5

病理专业医疗质量控制指标
（2015 年版）

一、每百张病床病理医师数

定义：平均每 100 张实际开放病床病理医师的数量。

计算公式：

$$每百张病床病理医师数=\frac{病理医师数}{同期该医疗机构实际开放床位数/100}$$

意义：反映病理医师资源配置情况。

二、每百张病床病理技术人员数

定义：病理技术人员是指进行病理切片、染色、免疫组化及分子病理等工作的专业技术人员。每百张病床病理技术人员数，是指平均每 100 张实际开放病床病理技术人员的数量。

计算公式：

$$每百张病床病理技术人员数=\frac{病理技术人员数}{同期该医疗机构实际开放床位数/100}$$

意义：反映病理技术人员资源配置情况。

三、标本规范化固定率

定义：标本规范化固定是指病理标本及时按行业推荐方法切开，以足量 10%中性缓冲福尔马林充分固定。有特殊要求者可使用行业规范许可的其它固定液。标本规范化固定率是指规范化固定的标本数占同期标本总数的比例。

计算公式：

$$标本规范化固定率=\frac{规范化固定的标本数}{同期标本总数}\times 100\%$$

意义：反映处理标本是否及时规范的重要指标。

四、HE 染色切片优良率

定义：HE 染色优良切片是指达到行业优良标准要求的 HE 染色切片。HE 染色优良切片优良率，是指 HE 染色优良切片数占同期 HE 染色切片总数的比例。

计算公式：

$$HE\ 染色切片优良率=\frac{HE\ 染色优良切片数}{同期\ HE\ 染色切片总数}\times 100\%$$

意义：反映病理科 HE 染色、制片质量的重要指标。

五、免疫组化染色切片优良率

定义：免疫组化染色优良切片是指达到行业优良标准要求的免疫组化染色切片。免疫组化染色优良切片优良率，是指免疫组化染色优良切片数占同期免疫组化染色切片总数的比例。

计算公式：

$$免疫组化染色切片优良率=\frac{免疫组化染色优良切片数}{同期免疫组化染色切片总数}\times 100\%$$

意义：反映病理科免疫组化染色、制片质量的重要指标。

六、术中快速病理诊断及时率

定义：在规定时间内，完成术中快速病理诊断报告的标本数占同期术中快速病理诊断标本总数的比例。规定时间是

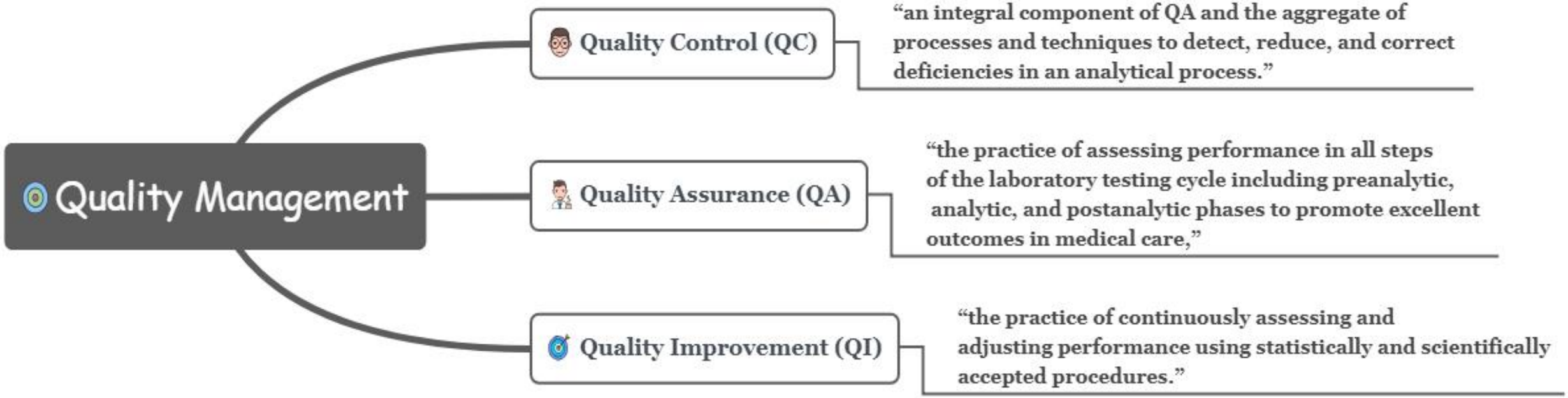
PART ONE

01

质量管理概述

Conception of Quality Management

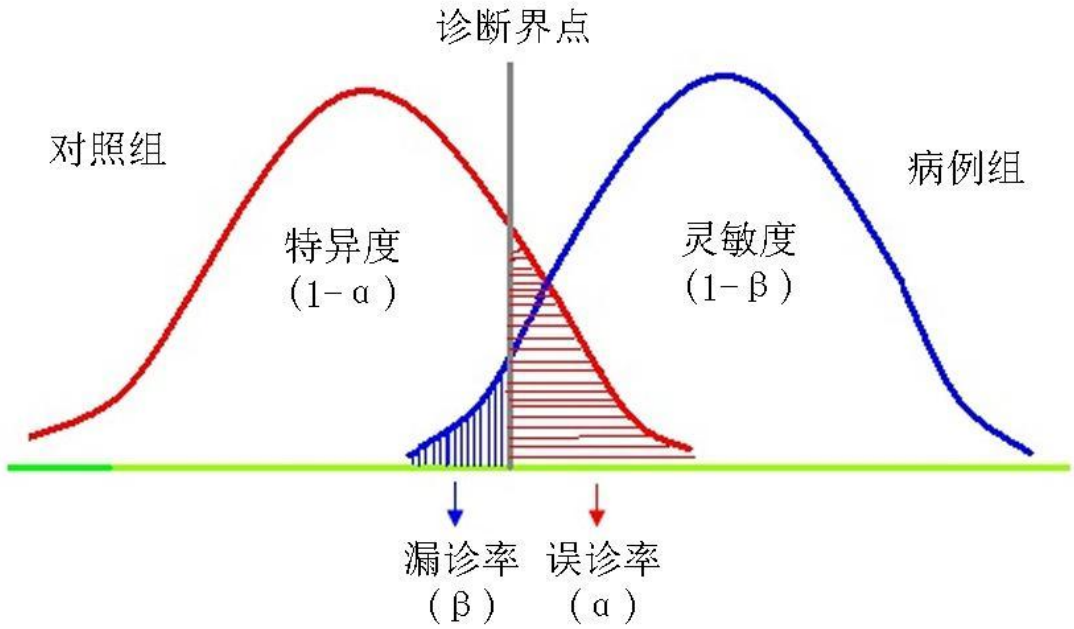
The College of American Pathologists (CAP)对质量控制的定义



免疫组化染色“质量”的基本考量

- 是否能检测到存在的抗原？——敏感性
- 如果抗原不存在是否能得到阴性结果？——特异性

最佳信躁比（ signal-to-noise ratio）



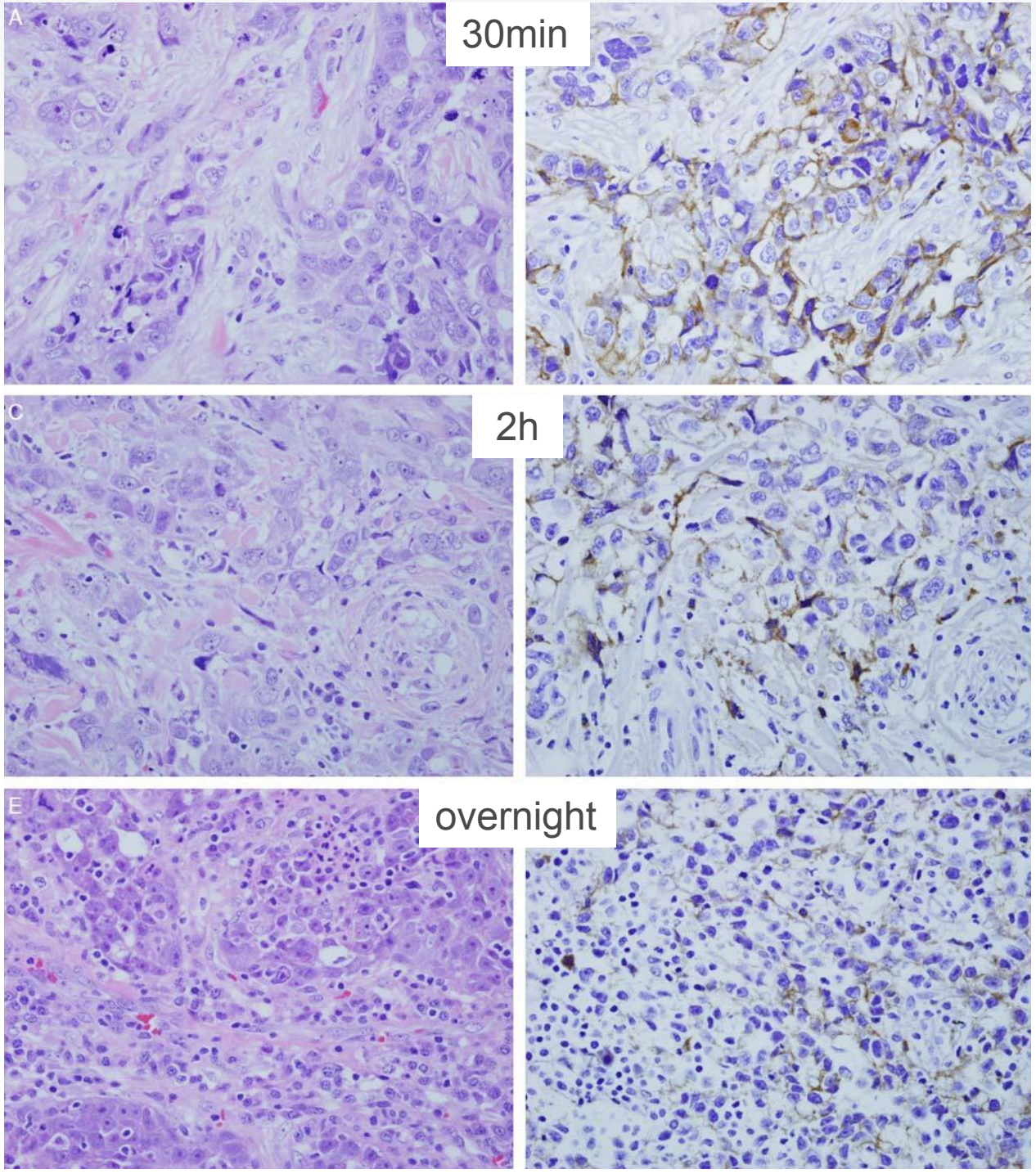
PART TWO

组织固定和处理

TISSUE FIXATION AND PROCESSING

组织固定

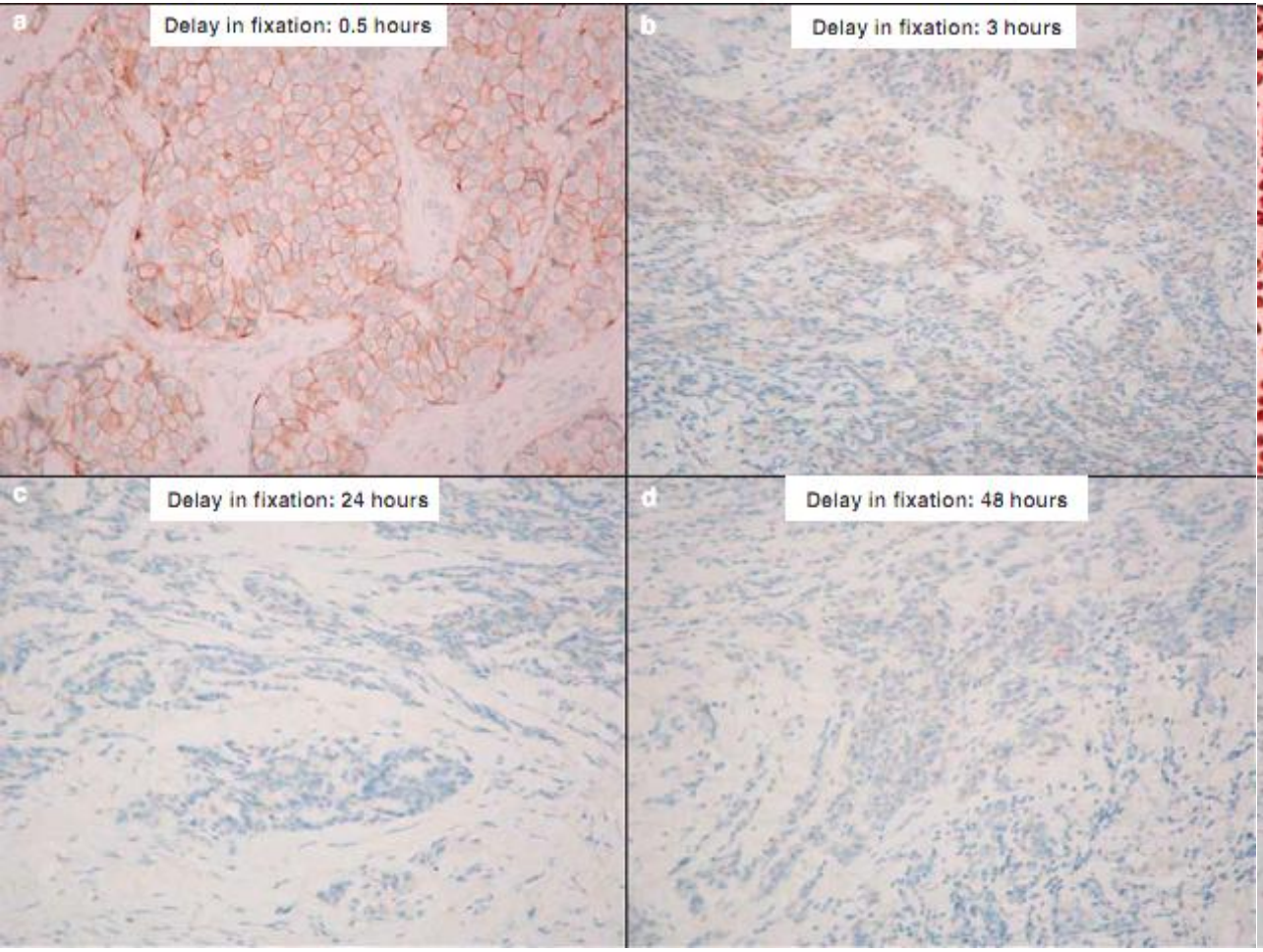
- 组织应尽快切开固定
- 冷藏会延迟、但不能防止组织自溶
  - 抗原降解导致假阴性
  - 非特异性结合导致高背景着色
- 冷缺血时间对膜抗原免疫组化染色影响尤其明显（<1h）



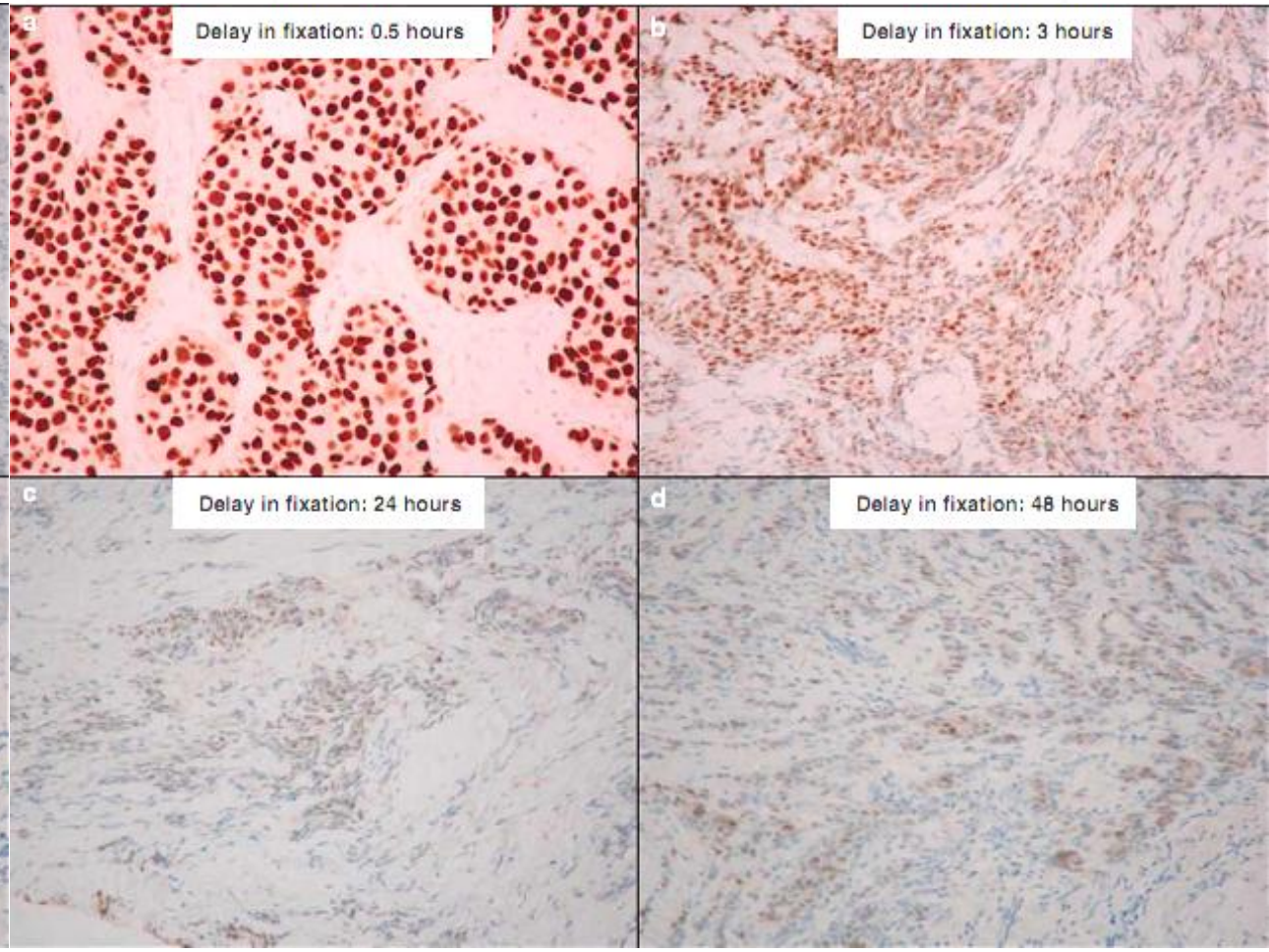
Thaer Khoury,Appl Immunohistochem Mol Morphol,2012

延迟固定对免疫组化的影响

HER2

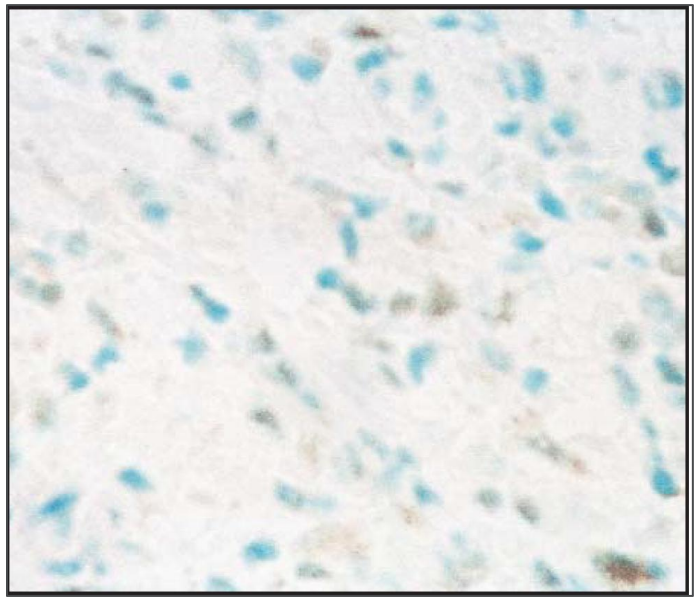


ER

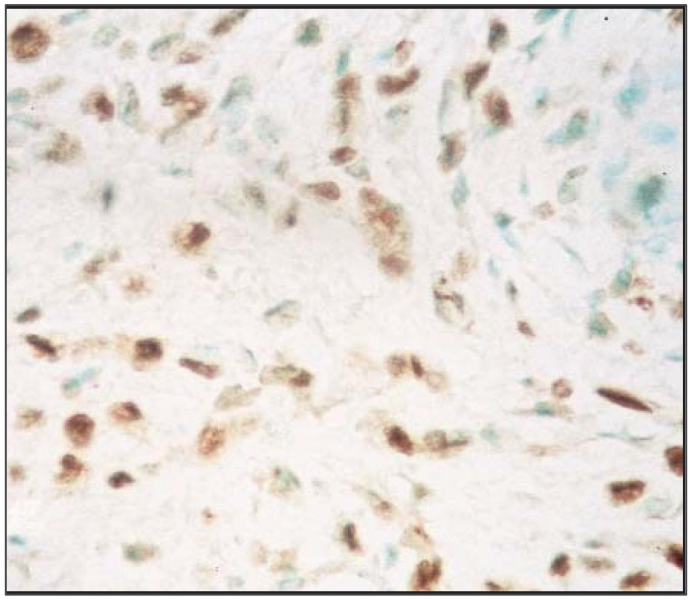


组织固定

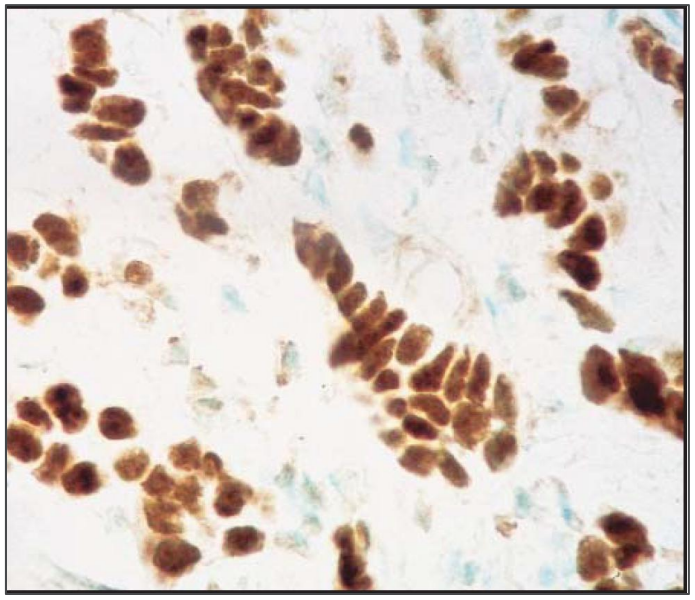
- 固定时间：推荐固定6-24小时
- 固定液种类：10%中性磷酸缓冲福尔马林



固定3小时



固定6小时



固定8小时

Goldstein NS,et al. Appl Immunohistochem Mol Morphol. 2007

PART THREE 03

抗体和检测系统评价

ANTIBODY AND DETECTION SYSTEM EVALUATION

抗体

- 开展新的检测项目之前，应查阅文献、外部质量控制报告和网络资源选择合适的抗体
- 单克隆抗体：特异性好，敏感性较差

  多克隆抗体：敏感性高，易产生交叉反应或非特异性着色

  即用型抗体（ready to use,RTU)：稳定、重复性好；在过程优化中灵活性较差

检测系统

- 检测系统对免疫组化染色质量的影响与抗体同样重要
- 亲和素-生物素检测系统已经逐渐被废弃
  - 非特异性着色
  - 假阳性
- 酶/抗体偶联聚合物检测系统

PART FOUR

04

流程优化和标准化

PROTOCOL STANDARDIZATION AND OPTIMIZATION

流程标准化

- 流程标准化的目的：增加准确性和可重复性，减少各轮检测间的差异
- 要求：制定详细的SOP，并严格执行
  - 最佳抗体稀释度
  - 一抗孵育时间和温度
  - 最佳抗原修复方法和时间
  - 检测系统
  - 推荐的对照组织
  - 预期的结果阳性模式

  } 具体到抗原克隆和货号

- 自动化能够达到较高的标准化程度
- 流程的标准化要求组织固定和处理的标准化，其他组织固定方法应予重新确证和优化

流程优化

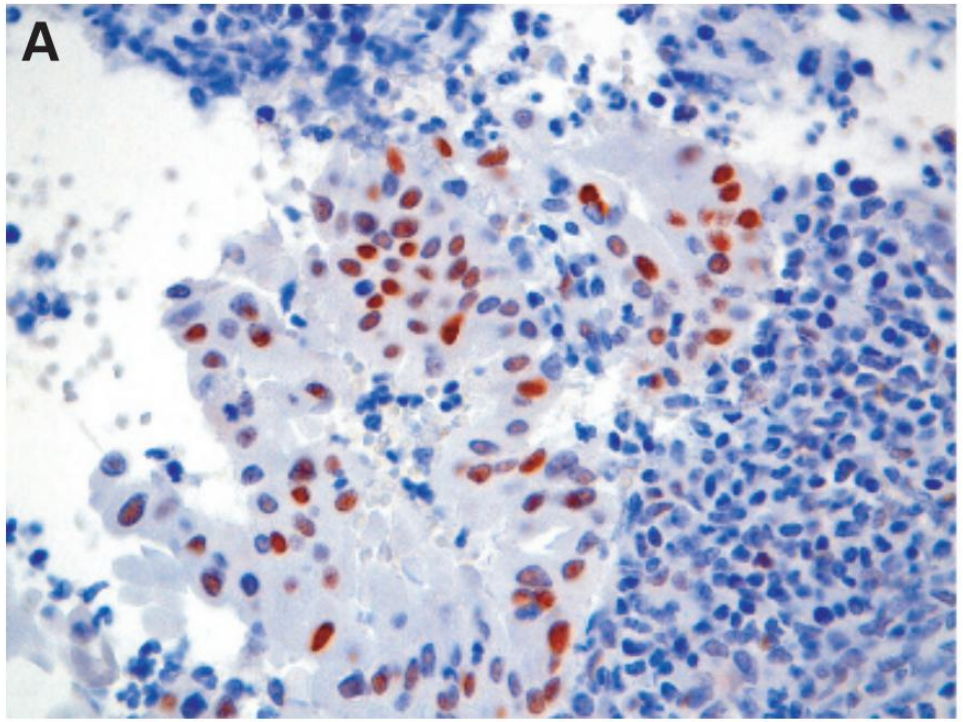
- 目标：稳定、高质量的染色
- 优化是一个经验性的过程
  - 参考产品说明书的推荐
  - 参考其他实验室、文献、外部质量控制报告
  - 各个实验室都有适合自己的最优方案，无法照搬
- 推荐使用弱表达抗原的组织来进行流程的优化

Goldstein NS,et al. Appl Immunohistochem Mol Morphol. 2007

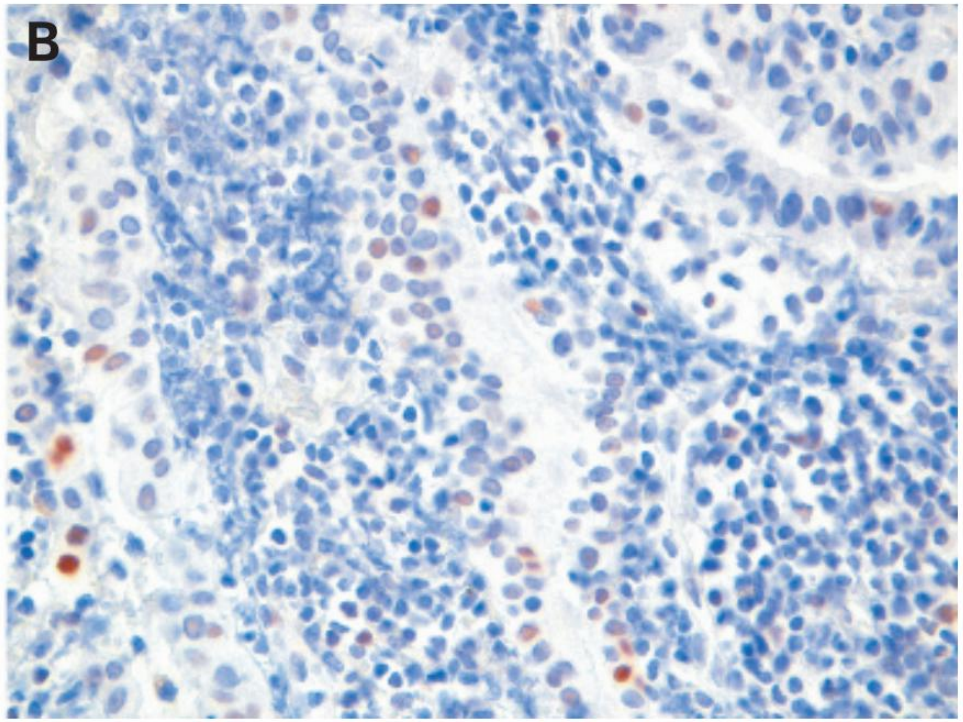
TABLE 2. List of Factors That Could be Adjusted During the Antibody Optimization

Parameter	Description
No pretreatment	Some antibodies still perform best without any type of pretreatment
Enzyme digestion	Few antibodies perform best only when enzyme digestion was used without the need for heat induced epitope retrieval
Retrieval buffer	The combination of the type of buffer (ie, citration, ethylenediaminetetraacetic acid, trishydroxymethylaminomethane), and pH level can result in dramatically different signal intensity and signal-to-noise ratio
Heating device	That is, pressure cooker, electronic water bath, microwave, steamer, hot plate
Primary antibody incubation time	This varies depending on the affinity of the antibody to its antigen target, the primary antibody concentration, incubation temperature, and antigen levels in target tissue
Detection system	Polymer detection systems may allow to further dilute the antibody titer, given their generally higher sensitivity than avidin-biotin systems. Tyramine amplification systems are the most sensitive, but also most cumbersome
Chromogen	Prolonging the application of chromogen often lead to more intense signal, but could also compromise the signal-to-noise ratio

ER免疫组化染色，热修复



抗原修复液：PH 6



抗原修复液：PH 3

Bussolati, et al. J Clin Pathol, 2008

05

PART FIVE

抗体验证

VALIDATION OF THE IHC ASSAY

- 目的：确保检测的准确性、可靠性、可重复性

  确保检测发挥预期的功效
- 新的检测项目、新抗体、新克隆号、新货号、新方法、新设备在投入临床诊断的使用之前均应进行验证
- 进行验证的标本数量没有统一要求
  - 对于仅评价为阴性/阳性的抗体：至少25例

    阴性5例，高表达至少10例，低表达至少10例
  - 对于结果评价更复杂的抗体，例数要求更多
  - 对于提示预后和治疗的关键性抗体，验证要求更为严苛

推荐采用的验证方法

Table 1. Acceptable Validation of Hormone-Receptor Assays[a]

ER and PgR IHC assays not subjected to direct clinical validation may be validated by showing 90% agreement for positive results and 95% agreement for negative results with any of the following:

1. Testing performed on the same blocks in another laboratory that has directly validated its assay against clinical outcome
2. Testing performed on the same blocks using a previously validated ligand binding assay
3. Testing performed on the same blocks in another laboratory that provides written attestation that it is in conformance with ASCO/CAP testing requirements *and* is using one of the following:
   a. An FDA-approved assay that has been fully validated using an 80-specimen challenge set as described in Table 3, *or*
   b. An LDT or LMT that has been validated according to all other requirements set forth in this document
4. Testing performed on the same blocks in another laboratory that uses an alternative, clinically validated method for measuring hormone-receptor expression (eg, a gene-expression assay)[17]
5. Testing performed on one of the following:
   a. Tissue challenges used in a formal PT program, provided that each case used in the validation study was graded by the PT vendor and ≥50 laboratories are included in the participant's peer group, *or*
   b. Validation tissues provided by an organization such as the CAP or the NIST, with established ER and PgR status determined through IHC testing using a technically validated assay[24]

Fitzgibbons, et al.Arch Pathol Lab Med, 2010

抗体验证表单

IHC Validation Study - *Name of Laboratory*

To be completed following individual instrument and methodology validations

Antibody	**INI1**				Technologist:	
Current Methodology:						
Current Instrument:					Pathologist reviewing slides:	
New Methodology:						
New Instrument:						
Date:					Protocol Number:	

Specimen #	Diagnosis	Expected Result	Actual Result	Acceptable or Unacceptable Performance	Comments	Date
	Epithelioid sarcoma	negative	negative	acceptable		
	Epithelioid sarcoma	negative	negative	acceptable		
	Epithelioid sarcoma	negative	negative	acceptable		
	Epithelioid sarcoma	negative	negative	acceptable		
	Epithelioid sarcoma	negative	negative	acceptable		
	normal tonsil	positive	positive	acceptable		
	normal tonsil	positive	positive	acceptable		
	normal tonsil	positive	positive	acceptable		
	normal tonsil	positive	positive	acceptable		
	normal tonsil	positive	positive	acceptable		
	normal tonsil	positive	positive	acceptable		
	normal tonsil	positive	positive	acceptable		
	normal tonsil	positive	positive	acceptable		
	normal tonsil	positive	positive	acceptable		
	normal tonsil	positive	positive	acceptable		

Conclusions: In the 5 cases available for which the expected result is negative, INI1 is appropriately negative. The stain is also appropriately positive in 10 tissue samples used as validation controls. Given the limited number of INI1-negative tumors available for validation, the number of specimens evaluated (N=15) is considered appropriate for this marker (concordance = 100%; minimum 95% CI, 80%). Precision is acceptable. In summary, the INI1 IHC stain is performing adequately for clinical use (100% sensitivity; minimum 95% CI, 57%; 100% specificity; minimum 95% CI, 72%), but it should be monitored prospectively.

This validation study has been reviewed and the performance of the method is considered acceptable for patient testing.

Medical Director | Date

PART SIX

日常质控

DAILY QUALITY CONTROL

日常质控

- 日常质控是免疫组化质量管理的关键
- 内容：
  - 设置对照
  - 发现并分析标本和对照的非预期的染色模式
  - 早期发现抗体敏感度和特异度降低的征象
  - 分析原因并及时解决
  - 记录问题和解决的方法及结果

如何发现？谁来发现？

对照

- 对照组织的固定和处理程序应该与待检样本的程序一致
- 阳性对照
  - 表达目标抗原，且表达强度明确的组织
  - 用弱阳性对照比强阳性对照更能反映敏感度、减少假阴性
  - 内对照
- 阴性对照
  - 已知不表达目标抗原的组织
  - 也可以使用阳性对照组织块中不表达目标抗原的部分
  - 春卷对照

◈ 空白对照：除一抗外，所有程序均与常规染色一致

用无关抗体、缓冲液等替代一抗

◈ 同一组织同时做不同标记时，可以互为空白对照

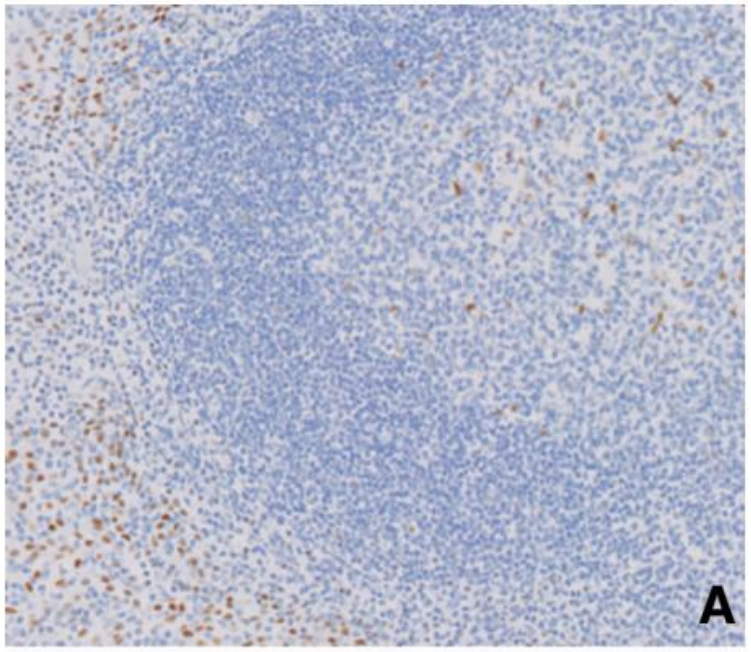
◈ 目的：观察非特异性背景着色

识别色素、含铁血黄素、脂褐素等易与阳性染色混淆的区域

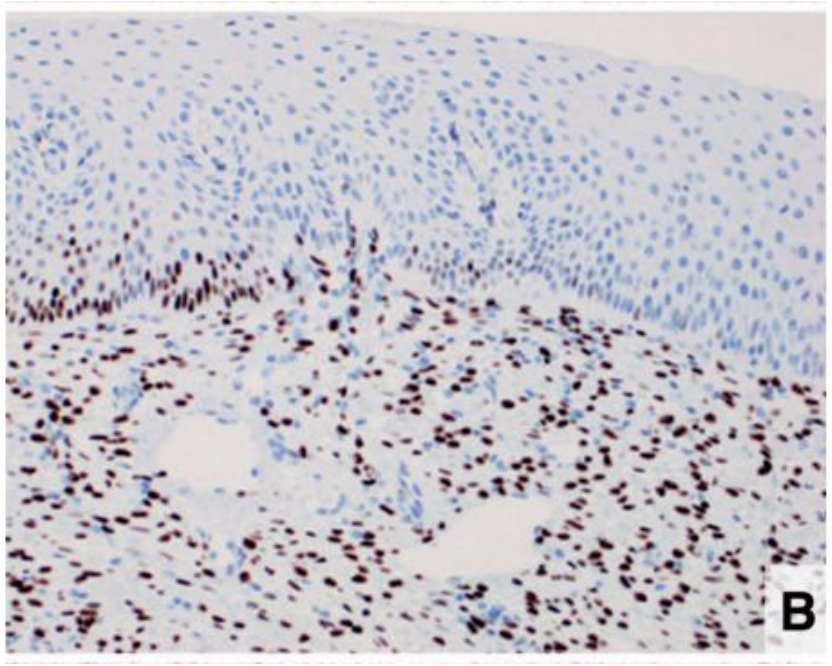
◈ 注意：对照蜡块提前切片储存可能会导致抗原丢失

建议每张切片设对照

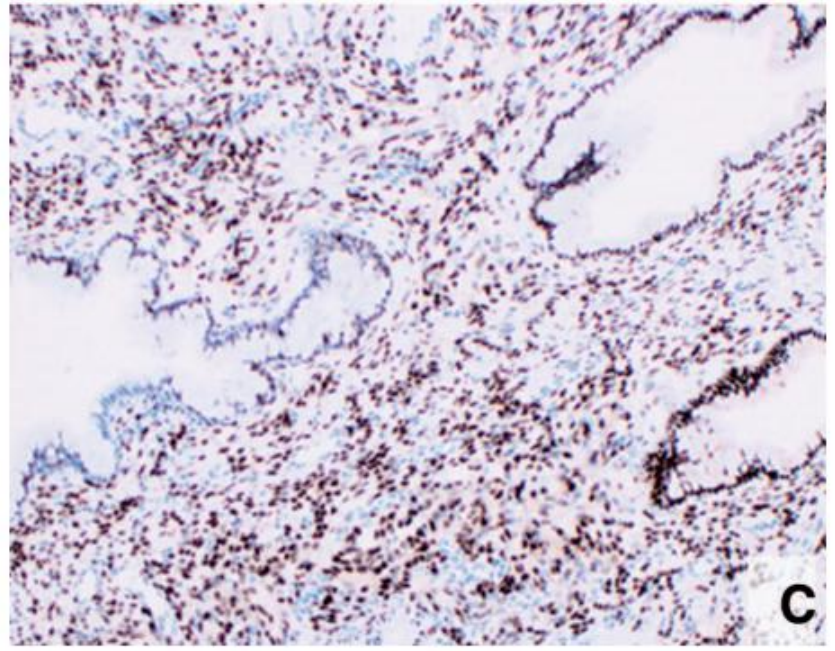
外对照



ER 扁桃体



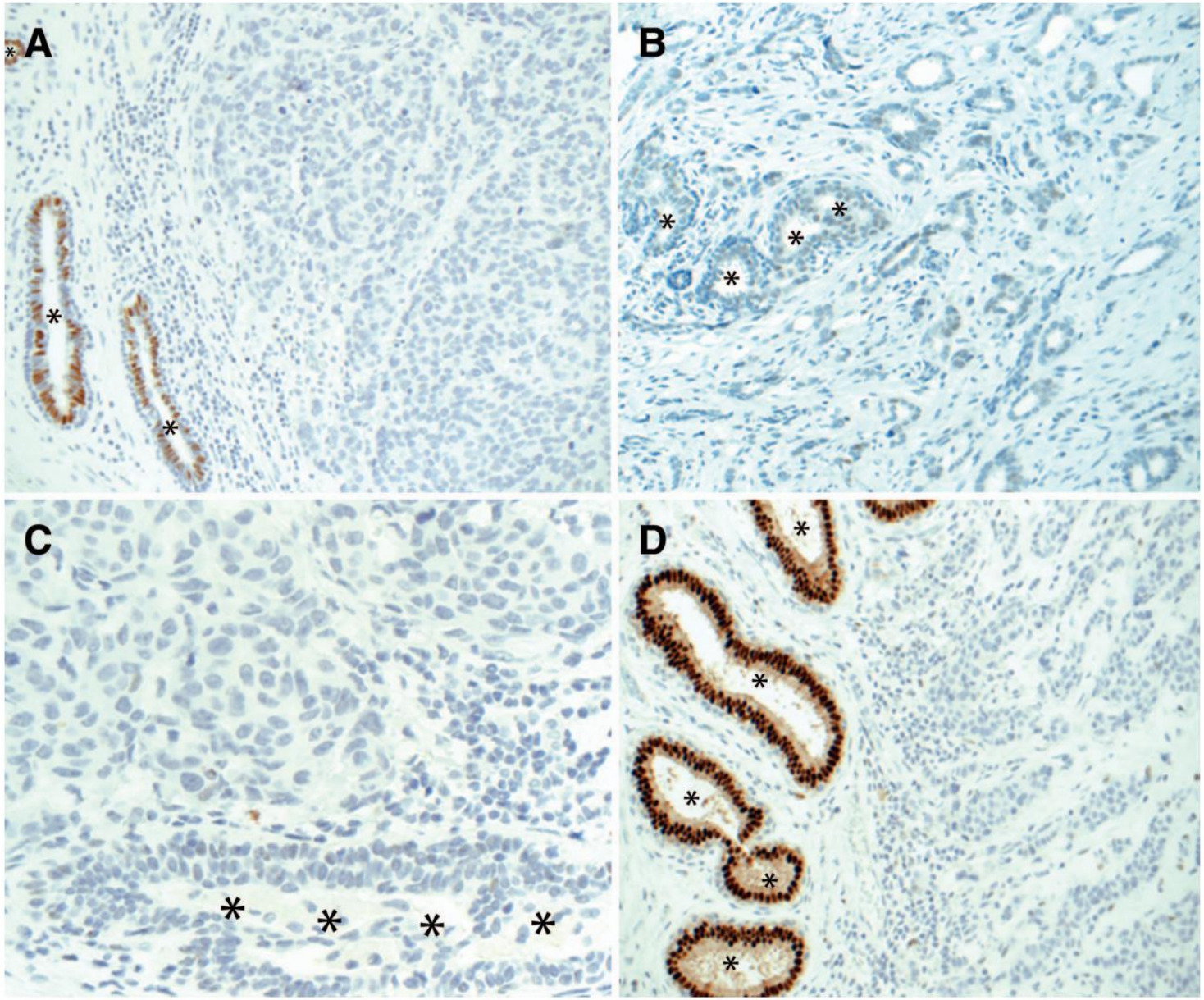
PR 宫颈管



PR 宫颈

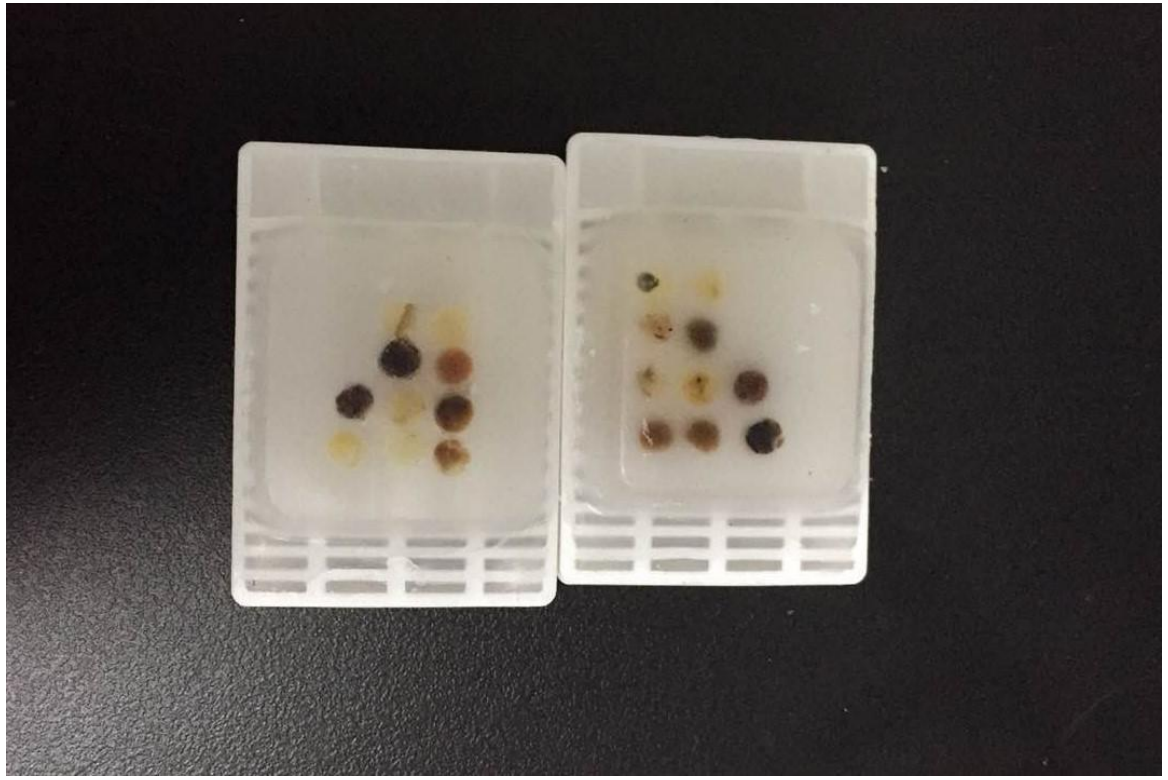
Allison, et al. Arch Pathol Lab Med, 2020

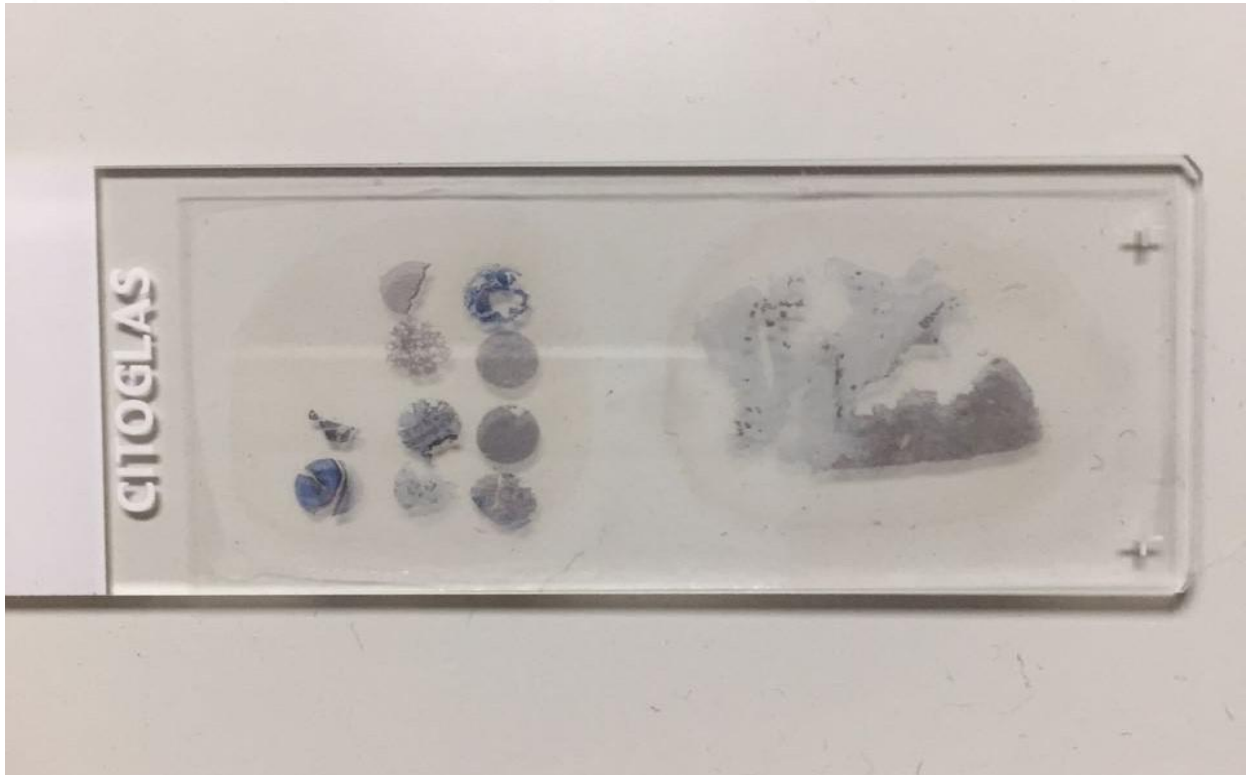
内对照 ER染色



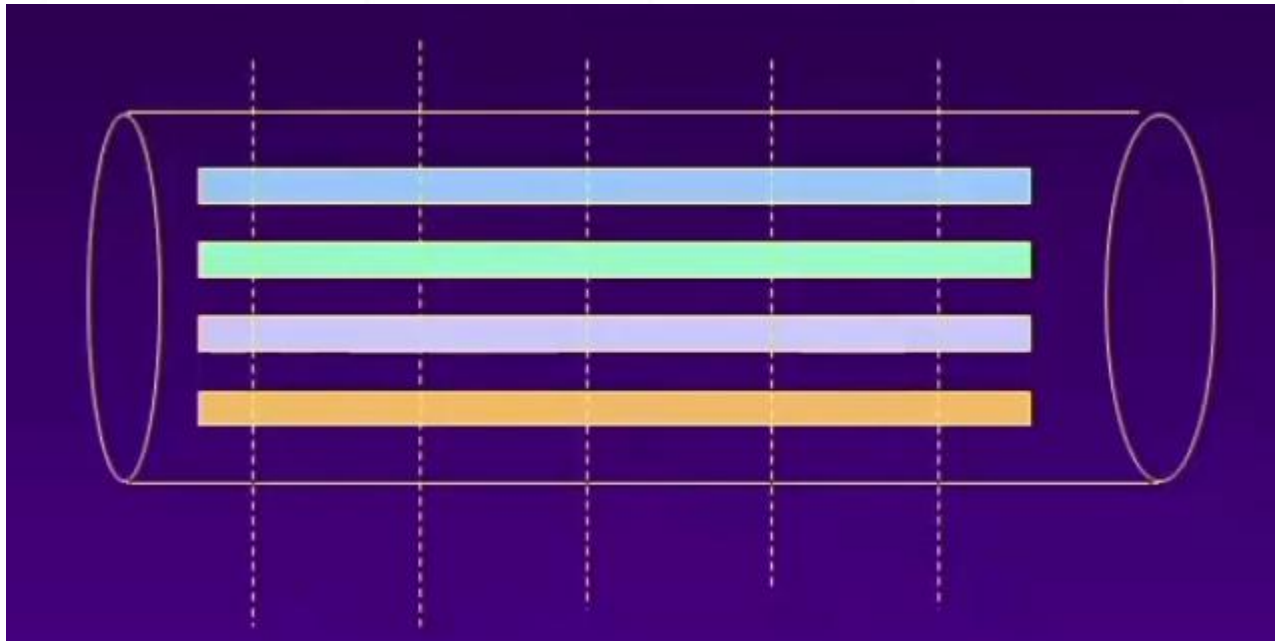
Allison, et al. Arch Pathol Lab Med, 2020

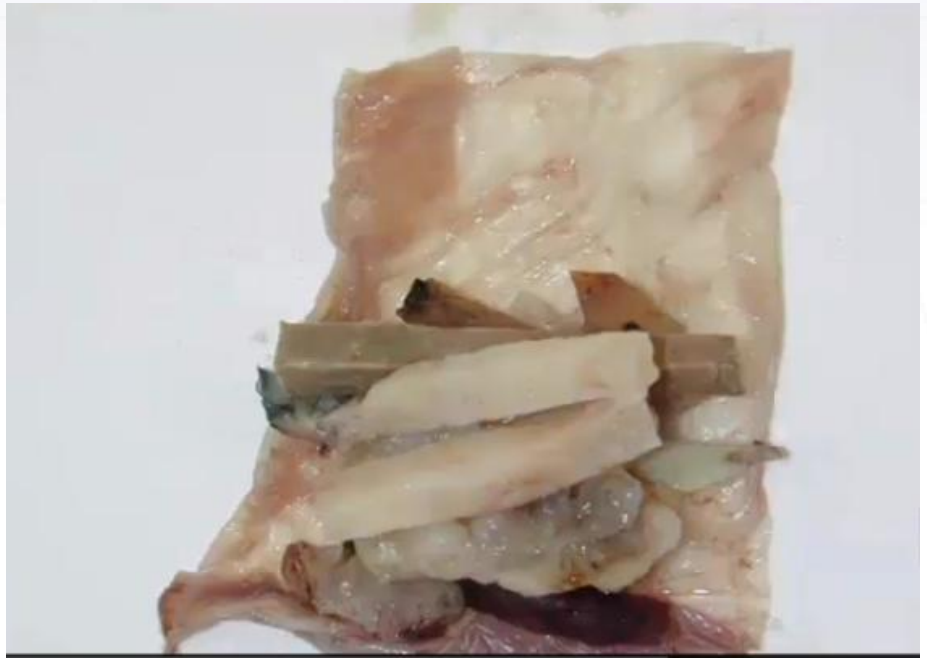
多组织对照





春卷多组织对照





推荐阳性对照

- 羊膜（GATA-3）
- 甲状腺（TG，TTF-1）
- 前列腺（PSA，p63）
- 肺（CK7，Napsin A，TTF-1）
- 子宫肌层（ER，PR，肌肉标记物）
- 大肠（部分CK，CGA，CEA，CDX2，CR）
- 扁桃体（部分CK，大多数CD抗体，p63，p53，Ki67）
- 脑（GFAP，SYN，S100，CD56，CDK4）
- 胸腺（TdT，CD1a）
- HBsAg+的肝（HBsAg，Arginase-1，Hep-Par1，部分CK，SMA，部分CD抗体）
- HER2+的乳腺癌（HER2，GATA3，Mammaglobin，GCDFP-15）

Standardization of Positive Controls in Diagnostic Immunohistochemistry: Recommendations From the International Ad Hoc Expert Committee

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Appl Immunohistochem Mol Morphol 2015 Jan;23(1):1-18

07

PART SEVEN

质量监测

QUALITY ASSURANCE MONITORS

- 实验失败（重复实验）的原因记录和分析
- 外部质量控制
- 对不常用的抗体进行监控管理
- 实验室所有的管理文件和操作流程都应每年进行整理

外部质量控制

- CAP (http://www.cap.org/apps/cap.portal)
- HistoQIP, jointly offered by CAP and the National Society for Histotechnology (http://www.nsh.org/content/all-abouthistoqip)
- The United Kingdom National External Quality Assessment Service (http://www.ukneqas.org.uk)
- NordiQC (http://www.nordiqc.org)
- The Canadian Immunohistochemistry Quality Control group
- PQCC
- 各级质控中心

PART EIGHT

结果报告

PATHOLOGY REPORTS

具有预后和治疗意义的标记

Estrogen and Progesterone Receptor Testing in Breast Cancer

American Society of Clinical Oncology/College of American Pathologists Guideline Update

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·共识与指南·

乳腺癌 HER2 检测指南（2019 版）

《乳腺癌 HER2 检测指南（2019 版）》编写组

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其他标记

- 依据免疫组化标记的诊断运用来报告
  - 阳性细胞种类：肿瘤、间质、肿瘤中的某种成分……
  - 阳性细胞百分比：p53、Ki-67……
  - 阳性细胞的分布：局灶、弥漫（S100）……
  - 阳性模式：胞膜、胞浆、胞核、核旁点状（β-catenin、EMA……）

TABLE 1. Quality Management Activities for the Immunohistochemistry Laboratory

	Quality Control	Quality Assurance	Quality Improvement
Daily	Review QC slides	Assess/investigate analytic problems Assess/investigate repeat stains Turn-around time log	
Weekly	Equipment maintenance	Report repeat stains and analytic problems/ resolutions to MD Prospective monitoring/review of IHC stains for recently validated antibodies	
Monthly		Assess turn-around time logs Surveillance program for infrequently ordered IHC assays	QA/QC report to AP QM Committee
Biannually		External Proficiency Testing Internal audit of pathology reports Review regulatory requirements (CAP, CLIA, etc.)	Report Proficiency Testing results to AP QM Committee Report audit results to AP QM Committee
Annually	Review laboratory technical equipment and staffing	Review and update QM program Review and update IHC procedure manuals	Continuing education Review volume of specific IHC assays (test inventory review) Compare IHC results with reported data
As needed	Optimization of new assays Validation of new assays Verification of new reagent lots	Troubleshoot analytic problems Implement corrective actions	Protocol standardization Evaluation of new platforms and reagents (antibodies, detection systems, etc.)

AP indicates anatomic pathology; CAP, College of American Pathologists; CLIA, Clinical Laboratory Improvement Amendments; IHC, immunohistochemistry; MD, medical director; QA, quality assurance; QC, quality control; QI, quality improvement; QM, quality management.

感谢聆听